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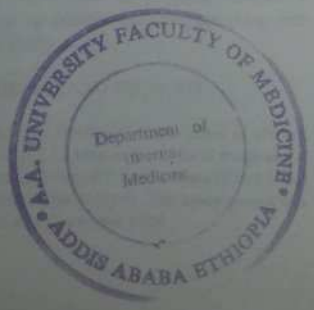
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CHRONIC LYMPHOCYTIC LEUKAEMIA IN ETHIOPIANS
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CHRONIC LYMPHOCYTIC LEUKAEMIA IN ETHIOPIANS

M. SHAMEBO and A. GEBREMEDHIN

SUMMARY

One hundred and two cases of chronic lymphocytic leukaemia (CLL) were seen at the Tikur Anbessa (Black Lion) Hospital, in Addis Ababa, Ethiopia, from January 1982 to December 1994. The age range was 35-91 (mean 55.6±11.08) years. The male to female ratio was 3.6:1. The commonest symptoms were weakness, weight loss, fever and sweating. The commonest signs were lymphadenopathy, splenomegaly and hepatomegaly. Fifty six per cent had Rai stage III and IV, only three patients were in stage 0. Of those treated with chemotherapy, 22.0% and 48.8% achieved complete and partial remissions respectively. Twelve patients are still alive and on follow up for 2-138 (median 18) months, 69 are lost to follow up after 0-132 (median 3) months and 21 are dead 0.5-84 (median 2.8) months after diagnosis. Of those that died, 13 were in stage IV and five in stage III. The main causes of death were septicaemia of undetermined origin in eight and pneumonia in seven. Thus CLL is not a rare disease in this centre. Its presentations are similar to cases reported in the literature. Optimal treatment is not possible due to lack of chemotherapeutic agents and supportive care. Therefore, we suggest that referral centres be equipped for better management of CLL patients.

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is a haematological malignancy characterised by monoclonal proliferation and accumulation of relatively mature looking small lymphocytes(1-5).

It is said to be the commonest type of leukaemia in Europe and North America but is rare in the Orient(1-6) and Papau New Guinea(7). Reports from Africa show that the prevalence of CLL is variable. In Nigeria(8,9), it accounted for 40% of all leukaemias, while in Zimbabwe(10), Kenya(11) and Sudan(12), it is the least common type of leukaemia at 20%, 19% and 13.0% respectively. The median age in Caucasians is 60-65 years (1-5,13) while in Africans it occurs about ten years earlier (8-12,14). In Caucasians and Africans, males are affected twice as often as females except in Nigeria where young females are affected more frequently(8,9). The dominant clinical feature in Caucasians is lymphadenopathy (1-5,13) while in Africans splenomegaly dominates (8-9,14). In Ethiopia, CLL accounted for 12-21% of all leukaemias (15-17); these studies, however, were not detailed. In this study, which includes the previously reported 38 cases, we present a detailed description of clinical, laboratory, treatment and follow up results and compare these with similar studies elsewhere.

MATERIALS AND METHODS

One hundred and two consecutive new cases of chronic lymphocytic leukaemia (CLL) seen in the medical wards and at the haematology referral clinics of Tikur Anbessa (Black Lion) Hospital, were included in the analysis. The study period was between January 1982 and December 1994.

The age, sex, address, date of diagnosis, clinical, haematological, biochemical, bone marrow aspiration results, treatment, outcome and follow up status were recorded for each patient. Antiglobulin (Coomb's) test was performed only on 31 patients.

The diagnosis of CLL was based on clinical and haematological findings: persistent lymphocytosis ≥10,000/mm^3 and bone marrow lymphocyte count of ≥30% of marrow cells (1-6) stained with the Wright's stain. Immunological cell markers were not performed due to lack of facilities.

The clinical staging was according to Rai *et al*(18). The indications for treatment were; Rai stages III, IV or bulky and symptomatic stages I or II (3-6,21). Chlorambucil 0.1-0.2 mg/kg/day or cyclophosphamide 2-3 mg/kg/day with or without prednisolone 40-60 mg/m^2/day were used (3-6,19). Four patients received radiotherapy over the spleen as used by Bayhardt *et al*(20). Treatment was given for at least six months before evaluating response(5).

Response criteria were as those used by Sawitsky *et al*(19). Complete remission (CR)=normalisation of all clinical and haematological parameters; Partial remission (PR)=reduction in the size of organomegaly to 50% of that at diagnosis and lymphocyte count less than 15,000/mm^3; No response (NR)=No change in the clinical or lymphocyte count; Progression=worsening over 28 days; increase in size of organs and in lymphocyte count.

RESULTS

During the study period of 12 years, 102 cases of chronic lymphocytic leukaemia (CLL) were seen in the adult medical wards and the haematology referral clinics of Tikur Anbessa Hospital, Addis Ababa. There were 80 males and 22 females giving a male to female ratio of 3.6:1. The age range was 35-91 (mean 55.6) years. Table

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1 shows the age and sex distribution of CLL in adults. In 36.3%, CLL occurred below the age of 51 years of age.

Table 1

Age and sex distribution of 102 chronic lymphocytic leukaemia patients in Tikur Anbessa Hospital

Age group (years)	Sex		Total	
	Male	Female	No	%
30-40	8	3	11	10.8
41-50	18	8	26	25.5
51-60	30	6	36	35.3
61-70	15	5	20	19.6
71-80	8	-	8	7.8
≥80	1	-	1	0.98
Total	80	22	102	100

Table 2

Presenting symptoms in 102 chronic lymphocytic leukaemia patient

Symptom	No.	%
Weakness	48	47.6
Weight loss	26	25.5
Fever and/or sweating	16	15.7
Cough	15	14.7
Abdominal mass	13	12.7
Incidental	5	4.9
Pruritis/skin lesions	5	4.9
Bleeding tendencies	3	2.9
Other	5	4.9

Table 3

Presenting signs in 102 chronic lymphocytic leukaemia patients

Sign No.	%	
Lymphadenopathy	94	92.2
Splenomegaly	57	55.9
Hepatomegaly	33	32.5
Pallor	38	37.3
Sternal tenderness	3	2.9
Skin lesions	2	1.9
Others	3	2.9

The clinical presentation at diagnosis are shown in Tables 2 and 3. Lumps in the neck and constitutional symptoms accounted for 89.2% and 88.8% respectively. In five patients (4.9%) the diagnosis was incidental, ie. patients were undergoing investigations for unrelated symptoms or for employment purposes. Lymphadenopathy was the predominant sign seen in 92.2% and was usually generalized. Splenomegaly and hepatomegaly occurred in 55.9% and 32.4% respectively. Sternal tenderness was noted in only three patients.

Table 4

Haematological parameters at diagnosis in chronic lymphocytic leukaemia patients

Parameter	Mean±2SD
Haemoglobin (g/dl)	10.77±3.78
Lymphocyte count (x10 mm^3)	150±183
Platelet count (x 10mm^3)	137.5±112

Table 4 shows the haematological parameters. The mean haemoglobin (Hgb) was 10.77 g/dl, range 3.1-16.4 g/dl. 37.3% of the patients had a Hgb of less than 10.0 g/dl. The lymphocyte count ranged between 16,340-864,000/mm^3, with a mean of 150,187/mm^3. 65.8% had lymphocyte count greater than 50,000/mm^3. The platelet count ranged from 10,000 to 550,000/mm^3 with a mean of 137,574/mm^3; 37.4% had a platelet count of less than 100,000/mm^3.

The antiglobulin (Coomb's) test was negative in the 31 patients it was tested. Serum protein electrophoresis was performed in only 23 patients; gamma globulins were depressed in 7(30.4%).

Table 5 shows the clinical stages of CLL patients at diagnosis according to Rai *et al*(18). The majority (56%) presented with stages III and IV. Only 12.7% were at stages 0 and 1.

Table 5

Rai clinical states in 102 patients with chronic lymphocytic leukaemia, Tikur Anbessa Hospital

Stage	No.	%
0	3	2.8
I	10	9.8
II	32	31.4
III	19	18.6
IV	38	37.4

Fifty five patients were treated with what was available at the time of diagnosis. Seventeen received chloroambucil and 34 cyclophosphamide, with or without prednisolone. Four received local radiotherapy over the spleen. Forty seven patients were not treated; 15 had early disease, 24 were lost to follow up and eight died shortly after the diagnosis of CLL.

Chloroambucil produced complete or partial responses in 26.7% and 46.7% respectively. Clinical improvement was seen in 26.7%. Cyclophosphamide produced complete or partial responses in 19.2% and 50% respectively. Clinical improvement was noted in 30.8%. Two patients in chlorambucil group and eight in cyclosphosphamide group were not evaluable as they were lost to follow up soon after intiation of therapy. Of the four who were treated with radiotherapy over the spleen, one attained partial remission, two had clinical improvement and one was lost to follow up.

As of December 1994, twelve patients were still alive

and on follow up 1-138 (median 18) months since diagnosis. Sixty nine were lost to follow up 0-132 (median 3.0) months and 21 were known to have died 0.5-84 (median 2.8) months after diagnosis. Of those that died 1,2,5 and 13 were at stages I, II, III and IV, with mean survival of 65, 31, 33.4 and 13.5 months respectively. The causes of death were; septicaemia in eight, pneumonia in seven, pulmonary tuberculosis in one, head injury in one, perforated peptic ulcer in one and unknown in three patients.

DISCUSSION

Chronic lymphocytic leukaemia (CLL) is said to be the most common form of leukaemia in the Western hemisphere accounting for 30% of all leukaemias in humans, but is rare in Orientals (1-6) and Papau New Guinea(7). In Africa, rates ranging from 12-40% were reported (8-12,15-17). The median age at presentation in Caucasians is 60-65 years (1-5), only 10-15% are below 50 years of age(6). For our patients the mean age was 55.6 years, 36.3% are below 51 years. This is also noted in other African series. The male to female ratio of 3.6:1 in our patients is higher than that observed in Caucasians, but unlike that in Nigerians where young females predominate(8,9).

The clinical features in our patients are similar to those reported in Caucasians, where lymphadenopathy is the dominant feature(1-5,13). Splenomegaly and hepatomegaly accounted for 55.9% and 32.4% unlike in Kenyans where these accounted for 80.9% and 76.5% respectively(14). Significant pallor was noted only in 37.3% of our patients, Oloo *et al*(14) reported it in 59.5% in their series. Stenal tenderness was observed in 2.9% of our patients in contrast to 27% reported by Boggs *et al*(13). Two patients had skin infiltration. The mean haemoglobin (Hgb) was 10.77 g/dl with 62.7% having less than 12.0 g/dl and 37.3% less than 10.0 g/dl. In Caucasians Hgb less than 10.0 g/dl was noted in only seven percent(13). In Kenyans anaemia was even more marked, Hgb less than 8.0 g/dl was found in 66.7%.

Like in Kenyans, no patient in our series had evidence of autoimmune haemolysis; this occurs in 10-15% in Caucasians(1,3,5). Lymphocytosis greater than 100,000/mm^3 was seen in 42.5% of our patients compared to 32% in Boggs *et al*(13) series. Very high lymphocyte counts are said to be associated with poor prognosis(3-6). Platelet counts less than 100,000/mm^3 and 50,000/mm^3 were seen in 37.8% and 20.5% respectively. Three patients presented with bleeding tendencies. Platelet count less than 50,000/mm^3 was noted in only one of 130 patients by Boggs *et al*(13).

In western hemisphere, 25% of patients were diagnosed incidentally and 40-60% were asymptomatic(1-5). In our patients, only 5 were diagnosed incidentally. Only three were in stage 0. Stages I and II, III and IV accounted for 41.2% and 56% respectively. Gale *et al*(3) reported stage 0=25%, I+II=50%, III+IV=25%. This shows that most patients in our series present at late stages like in the Kenyan series(14).

In patients that could be evaluated for outcome of treatment, overall responses of 73.4% and 69.2% for chloroambucil and cyclophosphamide were similar to those reported in the literature(3-6,19). As sixty nine patients were lost to follow up, it is not possible to determine the median survival for our patients. The median survival in reported series ranges from 6-9 years (1-6,13,19). Twenty one patients in our series are known to have died. The commonest causes of death were septicaemia and pneumonia. This is similar to the reported series in the literature(1-6,13,19).

Although it is not possible to cure CLL with the currently available chemotherapeutic agents, good palliation is possible. In Africa in general and in our centre in particular, these agents are not readily available, therefore, we suggest that referral centres be equipped for better management of CLL patients.

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REFERENCES

1. Wintrobe, M.M. Chronic lymphocytic leukaemia. *In: Clinical haematology* 8th edn., (eds), M.M. Wintrobe, G.R. Lee, D.R. Boggs et al), 1981, Chapter 67, pp. 1631-1647. Lea and Febiger, Philadelphia, PA. USA.
2. Gale, R.P. and Foon, K.A. Recent advances in the biology and treatment of chronic lymphocytic leukaemia. *Ann. intern. Med.* 1985; 103:101-120.
3. International Workshop on Chronic lymphocytic Leukaemia. Chronic Lymphocytic Leukaemia: Recommendations for diagnosis, staging and response criteria. *Ann. intern. Med.* 1989; **110:236-238.**
4. Kamirirs. T.D., Lister, T.A. and Rohatiner, A25. Chronic lymphocytic leukaemia. *Brit. J. Hosp. Med.* 1991; 46:379-385.
5. Rozman, C. and Montserrat, E. Chronic lymphocytic leukaemia. *N. Engl. J. Med.* 1995; 333:1052-1057.
6. Damshek, W. and Gunz, F. Epidemiology: geographic, racial and social distribution, *In: Leukaemia* 4th edn., Ed. F. Gunz, Chapter 3, pp. 18-34. Grune and Stratton, New York, 1983.
7. Booth, K. and Amato, D. Leukaemia in Papua New Guinea. *Trop. geogr. Med.* 1978; 30:343-349.
8. Essien, E.M. Leukaemia in Nigerians: The chronic leukaemias. *East. Afr. Med. J.* 1976; 53:96-110.
9. Kulkarni, A.D. Leukaemias in Guinea Savana area of North of Nigeria. *East Afr. Med. J.* 1986; 63:660-665.
10. Levy, L.M. The pattern of leukaemia in Zimbabweans. *Central Afr. J. Med.* 1984; 30:57-63.
11. Kasili, E.G. and Taylor, J.R. Leukaemia in Kenya. *East Afr. Med. J.* 1970; 47:461-468.
12. Ahmed, M.A.M., Kordofani, A.A.y, Hidaytalla, A. and Omer, A. Leukaemia in the democratic republic of Sudan. *East. Afr. Med. J.* 1982; 59:533-538.
13. Boggs, D.R. Sofferman, S.A., Wintrobe, M.M. and Cartwright, G.E. Factors influencing the duration of survival of patients with chronic lymphocytic leukaemia. *Amer. J. Med.* 1966; 40:243-254.
14. Oloo, A.J. and Ogada, T.A. Chronic lymphocytic leukaemia (CLL): Clinical study at Kenyatta National Hospital (KNH). Nairobi. *East Afr. Med. J.* 1984; 61:797-801.
15. Lester, F.T. Haematological malignancies in adults in an Addis Ababa hospital. *East Afr. Med. J.* 1981; 58:278-283
16. Milkias Shamebo. Pattern of haematological diseases among adult hospitalized Ethiopians. *Ethiop. Med. J.* 1987; 25:113-118.

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7. Milkias Shamebo. Leukaemia in adult Ethiopians. *Ethiop. Med. J.* 1990; 28:31-37.
8. Rai, K.R. Sawitsky, A. Cronkite, E.P. Chanana, A.D., Levy, R.N. and Pasternack, B.S. Clinical staging of Chronic Lymphocytic Leukaemia. *Blood.* 1975; 46:219-34.
9. Sawitsky, A., Rai, K.R. Glidewell, O. and Silver R.T. Comparison of daily versus intermittent chloroambucil plus prednisone therapy in the treatment of patients with chronic lymphocytic leukaemia. *Blood.* 1977; 50:1049-1059.
20. Bayhardt, R.W., Brace, K.L. and Wiernick P.H. The role of splenic irradiation in Chronic Lymphocytic Leukaemia. *Cancer.* 1975; 35:1621-1625.

ANNOUNCEMENTS

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Edemariam Tsega, Wondwossen Gebre, Paul Manley,
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HELICOBACTER PYLORI, GASTRITIS AND NON-ULCER DYSPEPSIA IN ETHIOPIAN PATIENTS

Edemariam Tsega, MD,FRCPC[1], Wondwossen Gebre MD[2],
Paul Manley,MD[3], Tesfaye Asfaw[2].

ABSTRACT: *The aim of this study is to find out the prevalence of* Helicobacter pylori *infection in patients with nonulcer dyspepsia (NUD) and asymptomatic controls and to see if there is an etiological association between gastritis, NUD and* Helicobacter pylori. One hundred thirty six patients with NUD and 71 controls had six endoscopic biopsies from different sites of the gastric mucosa for histological diagnosis. Helicobacter pylori *was looked for in all biopsy specimens utilizing half Gram, Giemsa and Gimenez staining techniques. Type B chronic gastritis was detected in 96% of the NUD cases and in 100% of the asymptomatic controls (P > 0.05).* Helicobacter pylori *was found in 82 (65%) patients with NUD and in 38 (56%) asymptomatic controls (P > 0.05). Type B chronic gastritis is almost universal in both NUD cases and asymptomatic controls. There is no difference in the prevalence of* Helicobacter pylori *infection between the two groups. The absence of* Helicobacter pylori *in a significant number of patients (36%) and controls (45%) with gastritis contradicts the etiological association between* Helicobacter pylori *and gastritis reported by others, suggesting that in Ethiopia there may be a chronic environmental gastritis which may not be helicobacter-related. There is no correlation between NUD and Type B gastritis, and between symptoms and* Helicobacter pylori *infection in this population.*

INTRODUCTION

Non-ulcer dyspepsia (NUD) is a common complaint among Ethiopians (1,2). A significant number of patients with NUD experience lactose intolerance but the role of lactase deficiency in NUD remains Histological gastritis does not correlate with symptomatology (4). Hot, spicy food, part of the staple diet of Ethiopians, has been implicated in the aetiology of NUD but the evidence is circumstantial (5). In recent years, *Helicobacter pylori* has been etiologically associated with NUD and

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peptic ulcers (6-8). This study was conducted to find the prevalence and role of *H. pylori* infection in patients with and without non-ulcer dyspepsia and gastritis.

PATIENTS AND METHODS

Patients and controls: There were 136 patients, 86 men and 50 women, with NUD. The mean age was 30.5 years (range: 14-77) and 95% of the patients were between 15 and 45 years of age. Non-ulcer dyspepsia was defined as epigastric pain of recurrent nature lasting for several months, often felt soon after meals, aggravated by eating and temporarily relieved by antacids and milk. Oesophagitis, gastric ulcer, duodenal ulcer, duodenitis and other specific lesions involving the oesophagus, stomach and duodenum were excluded by endoscopic examination. those suspected to have biliary, hepatic, pancreatic, etc. conditions were excluded by history and physical examination, stool examination for ova and parasites, oral cholecystography, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum protein electrophoresis, serum amylase and sigmoidoscopy. Patients with diabetes mellitus, cirrhosis of the liver, lower abdominal complaints and alcoholism were excluded from the study.

Seventy-one subjects, 42 men and 29 women, with a mean age of 27.9 years (range: 17-60) served as controls. The majority, 95% were between 15 and 45 years of age. These volunteers were nurses and patients, the latter admitted to hospital for non-abdominal complaints, cured and awaiting discharge. None of these subjects had abdominal complaints lasting for more than a week at any time. Fifty-nine per cent of the patients and controls came from Addis Ababa and its vicinity while the rest came from 13 different provinces of Ethiopia. A complete history and physical examination were documented before starting any investigation. The protocol, which included informed consent by the patients, was approved by the Research Committee of the Faculty of Medicine of Addis Ababa University.

Endoscopy: All patients with NUD, and asymptomatic controls had esophagogastroduodenoscopy (with OLYMPUS-GIF Type K) by one of us (ET). Every patient and control had two biopsies from the antrum 10 cm above the pyloric opening and as far apart as possible. Similarly, biopsies were taken from the lesser and greater curve side of the body of the stomach above the level of the incisura, two from each side. Thus, each subject had a total of six biopsies.

Histology: The fresh gastric mucosal specimens were submitted in 10% formalin for immediate processing and embedding in paraffin. Sections of 5 microns' thickness were prepared and stained with Haematoxylin and Eosin. The pathologist had no knowledge of the specimens in terms of patients, controls or symptomatology. Each coded slide was read "blind" by the same pathologist and then by a second pathologist. The final reading was recorded after all areas of significant discordances between the two observers were resolved. The histological grading was according to the classification of Whitehead and associates (9), using the pictorial criteria of Joske and co-workers (10). The biopsies from the antrum and both the greater and lesser curvatures of the body of the stomach were graded according to the extent and type of inflammation, loss of specialized cells and the degree and type of metaplasia. Each parameter was ranked in severity 0 to 3+.

The biopsy specimens were originally

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prepared sometime ago for histological studies in NUD patients (4), and hence the reason for not using the recent Sydney gastritis classification. *Helicobacter pylori* was looked for in the paraffin embedded specimens retrospectively. Sections from each of the six specimens obtained from every subject were stained with half-Gram (11), Giemsa (12) and Gimenez techniques (13) for *H. pylori*, identified by the typical curved bacilli lying beneath the mucous layer of the epithelium, especially in the regions of the gastric pits.

Neither the pathologists nor the endoscopist had knowledge of the type of subjects studied and the corresponding histological and endoscopic findings prior to completion of the study.

Statistical methods: The Chi-square test with Yates correction was utilised where applicable. Statistical results were at 5% level of significance.

RESULTS

Patients with NUD and asymptomatic controls were comparable in age, sex and geographic origin.

The histological findings are summarised in Table 1. Intestinal metaplasia was present in 116/136 (85%) NUD cases and in 60/71 (81%) controls ($P > 0.05$). Variable degrees of infiltration of the columnar epithelium and lamina propria by mononuclear cells were present in almost all biopsies from the antrum and the body of the stomach. Except for 10 patients and 1 control, all had full thickness antral gastritis. The different types of gastritis occurred in patchy distribution in several cases. For example, while 4 patients and 1 control demonstrated normal histology on one side of the body of the stomach, the other side revealed superficial gastritis. Similarly, a mixture of superficial and chronic atrophic gastritis were documented in the body of the stomach in 33 and 26 patients and controls, respectively. Considering all types of gastritis, the prevalences were comparable in the NUD and control groups. Thus, 128 (94%) of 136 patients with NUD had body superficial and/or chronic atrophic gastritis while 130 (96%) had full thickness antral gastritis. all 71 (100%) controls had body superficial and/or chronic atrophic gastritis and 70 (99%) had full thickness antral gastritis. Thus, there was no statistically significant difference in the prevalence of gastritis between the two groups, neither in the body nor in the antrum of the stomach ($P > 0.05$).

Helicobacter pylori was found in 82 (65%) body mucosa and in 80 (64%) antral mucosa of 126 patients with NUD and gastritis. Ten patients and 3 controls had inadequate biopsy specimens for the special stains. Among 68 asymptomatic controls with gastritis, *H. pylori* was detected in 37 (54%) body mucosa and in 38 (56%) antral mucosa (Table 2). There was no statistically significant difference in the prevalence of *H. pylori* infection between the two groups, either in the body ($P > 0.05$) or in the antrum ($P > 0.05$).

The Gimenez technique was found to be more sensitive than the half-Gram and Giemsa stains in identifying *H. pylori* in the paraffin sections.

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Table 1. Histological findings in gastric mucosal biopsies of 136 non-ulcer dyspepsia (NUD) patients and 71 asymptomatic controls 1993.

Biopsy sites and histological findings		Patients with NUD	Asymptomatic controls
Body:			
	Normal histology	8 (6%)	0 (0%
		128 (94%)*	71 (100%)
	Chronic gastritis	126 (85%)	60 (85%
	Neutrophils present		
Antrum:			
	Normal biopsy	2 (2%)	1 (1%
		126 (96%)**	70 (95%)
	Full thickness gastritis		

* Chi-square with Yates correction = 2.91; P = 0.0883
** Chi-square with Yates correction = 0.53; P = 0.4655

Table 2. Prevalence of *Helicobacter pylori* in non-ulcer dyspepsia patients and asymptomatic controls, 1993.

Biopsy site (gastric)	Patients with gastritis		Controls with gastritis	
	Number examined	*H. pylori* positive	Number examined	*H. pylori* positive
Body	126	83 (66%)	68	37 (54%)*
Antrum	126	80 (64%)	68	38 (56%)**

* Chi-square with Yates correction = 2.00; P = 0.1576
** Chi-square with Yates correction = 0.78; P = 0.3778

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DISCUSSION

Almost all of the patients and controls had full thickness antral gastritis. In a separate study of 67 of the above patients and 38 of the controls, basal and maximal acid secretion was normal and there was no case of achlorhydria (14). When anti-parietal cell antibodies were looked for in 107 patients and 71 controls, all from the above study population, all but one control were negative (15). It is also known that autoantibodies and auto-immune diseases are uncommon among this population (16). Thus, the gastritis seen in the patients with NUD and asymptomatic controls was Type B (17). The prevalence of body gastritis, almost identical to that of antral gastritis, is strikingly high compared to that reported from Europe, Australia, North America and the Far East (18-21). An important observation is the presence of neutrophils in the lamina propria of a significant number of patients (85%) and controls (81%), an indication of current active process in addition to the chronic inflammatory changes.

The remarkably high prevalence of chronic and currently active gastritis in the asymptomatic controls, almost the same as in the patients with NUD, indicates that there is no correlation between histological gastritis and symptomatology.

Helicobacter pylori was found in 64-65% of patients with NUD and in 54-56% of asymptomatic controls, both in association with active and chronic gastritis ($P > 0.05$). The involvement of the antrum and the body of the stomach by H. Pylori and Type B gastritis was comparable ($P > 0.05$) (Table 2). In contrast to other reports (22), the prevalence of antral gastritis associated with *H. pylori* in asymptomatic controls is high. Therefore, there is no correlation between symptoms and gastritis and between symptoms and *H. pylori* infection. On the other hand, based on a limited number of therapeutic trials, Marshall and others (23,24) have suggested that NUD is a consequence of *H. pylori* infection and, therefore, should be treated with bismuth and antibiotics. The resolution of symptoms, however, was never complete (24,25). Recent reports indicate that there are reasonable points both for and against the treatment of *H. pylori* infection in patients with NUD (26).

Of the three special stains used in this study (half-Gram, Giemsa and Gimenez) for the detection of *H. pylori* organisms in tissue sections, the Gimenez technique has been found more sensitive than the other two techniques and as good as culture (6). The Gimenez technique was also found to be better than the Warthin-Starry or silver stain in detecting *H. pylori* in paraffin sections as the latter is unreliable, time consuming and expensive (6,11-13). Although the combination of culture and Gimenez technique would be ideal, the latter has been recommended as reliable, quick and economical, especially in a retroactive study of this type. Furthermore, the findings of *H. pylori* with stains alone in this study, were comparable to recent culture results (68%) in 88 Ethiopian patients with NUD and gastritis (unpublished data). Therefore, the detection of *H. pylori* by the above stains is as valid as by culture.

The study does not convincingly confirm the belief that *H. pylori* is etiologically related to the symptomatology of non-ulcer dyspepsia. Furthermore, the absence of *H. pylori* in a significant number of patients with Type B gastritis suggests that there may be a chronic environmental gastritis which may not be *Helicobacter pylori*-related in this population. This view requires further and prospective studies.

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REFERENCES

1. Tsega E. Analysis of fibreoptic gastroduodenoscopy in 1084 Ethiopians. *Trop Geog Med* 1981;33:149-54.
2. Lester F, Tsega E. The pattern of adult medical admissions in Addis Ababa, Ethiopia. *East Afr Med J* 1976;53:620-39.
3. Tsega E, Endeshaw Y, Mengesha B, Tedla B. The role of mild and lactose intolerance in Ethiopian patients with non-ulcer dyspepsia: A controlled and prospective study. *Ethiop Med J* 1989;27:135-45.
4. Tsega E, Manley N, Asfaw T. A clinical and histological study of Ethiopian patients with non-ulcer dyspepsia. *East Afr Med J* 1985;62:621-39.
5. Tsega E, Manley PN, Asfaw T, Choremi H, Botazzo GH, Doniach D, Tegene N. Clinical, Functional, Histological and Immunological Evaluation of Ethiopian Patients with Non-ulcer Dyspepsia. in: Edemariam Tsega, ed. Non-ulcer Dyspepsia and Gastritis in Ethiopia. pp. 87-93. Addis Ababa Ethiopia: Artistic Printers, 1983.
6. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;1:1273-75.
7. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;1:1311-14.
8. Dooley P, Cohn H. The clinical significance of Campylobacter pylori. *Ann Int Med* 1988;108:70-9.
9. Whitehead R, Truelove SC, Gear WL. The histological diagnosis of chronic gastritis in fibreoptic gastroscope biopsy specimens. *J Clin Pathol* 1972;25:1-11.
10. Joske RA, Finckh ES, Wood IJ. Gastric biopsy - a study of 1,000 consecutive successful biopsies. *Ouart J Med* 1955;24:269-78.
11. Trowell JE, Young AKH, Ng L, Sherbaniuk RW, Jewell LD. Simple half-Gram stain for showing presence of Campylobacter pyloridis in sections. *J Clin Pathol* 1987;40:702.
12. Jones DM, Lessells AM, Eldrige J. Campylobacter like organisms of the gastric mucosa: culture, histological, and serological studies. *J Clin Pathol* 1984;37:1002-6.
3. McCullen M, Walter MM, Bain LA, Karim QN, Baron JH. Histological identification of Campylobacter using Gimenez technique in gastric antral mucosa. *J Clin Pathol* 1987;40:464-65.
14. Tsega E, Tegene N. Pentagastrin-stimulated gastric acid secretion in Ethiopian patients with dyspepsia and normal control. *East Afr Med J* 1981;58:192-8.
15. Tsega E, Choremi H, Botazza GF, Doniach D, Manley PN, Asfaw T. Autoantibodies in Ethiopian patients with non-ulcer dyspepsia and gastritis, in Non-ulcer Dyspepsia and Gastritis in Ethiopia (eds. Edemariam Tsega), 1983, pp 71-74, Artistic Printers, Addis Ababa, Ethiopia.

16. Tsega E, Choremi N, Botazzo GF, GF Doniach D. Prevalence of autoimmune diseases and autoantibodies in Ethiopia. *Trop Geog Med* 1989;32:231-6.
17. Strickland RG, Mackay IR. A reappraisal of the nature and significance of chronic atrophic gastritis. *An H Dig Dis* 1973;18:426-40.
18. Siurala M, Isokoshi M, Varis K, Kekki M. Prevalence of gastritis in a rural population. Bioptic study of subjects selected at random. *Scand J Gastroent* 1968;3:211-23.
19. Motteram R. Biopsy study of chronic gastritis and gastric atrophy. *J Pathol Bacteriol* 1951;63:389-94.
20. Williams A, Edwards F, Lewis THC, Coghill NF. Investigations of non-ulcer dyspepsia by gastric biopsy. *Br Med J* 1957;1:372-77.
21. Khosla SN, Chugh SN, Nehrotra GC, Srivastava SC, Jain AL. Gastric changes in non-ulcer dyspepsia. *Indian J Med Res* 1978;67:862-71.
22. Pettross CW, Appleman MD, Cohen H, Valenzuela JE, Chandrosoma P, Laine LA. Prevalence of Campylobacter pylori and association with antral mucosal histology in subjects with and without upper gastrointestinal symptoms. *Dig Dis Sci* 1988;33:649-53.
23. Marshall BJ, Armstrong JA McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric campylobacter. *Med J Aust* 1985;142:436-9.
24. McNulty CA, Gearty JC, Crumpylobacter pyloridis and associated gastritis: Investigator blind, placebo controlled trail of bismuth salicylate and erythromycin ethylsucinate. *Br Med J* 1986;293:645-9.
25. Lambert JR, Borromeo M, Korman MG, Hamsky J. .Role of Campylobacter poyloridis in non-ulcer dyspepsia- a randomized controlled trial (Abstract). *Gastroenterol* 1987;92:1483.
26. Matisko A, Thomson ABR. Helicobacter pylori: Beginning the second decade. *Can J Gastroenterol* 1995;9:333-43.

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HELICOBACTER PYLORI INFECTION IN ETHIOPIAN PATIENTS WITH DYSPEPSIA

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ABSTRACT: *Antral biopsies obtained by gastro-intestinal endoscopy from 200 Ethiopian patients with dyspepsia were studied histologically and bacteriologically for the presence of* Helicobacter pylori. Helicobacter pylori *was identified by culture, urease-screening, and Gram, Giemsa and Gimenez staining of the antral mucosa. There were 87 patients with duodenal ulcer, 80 (92%) of whom had antral gastritis and 7 normal histology; and 113 patients with non-ulcer dyspepsia, 88 (78%) with antral gastritis and 25 with normal mucosa.* Helicobacter pylori *was present in 83 (94%) patients with duodenal ulcer (76 associated with gastritis and 7 with normal histology), and in 82 (73%) patients with nun-ulcer dyspepsia (73 associated with gastritis and 9 with normal mucosa) ($p < 0.0002$). When the prevalence of* Helicobacter pylori *infection in the duodenal ulcer group (95%) and non-ulcer dyspepsia group (83%) with antral gastritis was compared, the difference was significant ($p = 0.0265$). The finding of* Helicobacter pylori *in 50% of normal antral mucosa (9 of 25 patients with non-ulcer dyspepsia and all 7 duodenal ulcer cases) was unusual. The total absence of gastric ulcer in the presence of gastritis and* Helicobacter pylori *infection may be due to strain difference in the organism involved or due to unknown factor(s) which may be protective to the gastric mucosa of Ethiopians.*

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INTRODUCTION

Non-ulcer dyspepsia (NUD) is a common condition among Ethiopians (1). Histological studies have revealed that Type B gastritis is present in over 90% NUD cases and asymptomatic controls (2). However, the cause of the gastritis remains unknown. Also, for unknown reasons, duodenal ulcer (DU) is common in this population whereas gastric ulcer and reflux oesophagitis are rare (3,4).

A number of recent reports suggest that there is an aetiological association between *Helicobacter pylori (H. pylori)* infection, Type B gastritis and peptic ulcer (5-7). This study was conducted to find out the prevalence of *H. pylori* in symptomatic patients undergoing diagnostic endoscopic examination.

PATIENTS AND METHODS

Patients: Two hundred patients who had upper gastro-intestinal endoscopic examinations for dyspeptic symptoms in the Gastroenterology Unit of the Black Lion Hospital, a teaching hospital of Addis Ababa University, were consecutively (without any exclusion criteria) studied for *H. pylori* during 1990. There were 123 males and 77 females with a mean age of 34.8 years (Range: 16-70 years). The majority (77%) were under 45 years of age. These patients came from 13 different provinces but 41% were from Addis Ababa and its vicinity. The majority were referred for investigation of dyspepsia with a mean duration of 5 years (3 weeks-18 years). None of them had upper gastro-intestinal endoscopic examination previously. The protocol, which included informed consent by the patients, was approved by the Research Committee of the Faculty of Medicine of Addis Ababa University.

Endoscopy: Oesophagogastroduodenoscopy was performed after sedation with intravenous pethidine and oral anaesthesia with 1% xylocaine spray. The endoscope and biopsy forceps were sterilized in savlon and alcohol (70% and 90%) concentration before use. Each patient had four biopsies from the antral mucosa, 5-10 cm proximal to the pyloric opening. Two of the biopsies were for histological examination and the other two for bacteriological studies.

Histology: The two biopsy specimens for histology were kept in 10% formalin until embedded in paraffin. For sections, each of 5 micron thickness, were prepared from these biopsy specimens. The first was stained with haematoxylin and eosin for routine histological study and the other three with Gram, Giemsa and Gimenez techniques ((8-10) for identification of *H. pylori*. The histological findings from the haematoxylin and eosin stain were read and scored according to the classification of Whitehead (11), using the pictorial criteria of Joske (12). The presence of polymorphonuclear cells were looked for to confirm the presence of active gastritis. *H. pylori* was identified as typical curved bacilli lying beneath the mucous layer of the epithelium, especially in the regions of the gastric pits.

Bacteriology: The two specimens for bacteriological studies were transported in 0.5 ml sterile normal saline and were plated on culture medium within one hour of the biopsy. These two specimens were divided

into four proportions: One portion was inoculated on chocolate agar (Difco) which had 1% isovatalex (BBL) and a second portion was inoculated on chocolate agar which had isovatalex and 2 mg% amphotericin B (SQUIB). These two plates were incubated at 37°C in a moist 10% candle jar and were inspected for six days for flat and colourless colonies. If such colonies appeared, they were: a) smeared and Gram stained to observe Gram negative curved bacteria, b) inoculated in urease test broth (Difco) to observe utilization of urea within 5 minutes, and c) were tested for oxidase and catalase positivity. If no bacterial colonies were observed, subculturing and observing for two more days were carried out. The third portion was used for direct smears which were stained with Gram stain to observe Gram negative spiral bacteria. The fourth portion was inoculated into urease test broth (Difco) to observe utilization of urea within 5 minutes of the inoculation. Subculture from the last specimen was carried out in BA plus MacKonkey to rule out other common urease-positive organisms. Lyophilized *H. pylori* organisms (received from Dr. B.J. Marshall, Perth, Australia) were used as quality control.

The bacteriologist had no knowledge of the endoscopic or histological findings and the pathologist did not know about the endoscopy and bacteriology reports. Likewise, the endoscopist did not have access to the bacteriological and histological results until the completion of the study. The diagnosis of *H. pylori* was accepted when one or more of the special stains, with or without positive culture, revealed the microorganism.

Statistical methods: The Chi-square test with Yates correction was used to compare statistically significant differences where applicable.

RESULTS

Based on endoscopic findings, there were 87 patients with DU, and 113 cases with NUD (9 associated with oesophageal varices and one with carcinoma of the body of the stomach). There was no single case of gastric ulcer.

Of the 87 patients (19 females and 68 males) with DU, 80(92%) had histological Type B chronic antral gastritis (79 (99%) with neutrophils, indicative of current, active gastritis) while the remaining 7 had normal mucosa. Of the 113 patients (58 females and 55 males) with NUD, 88 (78%) had gastritis (85 (97%) with neutrophils) and 25 had histologically normal antral mucosa.

There was no statistically significant difference in the mean ages or in the duration of symptoms between those with gastritis and those with normal histology among the DU and the NUD groups. Although the number of patients over 45 years of age was small, there was no tendency of histological gastritis to increase with age in the DU group or the NUD group.

The results of the culture and special stains for *H. pylori* are summarized in Table 1. They are analyzed in relation to the histological findings in the DU and NUD cases. Of the 80 DU cases with gastritis, 75 (94%) were positive for *H.*

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pylori. Sixty seven (84%) of these cases were culture positive and 8 (10%) were stain positive, but culture negative. The remaining 7 DU patients with normal histology were positive for *H. pylori* by both culture and stain. The overall *H. pylori* positivity in patients with duodenal ulcer with or without gastritis was 94%. Of the 88 NUD patients with gastritis, 73 (83%) were positive for *H. pylori*. Sixty (68%) of these cases were culture positive and 13 (15%) were only stain positive. Of the remaining 25 NUD patients with normal histology, 9 (36%) cases were positive for *H. pylori*, 6 by culture and 3 by stain alone. The overall *H. pylori* positivity in patients with NUD with or without gastritis was 73%.

Table 1. *Helicobacter pylori* positivity in 200 patients with ulcer and non-uler dyspepsia, Black Lion Hospital, 1990.

Histological diagnosis	Number studied	Culture +ve	Culture -ve stain +ve*	Total *H. pylori* positivity	
				Number	per cem
Duodenal ulcer**					
Normal	87	74	8	82	94.0
Gastritis	7	7	0	7	100
	80	67	8	75	94.0
Non-ulcer dyspepsia					
Normal	113	66	16	82	73.0
Gastritis	25	6	3	9	36.0
	88	60	13	73	83.0

* Duodenal ulcer was diagnosed endoscopically;
** Stain positivity indicates Gram and/or Giemsa and/or Gimenez stain positivity.

The prevalence of *H. pylori* infection was significantly higher in the DU patients than in the NUD cases ($p < 0.002$), although the two groups were comparable in mean age (35 years each) but differed in sex ratio (M:F = 68:19; 55:58, respectively). Also, when the prevalence of *H. pylori* infection in the DU (95%) group and NUD (83%) group with histological gastritis were compared, there was some difference ($p = 0.0097$).

While all 7 patients with DU and normal histology revealed both culture and stain positivity, only 9 (36%) of 25 patients with NUD and normal histology were culture and/or stain positive for *H. pylori*.

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DISCUSSION

The 92% antral gastritis and 94% colonization of the gastric mucosa by *H. pylori* in the 87 patients with DU are comparable to several reports from different parts of the world (7,11,13-16). The presence of *H. pylori* n association with normal antral histology in the 7 DU and 9 NUD cases is unusual (7,17,18). However, antral gastritis could not be excluded in all these cases with certainty as histological gastritis tends to be patchy in distribution in the gastric mucosa of Ethiopians (1,2), resulting in biopsy sampling error.

The association between antral gastritis, DU and *H. pylori* is impressive. Complete resolution of the gastritis and DU have been observed after eradication of *H. pylori*, leaving the patient with about a 1% per year risk of re-infection with *H. pylori* and about a 2% risk of the ulcer recurring, versus at least a 50% chance that the DU will recur over the next year if the *H. pylori* is not eradicated (16,19,20). In the 27 DU studies quoted by Tytgart (21), the annual ulcer relapse rate was 58% (571 of 988) in *H. pylori*-positive patients versus 2.6% (23 of 893) in *H. pylori*-negative patients. However, the recurrence rate of *H. pylori* infection in the developing countries is higher than in the developed countries (22). Thus, there are sufficient data to indicate that *H. pylori* is causally related to peptic ulcers and that eradication therapy reduces the risk of recurrence. Impairment of the mucus layer covering the surface epithelium by colonization of the gastric mucosa by *H. pylori* has been suggested as a possible pathogenetic mechanism for the development of peptic ulcer (23). The lower relapse rate at 2 years, of DU patients treated with sucralfate, an anti-ulcer agent that also acts by promoting mucosal defences, may support this view (24). Ammonia generated from hydrolysis of urea by urease in the stomach has also been implicated in the damage of gastric mucosa (25).

Helicobacter pylori was not found, by either culture or special stains, in 4 patients with DU and antral gastritis and in 15 patients with NUD and gastritis. The absence of 24 *H. pylori* may be due to one of the following reasons: a) spontaneous elimination of the micro-organism (26); b)swallowed local anaesthesia preceding the endoscopic biopsy and/or previous treatment with antimicrobial agents and/or H_2-receptor antagonists (16,27); and c) biopsy of areas with sparse bacteria. The combined use of a specific enzyme-linked immunosorbent assay and the C13- urea breath test have recently been recommended to differentiate between those who had past infection but have spontaneously eliminated the *H. pylori* organisms and those with active infection (26).

It remains unclear why some people with antral gastritis and *H. pylori* develop peptic ulcers while others, with the same findings, do not. In this study, of a total of 149 patients (both DU and NUD cases) with histological gastritis and *H. pylori* infection, 73 (49%) did not have peptic ulcer. Grabtree and associates (28) have suggested that infection with 120-kDa-positive strains of *H. pylori* are specifically associated with active gastritis and peptic ulceration and that the absence of such strains may explain why peptic ulceration develops in only a

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proportion of subjects infected with *H. pylori* organisms from different regions of the world. Once virulence factors of *H. pylori* organisms have been identified and confirmed in a clinical setting, one will be in a better position to determine who is at risk and who will require eradication therapy with or without ulcers.

There was no case of gastric ulcer among the 200 patients included in this study. This is in keeping with previous observations that duodenal ulcer is common while gastric ulcer and reflux oesophagitis are rare among the Ethiopian population (3,4). Yet, both patients with NUD and asymptomatic controls are known to have Type B gastritis, involving the antrum (over 90%) and the body of the stomach (over 80%) (1,2), and *H. pylori* infection (51-66%) (unpublished data). In western Europe and north America, gastric ulcer is common and it is associated with about 70% infection with *H. pylori*, which, when eradicated, the recurrence rates are 0-3% versus 48-51% without eradication therapy (16,19). Specific strain difference of *H. pylori* may account for the occurrence of DU and absence of GU. Also, there may be specific factors which may selectively protect the gastric mucosa from developing gastric ulcers in Ethiopian patients.

Irrespective of the normal histological findings in the 25 NUD cases, symptoms were present in both *H. pylori*-positive and *H. pylori*-negative patients. Conversely, in a previous study of 126 patients with NUD and 68 asymptomatic controls (see above), Type B gastritis occurred in the majority of each group (1,2) and the prevalence of *H. pylori* was not significantly different between patients and controls (unpublished data). Therefore, there is no correlation between symptoms and *H. pylori* infection. A recent report from the People's Republic of China also concluded that there was no significant association between *H. pylori* infection and symptoms (30).

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REFERENCES

1. Tsega E, Manley N, Asfaw T. A clinical and histological study of Ethiopian patients with non-ulcer dyspepsia. *East Afr Med J* 1985;62:621-39.
2. Tsega E. Non-ulcer Dyspepsia and Gastritis in Ethiopia. Artistic Printers, Addis Ababa, Ethiopia: 1983:pp 5-7, 87-93.

3. Tsega E. Analysis of fibreoptic gastroduodenoscopy in 1084 Ethiopians. *Trop Geog Med* 1980;33:149-54.
4. Tsega E, Mengistu M. Peptic diseases in Ethiopia: A prospective analysis of 200 endoscopically proven cases. *Trop Geog Med* 1985;37:150-55.
5. Warren JK, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;1:1273-5.
6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;1:1311-4.
7. Dooley P, Cohn H. The clinical significance of *Campylobacter pyori*. *Ann Int Med* 1988;08:70-9.
8. Trowell JE, Young AKH, Saul KJ, Gant PW, Bell GD. Simple half-Gram stain for showing presence of *Campylobacter pyloridis* in sections. *J Clin Pathol* 1987;40:702.
9. Jones DM, Lessells AM, Eldrige J. Campylobacter like organisms on the gastric mucosa: culture, histological, and serological studies. *J Clin Pathol* 1984;37:1002-6.
10. McCullen M, Walter MM, LA, Karim QN, Baron JH. Histological identification of Campylobacter using Gimenez technique in gastric antral mucosa. *J Clin Pathol* 1986;40:464-5.
11. Whitehead R, Truelove SC, Gear WL. The histological dignosis of chronic gastritis in fibreoptic gastroscope biopsy specimens. *J Clin Pathol* 1972;25:1-11.
12. Joske RA, Finckh ES, Wood IJ. Gastric bioposy - a study of 1,000 consecutive succesful gastric biopsies. *Quart J Med* 1955;24:269-94.
13. Shrager J, Spink R, Mitral S. The antrum in patients with duodenal and gastric ulcers. *Gut* 1967;8:497-508.
14. Hui WM, Lam SK, Ho J, et al. Chronic antral gastritis in duodenal ulcer: natural history and treatment with prostaglandin E1 *Gastroenterol* 1986;91:1095-1101.
15. Tatusta M, Iishi H, Okuda S. Location of peptic ulcers in relation to antral and dundal gastrits by chromoendoscopic folw-up examinations. *Dig Dis Sci* 1986;31:7-11.
16. Marshall BJ. *Helicobacter pylori*. *Am J Gastroenterol* 2994;89:S116-S128.
17. Drumm B, Sherman PO, Cutz E, Karmali M. Association of *Compylobacter pylori* on the gastric mucosa with antral gastritis in children. 1987;316:1557-61.
18. Dooley CP, Cohn H, Fitzgibbons PL, et al. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *New Eng J Med* 1989;321:1562-6.
19. McKenna D, Humphreys H, Dooley C, et al. *Campylobacter pyloridis* and histological gastritis in duodenal ulcers: a controlled prospective randomized trial. (Abstract) *Gastroenterol* 1987:92:1528.
20. Matisko A, Thompson ABR. *Helicobacter pylori*: Beginning the second decade. *Can J Gastroenterol* 1995;9:359-368.

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21. Tutgart GNJ. Treatments that impact favourably upon the eradication of *Helicobacter pylori* and ulcer recurrence. *Ailment Pharmacol Ther* 1994;8:359-68.
22. Megraud F, Brassens Rabble MP, Denise F, et al. Seroepidemiology of *Campylobacter pylori* infection in various populations. *J Clin Microbiol* 1989;27:1879-3.
23. Xia DH, Jiang SJ, Wang RN, Zhang ZH, Xu WW. The efficacy of furazolidine and metronidazole in the treatment of chronic gastritis associated with *Helicobacter (Compylobacter) pylori* - a randomized bouble-blind placebo-controlled clinical trial. *Hepatogastroenterol* 1990;37:503-6.
24. Lam SK, Hui WM, Lau WY, et al. Sucralfate overcomes adverse effect of smoking on duodenal ulcer healing and prolongs subsequent remission. *Gastroenterol* 1987;92:1193-1201.
25. Saita H, Matuo K, Asada T, Kita T. Generation of ammonia and mcosal lesion formation following hydrolysis of urea by urease in the rat stomach. *J lin Gastro enterol* (12 Suppl) 1990;1:104-9.
26. Meyer B, Werth B, Beglinger C, et al. *Helicobacter pylori* infection in healthy people: a dynamic process. *Gut* 1991;32:347-50.
27. Goodwin CS, Blincos ED, Warren Jr, Waters JE, Sanderson Cr, Easton L. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J Clin Pathol* 1985;38:1127-31.
28. Grabtree JE, Taylor JD, Wyatt JI, et al. Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration, and gastri pathology. *Lancet* 1991;338:332-335.
29. Vigneri S, Termini R, Scialabba A, DiMario F, Savarino V, Mela GS. One year relapse rate after *H. pylori* eradication with omeprazole plus antimicrobials. (Abstract) *Gastroenterol* 1993;104:A40.
30. Li YY, Hu PJ, Du GG, Hazell SL. The prevalene of *Helicobacter pylori* infection in the People's Democratic Republic of China. *Am J Gastroenterol* 1991;86:446-9.

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METHOD

Subjects

In Ethiopia, sera from 242 children out of a larger group of 281 children who had earlier been recruited for a vaccine trial were randomly selected for the study. The original sample consisted of children of employees of the Tikur Ambassa Hospital and Yekatit 12 Hospital, both in Addis Ababa. After informed consent from the parents, all children aged 2–14 years who had no previous hepatitis B vaccination, were negative for HBsAg, anti-HBc and anti-HBs, and who had no acute or chronic disease or a history of allergy to any vaccine preparation were included in the vaccine trial. The aim was to select randomly 20 stored sera from each year of age, but in some age groups this sum could not be attained. The samples were collected in 1988 and the results concerning hepatitis B were published in 1991 (22).

In Sweden, the study took place at the Department of Paediatrics at St Göran Hospital, Stockholm, and samples were collected during the period 1994–1995. After parents' informed consent, 5 ml of venous blood was collected from 295 consecutive children aged 1–15 years who came to the hospital for surgical intervention for any disease other than a stomach disorder. All children were born and brought up in Sweden and living in the area of Stockholm. Here also, the aim was to collect 20 consecutively sera from each age group, but in a few age groups this was not attained.

Serology

The antigen for the ELISA for the Ethiopian sera was prepared at the Department of Medical Microbiology, Lund University, according to a method described previously (23). One batch was prepared from the original Australian HP reference strain CCUG 17874 (NCTC 11637) and another from a strain retrieved at gastroscopy in Addis Ababa. All 242 Ethiopian samples were analysed with antigen from the reference strain and, for comparison of antigenic variability, 32 sera were randomly selected to be analysed also in an ELISA using antigen extracted from the Ethiopian strain. The 295 Swedish sera were analysed with antigen prepared from the supernatant of sonicated HP organisms, pooled from 4 patient strains and the reference strain (NCTC 11637) as described previously (24).

Antibodies were detected by means of an ELISA (23) at the Department of Microbiology, Black Lion Hospital, Addis Ababa, and the Division of Clinical Bacteriology, Huddinge Hospital, Stockholm.

The test results from the Ethiopian study are expressed as a corrected mean absorbance value in parts (%) of a reference standard (Human Gammaglobulin, KABI/Vitrum AB, Stockholm, Sweden). The cut-off value for seropositivity was set to a relative antibody activity (RAA) of ≥35 (23). To establish the degree of cross-reaction with antibodies to Campylobacter jejuni, 10 of the Ethiopian patient sera showing high and 10 of those showing low antibody activity in the HP ELISA were re-analysed after the sera had first been adsorbed with a suspension of C. jejuni.

The test results from the Swedish study are expressed as the optical density times the dilution factor. A positive standard serum was used as a reference. The cut-off value for seropositivity was set to an absorbancy of ≥0.35.

RESULTS

When analysing the 32 randomly chosen Ethiopian sera with the Australian reference HP strain, 22 sera (69%) were positive and 10 negative, whereas with the Ethiopian strain 3 of the positive sera became negative and 1 negative serum turned positive. In all 20 sera (63%) were positive and 12 negative when analysed with the Ethiopian strain. These differences could be due to strain variations, but also to slight imperfections in the ELISA method. After adsorption with C. jejuni antigen, all the originally positive sera remained positive and all the originally negative sera remained negative. We thus decided to use the RAA values obtained by using antigen extracted from the reference HP strain for the analysis of the serum samples from both countries.

When analysing the Swedish sera, a comparison was also made between the antigenic preparations from the Lund and Huddinge Hospitals. In all instances, there was agreement between the methods in identifying sera with high and low titres.

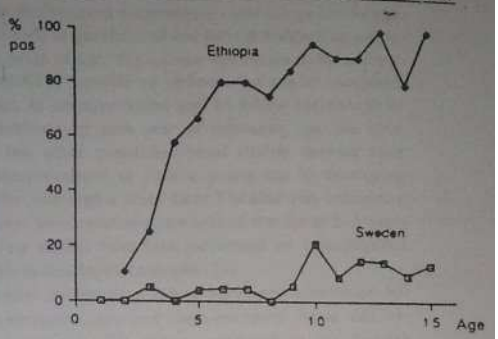


Fig. 1. Percentage seropositive to Helicobacter pylori by 1-year age groups in 1 sample of Ethiopian (n = 242) and 1 sample of Swedish children (n = 295).

Fig. 1 shows the percentage of seropositive children within each age group in Ethiopia and Sweden. In Ethiopia, the largest consecutive difference in seroprevalence was found between the ages of 2 and 4 years. Among the 4-year-olds, some 60% had already seroconverted, and among the 12-year-olds almost 100% had done so. In addition, the mean RAA titre among the seropositives increased with age. In the Swedish sample the largest consecutive difference in seroprevalence was found between the ages of 9 and 10 years. Among 10-year-olds the seroprevalence was 21%, and the values for older groups were similar. Mean RAA titres for the positive subjects did not increase with age.

DISCUSSION

We found that injection with HP is acquired at a very young age in Ethiopia, almost everybody having been infected before their teens. We also found that Swedish children acquire HP infection later in life, but still before their teens. Overall, the prevalence in children is thus much lower in Sweden than in Ethiopia. This is in accordance with the results of previous studies which used serological markers and showed that injection is acquired at a much

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younger age in developing countries, and that a greater portion of the population in those countries will eventually be infected during their lifetime (25, 26).

In the Ethiopian sample, seropositivity was higher in each consecutive age group up to 12 years, where it reached almost 100%. The increase in RAA titre with age may indicate repeated exposure to HP, with continuous natural boosters. In the Swedish sample, seroprevalence in children above 10 years of age was around 15–20%. A similar finding was made in a study in Belgium, in which the greatest difference in seropositivity occurred between 9 and 10 years of age, being 22% in the latter group and remaining at this level at least in the next highest age group (27).

All the findings cited here are from cross-sectional rather than longitudinal studies, but there is little reason to believe that the force of infection would change dramatically over the short periods covered by the lives of our study subjects. The findings thus suggest that in both developed and developing countries infection with HP is acquired mostly in childhood, and that few people experience primary infection after their teens. Longitudinal studies on adults from developed countries have generally shown a seroconversion rate of the order of 0.5%/year or below (28–31).

In the Ethiopian study the cut-off value was set to be the same as the one used for Swedish sera at the University of Lund. It is extremely difficult to determine a population-based cut-off value in Ethiopia, since there are almost no negative controls among adults in whom infection with HP could be excluded by culture, histopathology or the urea breath test. However, the overall distribution of the 242 RAA values in the Ethiopian study showed a bimodal shape with a shallow trough between RAA values of 30 and 40. If a RAA value of, say, 50 had been chosen instead, this would have lowered the seroprevalence values in Fig. 1, but the general shape of the distribution would have remained the same.

In Ethiopia we also included a local strain of HP for antigen preparation, which has previously only been done in a few studies (32). We found that, at least for seroepidemiological studies of populations, there seems to be little difference between using a local HP strain or a reference strain. Furthermore, when comparing a pooled antigen with an antigen from the reference strain, as was done in Sweden, there were no differences in identifying positive and negative sera.

In the Swedish study a cut-off value of Optical Density (OD) = 0.35 was chosen based on the frequency distribution of the 295 Swedish sera. The same distribution was seen in studies of children in Somalia and Estonia (Bengt Wretlind, personal communication). Altering the cut-off value in the range 0.30–0.40 gave rise to only marginal changes in the percentage of seroconversion, but did not change the age distribution.

The age-dependent acquisition of antibodies found here is in accordance with earlier studies from developing and developed countries (6, 7, 26, 33, 34). However, most of these studies involved considerably wider ranges for the age groups, and, in general, did not include children as young as 1–2 years of age. The narrow age ranges in the present study make it possible to estimate the age at maximum incidence of seroconversion and to follow the change in seroprevalence by each year of increasing age. We have found few other population-based studies showing such high seroprevalence at such a young age in developing countries, although a study from Thailand also indicates a maximum seroconversion rate around the age of 3–5 years (32). Few studies have been performed on asymptomatic children in developed countries (27).

Previous studies have shown an inverse correlation between seroprevalence and socio-economic status and hygiene conditions (35). When compared to the Swedish study, the high overall prevalence and the young age at seroconversion found in the Ethiopian study could be due to the generally lower socio-economic conditions in this developing country. Close contact with the other people or with animals, hygiene conditions and dietary habits are all factors that must be considered when discussing possible transmission routes, as are local eating traditions or the weaning of children. The water supply, if vulnerable to bacterial contamination, could be an important source of infection, as pointed out in an earlier study (3).

We have found that infection with HP seems to occur very early in life in Ethiopia and a few years later but still before the teens in Sweden. Any prevention of infection should thus be aimed at protecting young children. From a scientific point of view, the important conclusion is that studies on risk factors for infection should probably concentrate on circumstances applying to young children. Several previous such studies have included older children and adolescents, and it is conceivable that risk factors assessed in these age groups have a poor correlation to the actual exposures at the time when those children were infected. If factors such as overcrowding or diet change with increasing age, case-control studies of variables associated with seropositivity that include children who were infected several years previously may produce very dilute measures of the strength of association.

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REFERENCES

1. Marshall BJ, Warren JR. Unidentified curve bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet i: 131–134, 1984.
2. Rauws EAJ, Langenberg W, Houthoff HJ, Zanen HC, Tytgat GNJ. Campylobacter pyloridis-associated chronic active antral gastritis. Gastroenterology 94: 33–40, 1988.

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3. Vaira D, Holton J, Cairns SR, Falzon M, Polydoron A, Dowsett J, Salmon PR. Antibody titres to Campylobacter pylori after treatment for gastritis. Br Med J 297: 397, 1988.
4. Nomura A, Stemmerman GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 325: 1132–1136, 1991.
5. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 325: 1127–1131, 1991.
6. Al-Moagel MA, Evans DG, Abdulgani M, Adam E, Evans Jr DJ, Malaty HM, Graham DY. Prevalence of Helicobacter (formerly Campylobacter) pylori infection in Saudi Arabia, and comparison of those with and without upper gastrointenstinal symptoms. Am J Gastroenterol 85: 944–947, 1990.
7. Perez-Perez IG, Taylor GN, Bodhidatta L, Wongsrichanalai J, Baze WB, Dunn BE, Echeverria PD, Blaser MJ. Seroprevalence of Helicobacter pylori infections in Thailand. J Infect Dis 161: 1237–1241, 1990.
8. Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of Helicobacter pylori from human faeces. Lancet 340: 1194–1195, 1992.
9. Klein PH, Graham DY, Gaillour A, Opekun AR, O'Brian Smith E. Water source as risk factor for Helicobacter pylori infection in Peruvian children. Lancet 337: 1503–1506, 1991.
10. Hopkins RJ, Vial PA, Ferrecio C, Ovalle J, Prado P, Sotomayor V, Rusell RG, Wasserman SS, Morris Jr JG. Seroprevalence of Helicobacter pylori in Chile: vegetables may serve as one route of transmission. J Infect Dis 168: 222–226, 1993.
11. Ramsey EJ, Carey KV, Peterson WL, Jackson JJ, Murphy FK, Read NW, Taylor KB, Trier JS, Fordtran JS. Epidemic gastritis with hypochlorhydria. Gastroenterology 76: 1449–1457, 1979.
12. Marshall BJ. Campylobacter pyloridis and gastritis. J Infect Dis 153: 650–657, 1986.
13. Langenberg W, Rauws EAJ, Oudbier JH, Tytgat GNJ. Patient-to-patient transmission of Campylobacter pylori infection by fiberoptic gastroduodenoscopy and biopsy. J Infect Dis 161: 507–511, 1990.
14. Schembri MA, Lambert JR, Lin SK, Nicholson L, Korman MG, Coulepis A. Helicobacter prevalence in endoscopy staff. Microbiol Ecol Health Dis 4: 194, 1991.
15. Drumm B, Perez-Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of Helicobacter pylori infection. N Engl J Med 322: 363, 1990.
16. Vaira D, Holton J, Londei M, Beltrandi E, Salmon PR, D'Anastasio C, Dowsett JF, Bertoni F, Gravenfels P, Gandolfi L. Campylobacter pylori in abattoir workers: is it a zoonosis? Lancet 2: 725–726, 1988.
17. Morris A, Nicholson G, Lloyd G, Haines D, Rogers A, Taylor T. Seroepidemiology of Campylobacter pyloridis. NZ Med J 99: 654–659, 1986.
18. Albenque M, Tall F, Dabis F, Megraud F. Epidemiological study of Helicobacter pylori transmission from mother to child in Africa. Third Workshop of the European Helicobacter pylori Study Group. Enfermedades Digestivas 78 (Suppl. 1): 48, 1990.
19. Krajden S, Fuksa M, Andersson J, Kempston J, Boccia A, Petrea C, Babida C, Karmali M, Penner JL. Examination of human stomach biopsies, saliva and dental plaque for Campylobacter pylori. J Clin Microbiol 27: 1397–1398, 1989.
20. Perez-Perez GI, Dworkin BM, Chodos JE, Blaser MJ. Campylobacter pylori antibodies in humans. Ann Intern Med 109: 11–17, 1988.
21. DeGiacomo C, Lisato L, Negrini R, Licardi G, Maggiore G. Serum immune response to Helicobacter pylori in children: Epidemiologic and clinical applications. J Pediatr 119: 205–210, 1991.
22. Tsega E, Tafesse B, Horton J, Nordenfelt E, Wolde-Havariat G, Hansson B-G, Lindberg J. Immunogenicity, reactogenicity and comparison of two doses of recombinant DNA yeast-derived hepatitis B vaccine in Ethiopian children. Trop Geog Med 43: 220–227, 1991.
23. Guruge JL, Schalén C, Nilsson I, Ljungh Å, Tyszkiewicz T, Wikander M, Wadström T. Detection of antibodies to Helicobacter pylori cell surface antigens. Scand J Infect Dis 22: 457–465, 1990.
24. Morera-Brenes B, Sierra R, Mora F, Mora W, Palmgren A-C, Quiros E, Salas P, Solano L, Nord CE. Occurrence of Helicobacter pylori infections in Costa Rican patients. Accepted for publication.
25. Li YY, Hu PJ, Du GG, Hazell SL. The prevalence of Helicobacter pylori infection in the Peoples Republic of China. Am J Gastroenterol 86: 416–419, 1991.
26. Graham DY, Adam E, Reddy GT, Agarwal JP, Agarwal R, Evans Jr DJ, Malaty HM, Evans DG. Seroepidemiology of Helicobacter pylori infection in India. Comparison of developing and developed countries. Dig Dis Sci 36: 1084–1088, 1991.
27. Blecker U, Hauser B, Lanciers S, Peeters S, Suys B, Vandenplas Y. The prevalence of Helicobacter pylori-positive serology in asymptomatic children. J Paediatr Gastroenterol Nutr 16: 252–256, 1993.
28. Veldhuyzen van Zanten SJO, Pollak PT, Best LM, Bezanson GS, Marrie T. Increasing prevalence of Helicobacter pylori infection with age: continuous risk of infection in adults rather than cohort effect. J Infect Dis 169: 434–437, 1994.
29. Cullen DJE, Collins BJ, Christiansen KJ, Epis J, Warren JR, Surveyor I, Cullen KJ. When is Helicobacter pylori infection acquired? Gut 34: 1681–1682, 1993.
30. Parsonnet J, Blaser MJ, Perez-Perez GI, Hargrett-Bean N, Tauxe RV. Symptoms and risk factors of Helicobacter pylori infection in a cohort of epidemiologists. Gastroenterology 102: 41–46, 1992.
31. Lindkvist P, Wadström T, Giesecke J. Helicobacter pylori infection and foreign travel. J Infect Dis in press, 1995.
32. Bodhidatta L, Hoge CW, Chumratanakul S, Nirdnoy W, Sampathanukul P, Tungtaem C, Raktham S, Smith CD, Echeverria P. Diagnosis of Helicobacter pylori infection in a developing country: comparison of two ELISAs and a seroprevalence study. J Infect Dis 168: 1549–1553, 1993.
33. Megraud F, Brassens-Raabe M-P, Denise F, Belbouri A, Hoa DQ. Seroepidemiology of Campylobacter pylori infection in various populations. J Clin Microbiol 27: 1870–1873, 1959.
34. Mitchell HM, Li YY, Hu PJ, Liu Q, Chen M, Du GG, Wang ZJ, Lee A, Hazell SL. Epidemiology of Helicobacter pylori in Southern China: identification of early childhood as the critical period for acquisition. J Infect Dis 166: 149–153, 1992.
35. Sitas F, Yarnell JWG, Burr ML, Elwood PC, Pedley S, Marks KJ. Helicobacter pylori infection rates in relation to age and social class in a population of Welsh men. Gut 32: 25–28, 1991.

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COMPARISON OF DIAGNOSTIC METHODS FOR DETECTION OF *HELICOBACTER PYLORI*
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COMPARISON OF DIAGNOSTIC METHODS FOR DETECTION OF *HELICOBACTER PYLORI*

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SUMMARY

Of two hundred Ethiopian patients with dyspepsia, multiple biopsies were taken from the antrum of the stomach. *Helicobacter pylori* was cultured from 85% of duodenal ulcer and in 75% of chronic antral gastritis patients. The overall *Helicobacter pylori* positivity was 70%. The sensitivity, specificity, positive and negative predictive values of the tests as compared to culture were as follows, respectively: direct urease test 100%/87%/95%/100%, direct gram stain 60%/98%/99%/51%, histological gram stain 66%/97%/98%/56%, Giemsa stain 100%/97%/99%/100% and Gimenez stain 100%/87%/95%/100%. It is concluded that gram staining of direct tissue smear or histology is an insensitive method in the diagnosis of *Helicobacter pylori*. All the other tests, are shown to be valid. Urease test is an excellent test for provision of presumptive diagnosis of *Helicobacter pylori* while awaiting confirmation either by culture or histology.

INTRODUCTION

Helicobacter pylori (HP) is found only in gastric mucosa and has been shown to be associated with chronic active gastritis and gastroduodenal ulceration(1,2). Tsega *et al*(3), reported the presence of HP in 94% of duodenal ulcer(DU) and 73% of non-ulcer dyspepsia in Ethiopian patients. Recent studies indicated that HP is no more a medical curiosity and is believed that more than 90% of DU and 70% of gastric ulcers are caused by HP(4,5). The correct diagnosis of HP infection, therefore, is an important goal in the management of patients with peptic ulcer diseases.

Invasive and non-invasive diagnostic methods are now available to identify HP infection(6). The invasive diagnostic method utilises endoscopy to obtain biopsy which could be used for direct urease test, gram stain, culture and histology. In spite of the fact that, direct gram stain provides rapid diagnosis with excellent specificity, there is controversy regarding its sensitivity(7-12). Likewise, the sensitivity of histology, depending on the type of stain used, and urease test varies(8,13-15). In the present study, we compared direct urease test, direct gram stain and histology using gram, Giemsa and Gimenez stains with culture which is generally used as a "gold standard".

MATERIALS AND METHODS

Patients: Two hundrd Ethiopian patients with dyspepsia who underwent endoscopic examination of the upper gastrointestinal tract in the gastroenterology unit of Tikur Anbessa Hospital, a teaching and referral hospital of Addis Ababa University, were consecutively studied for HP. There were 123 males and 77 females with a mean age of 34.8 years (range 16-70 years). The protocol was approved by the research and ethics committee of the Faculty of Medicine of Addis Ababa University.

Endoscopy: Upper gastrointestinal endoscopy was done after sedation with intravenous pethidine and oropharyngeal anaesthesia with one percent xylocaine spray. The endoscopes (Olympus GIF, Q10) and the biopsy forceps were cleansed with a detergent (30% savlon) and disinfected with 70% alcohol and 2% glutaraldehyde (cidex by soaking for 5-10 min and finally rinsed with water after each examination. Four biopsies (two for bacteriology and two for histology) were obtained from the antrum of the stomach of each patient.

Bacteriology: The two biopsy specimens for bacteriological studies were transported in 0.5 ml of sterile normal saline and were divided into four portions using sterile blades. One portion was directly smeared, gram stained and examined under oil immersion for 3-5 min or until gram negative curved rods were observed. The second portion was inoculated into urease broth (difco) containing percent isovatalex (BBC). Both plates were incubated at 37°C in a moist 10% candle jar. They were inspected daily for six days for flat and colourless colonies. If such colonies appeared, they were smeared, gram stained and tested for urease, catalase and oxidase positivity. A curved gram-negative rod shaped bacteria that was oxidase, catalase and urease positive was identified as HP.

Histology: The two biopsy specimens for histology were kept in 10% formalin until embedded in paraffin. Four sections, each of five micron thickness, were prepared from these specimens. The first section was stained with Haematoxylin and eosin (H&E). The histological findings from the H&E stain were read and scored according to the classification of Whitehead(16) using the pictorial criteria of Joske(17). The remaining three sections were stained with gram Giemsa and Gimenez techniques(18-20). HP was identified as curved rod shaped bacteria lying beneath the mucous layer of the epithelium, especially in the region of the gastric pits.

The endoscopic remained blinded to the bacteriological and histological results until the end of the study. Likewise, the bacteriologist had no access to the endoscopic of histologic findings and the pathologist did not have knowledge about the endoscopic and bacteriology results until the completion of the study.

Statistical method: The validity of the tests were assessed

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using their sensitivity, specifivity, positive and negative predictive values (PPV and NPV) employing culture as a "gold standard".

RESULTS

Endoscopy revealed duodenal ulcer in 87 patients, gastritis in 93 patients, oesophageal varices in nine patients, carcinoma of the body of the stomach in one patient and normal findings in ten patients. Histology, on the other hand, showed adenocarcinoma of the stomach in one patient and chronic antral gastritis (CAG) in 168 patients with neutrophil infiltration in almost all of them indicating active gastritis. The remaining thirty two patients however, had normal histological finding. Of the 87 patients with duodenal ulcer, 80 patiens had histologic gastritis and seven patients had normal histology. 72.5% of the patients had gastritis diagnosed both by endoscopy and histology.

Table 1 summarises the results of the various tests in the diagnosis of HP infection. Culture was positive for HP in 140 patients, direct urease test in 148 patients, direct gram stain in 85 patients, histological gram stain in 94 patients, Giemsa stain in 142 patients and Gimenez stain in 147 patients. Eight urease test, one direct gram stain, two histological gram stain, two Giemsa stain and seven Gimenez stain positive patients with culture negative. On the other hand, there were no patients who were culture positive but urease, Giemsa or Gimenez stain negative. Except for one, all urease test positive patients were Giemsa and/or Gimenez stain positive. This indicates the fact that urease test positivity had an excellent correlation with histology when stained with Giemsa or Gimenez stains.

Table 1

Results of the various diagnostic tests in the detection of H. pylori in patients with dyspepsia

Type of test	Positive	Negative	Total
Culture	140	60	200
Direct urease test	148	52	200
Direct gram stain	85	115	200
Histology gram stain	94	106	200
Giemsa stain	142	58	200
Gimenez stain	147	53	200

Table 2

The sensitivity, specificity, positive and negative predictive values of the various diagnostic tests as compared to culture in the detection of H. pylori

Type of test	Sensitivity %	Specificity %	PPV %	NPV %
Direct urease test	100	87	95	100
Direct gram stain	60	98	99	51
Histology gram stain	66	97	98	55
Giemsa stain	100	97	99	100
Gimenez stain	100	87	95	100

Note: PPV=positive predictive value
NPV=negative predictive value

Table 2 summarises the sensitivity, specificity, positive and negative predictive values of direct urease test, direct gram stain and special histological stains as compared to culture.

Direct tissue smear and histological gram stains had the least sensitivity (60% and 66%, respectively) and NPVs (51% and 55%, respectively, of all the tests. However, they had excellent specificity (98% and 97%, respectively) and PPVs (99% and 98%, respectively). There was no remarkable difference in sensitivity, specificity, positive and negative predictive values between the two grams tains. Urease test, Giemsa and Gimenez stains, on the other hand, had a sensitivity and NPV of 100%. Although the specificity and PPV of urease test (86.7% and 94.6%) and Gimenez stain (86.7% and 95.2%, respectively) were almost the same, it was lower than that of Giemsa stain (96.7% and 98.6%, respectively).

DISCUSSION

The finding of culture positivity in 70% of our patients is comparable to many reports from other parts of the world (19,21-24). Direct tissue smear gram stain was reported as good and rapid test in the diagnosis of HP by several investigators(7-9,25). Queiroz *et al*(26), compared the sensitivity of gram stain, urease test and culture and found no difference among the tests. On the other hand, Nicholas *et al* (27), reported a sensitivity of 69% for direct gram stain, 90% for culture and 93% for histologic examination, showing a clear difference. In addition, Magraud, Anderson *et al*, Marshal and Moris *et al* had shown unacceptably low sensitivity for direct gram stain (10-12,23). The low sensitivity of 60% for direct gram stain in our study is similar with the latter reports. Trowell *et al*(18) reported the correlation of histological half gram stain with culture in 91% of cases. This contrasts with the low sensitivity of 66% for histological gram stain in this study. When all the test criteria were compared between direct gram stain and histological gram stain, the difference was not striking. Our finding suggests that gram stain is of less importance in the diagnosis of HP infection.

Although urease test and Gimenez stain had a 100% sensitivity, their specificity of 87% and 88% respectively, was lower than the specificity of 98% reported by other investigators(5-6,15). Taking the 99% concordance rate between urease test and Gimenez stain, their low specificity could be due to the relatively low sensitivity of culture which was used as a "gold standard" in this study. McCullen compared Gimenez stain, gram stain and culture for detection of HP infection and reported the highest for Gimenez stain and the lowest for culture(20). Furthermore, Megraud compared urease test, histopathology, gram stain, culture and found out that culture and gram stain had a low sensitivity(23). This suggests the fact that the false positive urease test and gimenez stain, accounting for the low specificity, may actually be a true positive. Since the subculture for other urease producing organisms did not grow, it was unlikely for these organisms to cause a false

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positive urease test.

Despite the relatively low specificity, urease test and Gimenez stain had a very good positive and negative predictive values. Because of the high sensitivity, the excellent negative predictive value and the rapid result that urease test provides, it is an important test for presumptive diagnosis of HP infection.

Giemsa stain with its excellent sensitivity(100%), specificity(97%), positive and negative productive values (99% and 100%, respectively), is the best staining technique for histological diagnosis of HP infection. This finding is incomplete agreement with other reports(5,6).

In conclusion our finding suggests that both direct tissue smear and histological gram stain are of less importance in the diagnosis of HP infection. All the other tests, as compared to the gold standard, are shown to be valid. Furthermore, urease test is an excellent test for provision of presumptive diagnosis of HP infection while awaiting confirmation be either culture or histology.

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REFERENCES

1. Rauws, E., Langenberg, W., Houthoff, H., Zanen, H. and Tytgut, G. Campylobacter pyeloridis associated chronic active gastritis. *Gastroenterology*. 1988; 94:33-40.
2. Glupezynki, Y., Durette, A. ad Nyst, J.F. *Campylobacter pylori* associated gastritis. *Acta. Gastroenterol. Belg*. 1989; 51:329-33.
3. Tsega, E., Gebre, W., Hathaway, A.E. and Kassa, E. *H. pylori* in Ethiopian patients with dyspepsia. *Ethiop. Med. J*. 1992; 30:241.
4. Rauwsea, E.A. and Tytgat, G.N. Eradication of *Helicobacter pylori* cures duodenal ulcer. *Gastroenterology*. 1990; 199:98:A111.
5. Marshall, B.J. *Helicobacter pylori:* a review. *Amer. J. Gastroenterol*. 1994; 89:160-128.
6. Brown, K.E. and Peura, D.A. Diagnosis of *Helicobacter pylori* infection. *Gastroent. Clin. N. Amer*. 1993; 22:105-15.
7. Pickard, K.J., Harrison, B. and Castick, J.A. Detection of Campylobacter pyeloridis in gastric mucosa by phase contrast microscopy. *J. Clin. Pathol*. 1986; 39:112.
8. Montgomery, E.A., Martin, D.F. and Peura, D.A. Rapid diagnosis of Campylobacter pylori by gram stain. *Amer. J. Clin. Pathol*. 1988; 90:606.
9. Malan, E., Kemp, J. and Westblom, U. Evaluation of staining method for identifying Campylobacter pyeloridis. *Amer. J. Clin. Pathol*. 1988; 90:450.
10. Anderson, L.P., Holck, S. and Povlsen, C.O. Campylobacter pylori in peptic ulcer diseases: Histologic and microscopic findings. *Scand. J. Gastroenterol*. 1987; 22:219.
11. Marshall, B.J. and Warren, J.R. An identified curved bacilli in stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984; 1:311.
12. Morris, A. Arthur, J. and Nicholson, G. Campylobacter pyeloridis infection in Auckland patients with gastritis. *N. Z. Med*. 1986; 99:353.
13. Aymard, Labouyrie and de Korwin, J.D. Histological staining methods for detection of gastric Campylobacter-like organisms: A comparative study. *Gastroenterolgy*. 1988; 94:A16.
14. Morris, A. Mcintyre, D., Rose, T. and Nicholson, G. Rapid diagnosis of Campylobacter pyeloridis infection. *Lancet*. 1986; 1:149.
15. Borsch, G., Adamek, R. and Sandan, M. Comparison of urease test and histologic examination for detection of *Campylobacter pylori* in duodenal, antral and fundic biopsies. *Hepatogasteroenterology*. 1987; 34:236-238.
16. Whitehead, R., Truelove, Sc. and Gear, W.L. The histological diagnosis of chronic gastritis in fibroptic gastroscope biopsy specimens. *J. Clin. Pathol*. 1972; 25:1-11.
17. Josek, R.A., Genkch, E.S. and Wood, I.J. A study of 1000 consecutive successful gastric biopsies. *Quart. J. Med*. 1955; 24:269-294.
18. Trowell, J.E., Young, A.K.H. ans Saul, K.J. Simple half gram stain for showing presence of Campylobacter pyeloridis in sections. *J. Clin. Pathol*. 1987; 40:702-705.
19. Jones, D.M., Lessells, A.M. and Eldrige, J. Campylobacter like organisms on the gastric mucosa: Culture histological and serological studies. *J. Clin. Pathol*. 1984; 37:1002-1006.
20. McCullen, M., Walter, M.M. and Bain, L.A. Histological identification of Campylobacter using Gimenez technique in gastric antral mucosa. *J. Clin. Pathol*. 1987; 40:464-465.
21. Mohammed, A.E., Al-Karawi, A. and Al-Jumah, A.H. Pylori: Incidence and comparison of three diagnostic methods in 196 Saudi patients with dyspepsia. *Hepatogastroenterology*. 1994; 41:48-50.
22. Takagi, A., Ohta, U., Shirai, T. and Kobda, K. Evaluation of polymerase chain reaction for diagnosis of *Helicobacter pylori* infection. *Nippon Shokakibuo Gakkai Zasshi*. 1994; 91:1277-82.
23. Megraud, F. Direct and indirect diagnostic methods for H. pylori infection. *Gastroenterol. Clin. Biol*. 1994; 18:217-22.
24. Hernardz, F. Rivera, P. and Sigaran, M. Comparison of urease test histology and culture. *Rev. Inst. Med. Trop. Sao. Paulo*. 1991; 33:80-83.
25. Humphreys, H. and o'Morainc. Culture of organisms and histochemical identification. *Scan. J. Gastroenterol*. 1988; 23:16-19.
26. Queiroz, D.M., Rocha, G.A., Mendes, E.N. Difference in distribution and severity of *Helicobacter pylori* in gastritis in children and adult with peptic ulcer disease. *J. Paediat. Gastroenterol. Nutr*. 1991; 12:178-81.
27. Nichols, L., Sughayer, M. and Degirolami, P.C. Evaluation of diagnostic methods for Helicobacer gastritis. *Amer. J. Clin. Pathol*. 1991; 95:769-73.

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HIV-1 INFECTION IN RELATION TO EDUCATIONAL STATUS, USE OF HYPODERMIC INJECTIONS AND OTHER RISK BEHAVIOURS IN ETHIOPIAN SAILORS

K. DEMISSIE, D. AMRE and E. TSEGA

SUMMARY

Information on the prevalence and risk factors for HIV infection among sailors is generally scarce. The objectives of the study were to determine the prevalence and risk factors for HIV-1 infection among sailors in Ethiopia. A cross-sectional study was carried out in a population of sailors identified from their employment records. Two hundred and sixty sailors were personally interviewed to obtain information on risk factors. Blood samples were collected for the determination of antibodies against HIV-1 infection by ELISA with confirmation by Western blot. The prevalence of HIV-1 infection was 9.6% and the prevalence was observed to decrease with increasing level of education. The risk of acquiring infection was also found to increase with the use of hypodermic injections (OR=3.42, 95% CI: 1.19 to 9.80). Fourteen percent of the studied population reported condom use. However, the use was irregular. We did not find marital status and consumption of alcohol to be associated with HIV-1 infection. The high prevalence of HIV-1 infection in this population is alarming. Specific education programmes targeted to this group need to be established in order to reduce the increasing risk of infection in this population and the spread of infection to other segments of the population.

INTRODUCTION

The pandemic of HIV (type 1) infection is rapidly spreading, the sub-Saharan Africa alone accounting for approximately 60% of the estimated 11 million or more infections worldwide(1,2,3). Currently it is believed that acquired immuno-deficiency syndrome (AIDS) kills about 100,000 people a year worldwide and about 12 million children may have been infected with HIV. By the year 2000, as many as 40 million people could be infected, 90% of them are expected to occur in the developing world(4).

The prevalence of HIV infection in Africa varies from 2% to 85%(5,6). Type-1 is more common, with type 2 reported mainly from West Africa(7). The main risk factors identified for the heterosexual transmission were sexually transmitted diseases, especially the ulcerative varieties such as chancroid and syphilis, prostitution, multiple sexual partners, lack of male circumcision, and being separated, divorced or widowed(3,6,8,9).

In Ethiopia, the first HIV carriers were detected in 1984 (10) and the first few cases of AIDS were reported in 1986(11). By 1993, the mean prevalence of HIV infection was 7.7% and the number of AIDS cases was 3,739(12). These figures are likely to be an underestimate with the projected HIV infected persons from 1984 to 1993 being as high as 810,550 and the projected AIDS cases being about 45,458.

Of the first hundred cases admitted to the Central Referral Hospital (Tikur Anbessa Hospital) in Addis Ababa, the capital city of Ethiopia, the majority of the cases were from the port town of Asseb(13). Asseb is situated in the north eastern part of Ethiopia and is frequented by a large number of foreign and Ethiopian sailors. These sailors visit different European cities for about six months in a year and reside in Asseb city for the rest of the year. During the six months of residence in the port city, they interact with a vast segment of the population and hence are likely to be at high risk of being infected with HIV and are also capable of transmitting it. It is speculated that these sailors are perhaps the main reservoir of infection.

Todate there are no reports describing the prevalence of HIV infection in this population and neither is there any information on their risk behaviours. In order to obtain baseline information useful for the planning of preventive strategies, a cross-sectional study was carried out among Ethiopian sailors.

The main objectives of this study were thus: (i) to investigate the prevalence of HIV-1 infection in a population of sailors and (ii) determine the risk factors and behaviours associated with infection.

Amir sultan (MD), 2014

MATERIALS AND METHODS

Selection of subjects: During the initial discussions held with the management and representatives of the sailors labour union, the main objectives of the study were explained. A list of 300 currently employed sailors was provided by the employer. Over a period of one year, we were able to study 260 sailors. The rest (forty) were on board the ship and were sailing abroad and had not returned till the completion of the study.

Interview: Prior to the start of the interview, each subject was informed on the major objectives of the study and verbal consent was obtained from them.

Interviews were carried out by trained interviewers, using the research questionnaire prepared by the World Health Organisation for their Global Programme on AIDS(14). The questionnaire has been designed to survey patterns of sexual behaviour in different populations around the world. It is meant to be a prototype instrument for research, that can be translated and adapted according to the needs of different national situations and interests. A number of behavioural scientists were involved in the development of the questionnaire(14).

Information was collected for the following variables: socio-demographic characteristics such as age, sex, ethnicity, religion, marital status (currently married for the last one year, single, divorced, separated and widowed), number of children and educational status (literate: being able to read and write the Ethiopian language; Elementary education: ever attended grade 1-5; Junior high school: ever attended grade 6-8; Senior high school: ever attended grade 9-12; and University: ever attended above grade 12).

The questions asked on sexual behaviours were: 'have you had penetrative sexual intercourse with someone other than your spouse , in the last 12 months ?' (extramarital sex); 'did you or your partner use condom when having sexual intercourse?' (condom use); if the response is yes, 'was it each time or some times?'; 'in the last 12 months, did you have gonorrhoea, syphilis or chancroid?' (sexually transmitted diseases, STD).

Information was also collected on other risk factors such as blood transfusions, history of hypodermic injections and source of such injections (hospitals, private homes or private clinics) and circumcision. Habits such as intravenous drug abuse and consumption of alcohol were enquired into. For alcohol use, type and quantity of drinks consumed in a week was recorded. Based on the above information we were able to quantify the amount of alcohol consumed by each subject in the last one year and classify them into those who usually consumed alcohol and those who did not (alcohol consumption).

Serological tests: Five to 8 cc of blood was drawn from each subject and stored at -20°C pending determination of HIV-1 antibodies. HIV-1 antibodies were determined by the Wellcozyme ELISA method (Wellcozyme anti HTLV-III, Wellcozyme Diagnostic, Dartfield, U.K). ELISA was repeated on the positive sera and this was confirmed by Western Blot (BIO/RAD Clinical Division, Hercules Ca, Milan, Italy). Tests were performed by an experienced senior laboratory technologist.

Ethical issues: The protocol of this study was approved by the Research Committee of the Faculty of Medicine, Addis Ababa University. Oral consent was obtained from participants after explanation of the objectives of the study and strict confidentiality of the final results. Decoding of the results of the tests was done by the physician investigators. Pre-test, study participants were counselled by a medical doctor. Those participants who were found to be positive for HIV-1 infection were counselled by trained counsellors.

Statistical methods: The outcome or dependent variable in this study was HIV infection. The independent variables were age, educational level, level of income, marital status, extra-marital sex, profession of extramarital sexual contact, use of hypodermic injections, source of injections, use of condoms, blood transfusions, circumcision, STD's and alcohol consumption.

The relationships between these independent variables and the outcome variable were analysed using multiple logistic regression(15). Statistical significance was defined as a two-tailed, $p<0.05$. Analysis was carried out using the SAS statistical software(16).

RESULTS

All the subjects in the study were male with a mean age of 34 years (S.D= 8.1). Eighty percent (205/256) were less than forty years old. Only 4.2% (11/260) were illiterate while 49% (11/260) of them had high school education. Their mean monthly income was US$ 259 (S.D=183). The distribution of sailors in the different income groups was similar. Forty-five percent (119/260) of the sailors were married at the time of the study, 51% had never married while the rest were either divorced, separated or widowed.

Table 1

Socio-economic characteristics of the study population

	Number	*Percentage(%)
Age (years)		
18-30	95	37
31-40	110	43
41-57	51	20
Education (grade)		
Illiterate	11	4.2
Elementary and junior high school (1-8)	51	19.6
Senior high school (9-12)	127	48.8
University	71	27.3
Income (U.S. $)		
less than 145	69	26.8
145-210	73	28.4
210-314	60	23.3
more than 314	55	21.4
Marital status		
Married	119	45.8
Single	133	50.8
Divorced	6	2.3
Separated	1	1.2
Widowed	1	1.2

*may not add upto 100 due to rounding

Extramarital sex was reported by 13.4% of the married sailors. Ninety two percent of these extramarital contacts were with prostitutes. Twenty-eight percent (73/260) reported a history of STD in the past one year. Of these 11% (9/73) reported either chancroid or syphilis and 86% (62/73) reported gonorrhoea.

Table 2

Prevalence of risk factors for HIV infection.

	Number	Percentage(%)
Extramarital sex	16	13.4
Extramarital sexual contact with:		
Prostitutes	110	92.4
Others*	9	7.6
Circumcision	257	99.0
Blood transfusion	6	2.3
Use of Injections	201	77.3
Injection Source		
Hospitals	52	25.8
Private homes	56	27.9
Private clinics	93	46.3
Condom use	37	14.2
STD		
None	187	71.5
Gonorrhoea	62	23. 8
Syphilis	4	1.5
Chancroid	5	1.9
Alcohol consumption	136	53.3

*includes office girls, students and housemaids.

Ninety-two percent of the sailors were circumcised (257/260). Only 2.3% (6/260) had received blood transfusion. In the year preceding the study, hypodermic injections were taken by 77% (201/260) with 75% (151/201) of these taken at the private clinics or the private homes. About 14% (37/260) reported the use of condoms. None reported the use of intravenous drugs and 53% (136/260) usually consumed alcohol.

The prevalence of HIV infection was 9.6% (25/260). All age groups between 18 and 57 years were affected, with 84% (21/25) occurring in those less than 40 years old. There was a tendency for the prevalence to decrease with increasing age, however it did not achieve statistical significance.

Table 3

Relationship of risk factors and behaviours with HIV infection.

	Unadjusted Odds ratios(95% CI)	Adjusted† Odds ratios(95% CI)
Age (years)		
41-57	Reference category	Reference category
31-40	1.20(0.30-4.80)	1.30(0.33-5.00)
18-30	2.15(0.40-9.10)	2.25(0.50-10.1)‡
Education		
University	Reference category	Reference category
High school	2.15(0.58-8.00)	2.11(0.50-9.34)
Elementary & junior	4.90(1.32-18.44)	6.95(1.51-31.94)‡
Income (U.S.$)		
more than 314	Reference category	Reference category
211-314	0.51(0.13-2.08)	1.33(0.30-6.90)
145-210	0.81(0.24-2.70)	1.80(0.31-4.41)
less than 145	1.41(0.50-3.93)	2.33(0.80-6.90)
Injection use	3.22(1.16-8.94)	3.42(1.19-9.80)‡
Injection source		
Hospitals	Reference category	Reference category
Private homes	2.33(0.57-9.55)	2.09(0.50-8.75)
Private clinics	2.65(0.72-9.80)	2.43(0.65-9.14)
Condom use	0.50(0.11-2.20)	0.58(0.12-2.45)
Extramarital sex	0.70(0.33-1.73)	0.61(0.24-1.55)
Marital status	0.76(0.33-1.73)	0.61(0.24-1.55)
STD	0.90(0.34-2.36)	0.80(0.30-2.40)
Alcohol consumption	0.58(0.25-4.01)	0.67(0.26-1.75)

† Adjusted for other terms in the table; ‡ p<0.05

The prevalence of HIV infection increased significantly with decreasing level of education. The highest risk of infection was among sailors who had elementary level of education. The risk of infection increased with the increasing level of income. However, it did not achieve statistical significance.

Use of hypodermic injections significantly increased the risk of HIV infection and this risk was higher when the injections were given either at the private clinics or the private homes as compared to the hospitals. The prevalence of HIV infection did not differ significantly amongst those who were married and those who were not. Of the 141 married sailors, only 31(22%) reported having extramarital sex in the last one year prior to the interview. Of these, only two had infection and because of the small numbers involved meaningful analysis on this variable was not possible. Condom use was associated with a decreased risk for infection although the risk was not statistically significant. As can be seen from Table 2, the number of sailors who reported having syphilis and chancroid were small. Comparison of HIV infection between those who had either gonorrhoea, syphilis or chancroid (STD) on one hand and those without any history of these infections on the other, did not reveal any significant differences. Consumption of alcohol also did not increase the risk of infection.

DISCUSSION

We found HIV infection in 9.6% of the sailors in our study sample. The prevalence of HIV infection was observed to decrease with increasing level of education. The risk of acquiring HIV infection was also found to increase with increasing use of hypodermic needles. This risk was highest when the injection was taken in private clinics and private homes as compared to hospitals. To our knowledge there was no publication reporting the prevalence and risk behaviours among sailors and as a result we were not able to compare our results with others.

We were able to obtain information on socio-demographic features in the 40 sailors (13%) who were not studied in our sample. Comparison with sailors included in our sample did not show meaningful differences in age, educational status and level of income.

About 84% of the sailors who had HIV infection were less than 40 years of age. This finding is expected as this age group is likely to be sexually more active. The risk of HIV infection had a tendency to increase in the lower age groups. However it did not achieve statistical significance.

Contrary to many reports from Africa(17-20), in our sample, higher education was found to protect against the likelihood of acquiring HIV infection. In those studies that reported higher prevalence of HIV infection among the most educated, greater opportunity for travel and social interaction was suggested as a probable explanation. In Ethiopia, the National AIDS Control Programme was established at a very early stage of the epidemic and the educational campaign though not targeted to any specific

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population subgroup, it is likely that the more educated to have benefited most because of better access to mass media such as television. Furthermore, perhaps the culture of the educated class in Ethiopia might be different from the rest of Africa.

Similarly, HIV infection was found to be more common in higher income groups in other African studies(21). In our study population, there was no relationship between HIV infection and income. Increased alcohol intake was suggested as a possible explanation for the increased HIV infection among those with higher income. However those with high income are likely to have higher education and are less likely to consume alcohol and involve in high risk behaviour.

A higher risk of infection was observed in sailors who had hypodermic injections, a finding in conformity with other reports(22,23). However, others have reported no association between the risk of HIV infection and use of hypodermic injections(24,25,26). The finding that HIV infection was higher when injections were taken at private homes and private clinics as compared to those taken at the hospitals could be due to the use of non-disposable needles in the private homes and private clinics and the use of stringent sterilisation techniques in hospitals since the establishment of the National AIDS Control Programme(12).

We did not find history of STD to be associated with HIV infection. However, only 2.3% of the sailors had ulcerative type of STD. These small numbers may have resulted in the lack of association.

Sailors who were single, divorced, separated or widowed were not found to be at higher risk of acquiring HIV infection compared to those who were married. Most of the sailors are away from their homes for long periods of time and they are likely to engage in risky sexual behaviours irrespective of their marital status.

Use of condoms seemed to protect against infection though the risk was not significantly decreased. Most of the sailors who reported using condoms did so infrequently and this may have resulted in the nonsignificant association.

The population was homogenous with respect to circumcision. Only two sailors were noncircumcised and hence no conclusions could be drawn from the analysis of this variable.

The reported history of blood transfusion was quite low. This might be because healthy workers are selected to be sailors and hence they are less likely to suffer from illnesses requiring blood transfusion.

Alcohol consumption did not show an association with a risk for acquiring HIV infection. The type of question asked may have resulted in under-reporting.

In conclusion, the high prevalence of infection in this population of sailors is alarming and they are probably the main reservoirs of infection besides prostitutes. Presently, there are no specific AIDS Educational Programmes targeted to sailors. It is necessary that such preventive strategies be established to reduce the increasing rate of infection in this population and the spread of infection to other segments of the population.

REFERENCES

1. Haneberg, B. and Howlett, W.P. Aids in the Third World. Africa suffers most. *Tidsskr Nor Laegeforen.* 1991; 111: 1493.
2. Estebanez, P., Fitch, K. and Najera, R. HIV and female sex workers. *Bull Wld. Hlth. Organ.* 1993; 71:397.
3. Berkley, S. AIDS in the developing world: An epidemiologic overview. *Clin. Infect. Dis* 1993; 17: 329.
4. ISSN 0954-6510. The Hidden Cost of AIDS. *World AIDS* 1993; No 25.
5. Abdool-Karim, Q., Abdool Karim, S.S., Singh, B.,Short, R. and Ngxongo, S. Seroprevalence of HIV infection in South Africa. *AIDS.* 1992; 6:1535.
6. Cameron, D.W., Simonsen, J.N., D'Costa, L.J., Ronald, A.R., Maitha, G.M., Gakinya, M.N., Cheang, M., Ndinya-Achola, J.O., Piot, P. and Brunham, R.C. Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. *Lancet.* 1989; 2:403.
7. De Cock, M., Adjorlolo, G., Ekpini, E., Sibailly, T., Konadio, J., Maran, M., Brattegaard, K., Velter, K.M., Doorly, R. and Gayle, H.D. Epidemiology and 15 transmission of HIV-2. Why there is no HIV-2 pandemic? *J. Ass. Med. Amer.* 1993; 270: 2083.
8. Diallo, M.O., Ackah, A.N., Lafontaine, M.F., Doorly, R., Roux, R., Kanga, T.M., Heroin, P. and De Cock, K.M. HIV-1 and HIV-2 infections in men attending sexually transmitted disease clinics in Abidjan, Cote d'Ivoire. *AIDS.* 1992; 6:581.
9. Larsen, C., Asefa, M., Aboud, F. and Sheferaw, T. Risk behaviours for HIV infection; their occurrence and determinants in Jimma-town, Southern Ethiopia. *Ethiop Med J.* 1991; 29: 127.
10. Tsega, E., Mengesha, B., Nordenfelt, E., Hanson, B.G. and Linberg, J. Serological survey of human immunodeficiency virus infection in Ethiopia. *Ethiop Med J.* 1988; 26:179.
11. Lester, F.T., Ayehunie, S. and Zewdie, D. Acquired immunodeficiency syndrome: seven cases in an Addis Ababa hospital. *Ethiop Med J.* 26:139, 1988.
12. National AIDS Control Programme. AIDS Case Surveillance in Ethiopia. Ministry of Health, Addis Ababa 1993.
13. Tsega, E. The demographic, social and clinical presentations of one hundred Ethiopian patients with human immunodeficiency virus (HIV) infection. *Ethiop Med J.* 1990; 16 28: 81.
14. World Health Organisation (W.H.O.), Global Programme On AIDS. Survey on Partner relations and risk of HIV infection, 1987.
15. Kleinbaum, D.G., Kupper, L.L. and Morgenstern, H(Eds). *Epidemiologic research; Principles and quantitative methods.* Belmont, CA: Lifetime learning, 1982; 419.
16. SAS Institute Inc. Statistical Analysis System. Cary, NC. SAS Institute Inc., 1989.
17. Van de Perre, P., Rouvroy, D., Lepage, P., Bogaerts, J., Kestelyn, P., Kayihigi, J., Hekker, A.C., Butzler, J.P. and Clumeck, N. Acquired immunodeficiency syndrome in Rwanda. *Lancet.* 1984; 2: 62.
18. Piot, P., Quinn, T.C., Taelman, H., Feinsod F.M., Milangie K.B., Wolsin, O., Mbendi, M., Mazebo, P., Ndangi, K. and Stevens, W. Acquired immunodeficiency syndrome in a heterosexual population in Zaire. *Lancet.* 1984; 2: 65.
19. Biggar, R.J. The AIDS problem in Africa. *Lancet.* 1986; 1: 79.
20. Mads, M., Bayley, A., Mukelabai, K., Njelesani, E.K., Mannwele, J.K. and Bowa, F.J. Evidence for heterosexual transmission and clinical manifestations of Human Immunodeficiency Virus infection and related conditions in Lusaka, Zambia. *Lancet.* 1986; 2: 1113.
21. Allen, S., Lindan, C., Serufilira, A., Van de Perre, P., Rundle, A.M., Nsengumuremyi, F., Caradel, M., Schwalbe, J. and Hulley, S. Human Immunodeficiency Virus Infection in Urban Rwanda. *J. Amer. Med. Ass. 1991;* 266(12): 1657.
22. Mann, J.M., Davachi, F., Quinn, T.C., N'Galy, B., Piot, P., Asita, P.K., Francis, H., Baudoux, P., Nzilambi, N., Colebunders, R.L., Kabote, N., Malonga, M. and Cursan, J.W. Second International Conference on AIDS, Paris, S17e: 105, 1987.
23. Centre for Disease Control. *Morbid. Mortal. Wkly. Rep.* 1987; 36: 285.
24. Kreiss, J.K., Koech, D. and PLummer, F.A. Aids virus infection in Nairobi prostitutes. *N. Engl. J. Med.* 1986; 314: 414.
25. Plummer, F.A., Simonsen, J.N., Ngugi, E.N., Cameron, D.W., Piot, P. and NdinyaAchola, J.D. Third International Conference on AIDS. Washington D.C, M8.4, 6 1987.
26. Mann, J.M., Francis, H., Quinn, T.C., Miathdila, M., Piot, P. and Curran, J.W. Risk factors for HIV seropositivity among children 1-24 months in Kinshiasa, Zaire. *Lancet.* 1986; 2: 654.

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Phylogenetic Analysis of Hepatitis D Viruses Indicating a New Genotype I Subgroup among African Isolates

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Genetic analysis was performed on 13 hepatitis D virus (HDV) isolates from Ethiopia, Somalia, Jordan, Kuwait, Bulgaria, Moldavia, and Sweden. The complete nucleotide sequence and genomic organization are described for the first time for two African HDV isolates. Phylogenetic analysis showed all the African isolates to be intrarelated and to form a novel group within HDV genotype I; the suggested designation for this group is IC. The genetic distance to previously described type I isolates was about 0.15. The HDV genotype I isolates (total of 22 examined) phylogenetically formed three clusters, each of them corresponding to certain geographic regions; the "western" group consisted of six HDV isolates from western Europe and the United States plus one from Kuwait; the "eastern" group consisted of two isolates from Moldavia and one each from Bulgaria, Nauru, mainland China, and Taiwan; and the "African-Middle East" group consisted of six HDV isolates from Ethiopia and one each from Somalia, Jordan, and Lebanon.

Hepatitis D virus (HDV) is a 36-nm subviral agent, and the establishment of an HDV infection depends upon the presence of hepatitis B virus to provide the hepatitis B surface antigen as its envelope protein (29, 31).

Through analysis of HDV RNA from isolates from all continents but Africa, nucleotide sequence diversities that justify grouping the isolates into three major genotypes have been found (4, 9a, 17, 34, 35). It has been noticed that the severity of liver disease caused by HDV infection varies between different geographic areas from fulminant hepatitis to asymptomatic chronic liver disease (8, 10, 24, 28), and it has been discussed whether viral genetic factors might influence the pathogenesis of HDV infections. HDV genotype III, the type most distantly related to the other genotypes, is found in patients from northern South America, where outbreaks of severe hepatitis with particular high morbidity and mortality have been described (4, 21, 27). This has spurred efforts to characterize more HDV isolates from different parts of the world.

So far, there has been limited genetic information about HDV isolates from Africa, the Middle East, and east Europe, where a high prevalence of HDV infection among patients with chronic liver disease has been reported (30, 33). We have performed phylogenetic analysis of 13 HDV isolates from the above-mentioned regions, and for the first time the full sequence and genetic organization of two African isolates are described in detail.

MATERIALS AND METHODS

Patients. Serum samples from 13 HDV-infected patients, including 6 from Ethiopia, 2 from Moldavia, and 1 each from Somalia, Jordan, Kuwait, Bulgaria, and Sweden, were employed in this study. The clinical data are summarized in Table 1.

Strategy for amplification and sequencing of HDV genomes. Two isolates, one from Somalia and one from Ethiopia (E1), were fully sequenced. Five overlapping segments (here designated A to E) for amplification and sequencing were selected to cover the whole HDV genome. Conserved stretches where the primers could be placed were found by alignment analysis of 13 available full-length sequences, including all three major genotypes of HDV (2, 4, 6, 7, 9a, 12, 13, 15, 16, 20, 23, 32, 34, 35). Between segments B and C there was an 11-base overlap of the inner primers, prompting us to custom-make primer pairs flanking this junction (segment F), based on the sequence information available for the two African HDV isolates.

The sequence analysis of the rest of the samples was focused on the 777-base fragment between nucleotides (nt) 872 and 1648 (numbering according to reference 34), which covers the autocleavage site domain on the antigenomic strand and the whole hepatitis D antigen (HDAg)-encoding region through fragments E and A.

The primers were designed with the OLIGO program (version 5.0; National Biosciences, Inc., Plymouth, Minn.). All inner primers were also used as sequencing primers (Table 2).

RT-nested PCR for HDV RNA amplification. Reverse transcription (RT) and the first PCR were carried out in a single tube, but in separate steps. Briefly, 10 μl of extracted RNA was mixed with 2.5 μl of outer antisense primer (final concentration, 0.5 μM), denatured for 5 min at 98°C, and annealed for 5 min at 62°C before addition of 35 μl of the RT-PCR mixture (final concentrations: Tris buffer [pH 8.4], 20 mM; magnesium chloride, 2 mM; potassium chloride, 60 mM; each of the deoxyribonucleoside triphosphates, 200 μM) and 4 U of avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wis.). RT was carried out at 42°C for 60 min, and then 2.5 μl of outer sense primer (final concentration, 0.5 μM) and 1 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Emeryville, Calif.) were added to the tube to complete the first PCR (35 cycles for 1.5 min at 95°C, 2 min at 62°C, and 3 min at 72°C). The second PCR was carried out by transferring 5 μl of the first-PCR product to a new tube containing 45 μl of the same PCR mixture with 1 U of *Taq* DNA polymerase and 0.5 μM concentrations of each of the inner primers, using the same temperature cycling conditions as for the first PCR in order to generate a sufficient amount of PCR amplimer for the sequence analysis.

Sequencing. The sequencing reaction was done with the Dyedeoxy terminator cycle sequencing kit (PRISM Ready reaction), which contains dye-labelled deoxyribonucleotides (Applied Biosystems Inc., Foster City, Calif.), according to the manufacturer's instructions but with a modification of the annealing temperature to 62°C instead of 50°C. The sequencing gels were run on an ABI 373A sequencer (Applied Biosystems). For each segment both strands were analyzed to obtain the consensus sequence. In order to compensate for the potential infidelity of the *Taq* polymerase, the full HDV cDNA sequences of the Somalian and the E1 Ethiopian samples were RT-PCR amplified and sequenced twice.

Sequence data analysis. Editing of raw sequence data and assembly of the segments into the continuous genome were accomplished by the programs Sequence Navigator (version 1.0.1; Applied Biosystems) and AutoAssembler (version 1.3.1b1; Applied Biosystems). Sequence alignments were done by use of the program MacMolly (Tetra, version 2.5; Soft Gene GmbH, Bocholt, Germany). RNA secondary structures were predicted by the program FoldRNA from the Genetics Computer Group program package (University of Wisconsin, Madison). Phylogenetic analysis of nucleotide sequences was conducted with the program DNA ML (maximum likelihood) (version 3.57c), with global rearrange-

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TABLE 1. Characteristics of HDV-infected patients investigated

Sample code	Origin	Age (yr)	Sex[a]	Diagnosis
E1	Ethiopia	25	M	Cirrhosis
E2	Ethiopia	18	M	Hepatoma
E3	Ethiopia	45	F	Cirrhosis
E4	Ethiopia	36	F	Cirrhosis
E5	Ethiopia	35	M	Cirrhosis
E6	Ethiopia	32	M	Cirrhosis
3239/94	Somalia	42	M	Chronic active hepatitis
H104	Jordan	NA[b]	NA	NA
K947	Kuwait	NA	NA	NA
15117/93	Bulgaria	32	M	Chronic hepatitis
M49	Moldavia	NA	NA	Chronic hepatitis
M64	Moldavia	NA	NA	Chronic hepatitis
236/95	Sweden	26	F	Acute hepatitis

[a] M, male; F, female.
[b] NA, not available.

ments. The programs ProtDist and Neighbor (version 3.57c) were used to analyze amino acid sequences. Unrooted trees were plotted by the program DrawTree and are presented with branch lengths drawn to scale. All programs used in the phylogenetic analysis are part of the PHYLIP program package, which was kindly provided by J. Felsenstein.

RESULTS

cDNA sequences and genomic organization of the Somalian and Ethiopian HDV isolates. Complete agreement between the results from the repeated RT-PCR sequence analyses of the two full-length sequences was found.

The complete HDV cDNA sequence of the Somalian and one Ethiopian (E1) isolates were shown to be 1,674 and 1,679 nt long, respectively. Although the two isolates did not contain the unique *Hin*dIII site which has been used as the start site in HDV sequence numbering (34), the similarity between the isolates in the sequence around this site allowed alignments with published sequences and corresponding numbering.

Overall, the Somalian and Ethiopian isolates have 83 to 85% and 84 to 86% sequence similarity with nine genotype I isolates (2, 6, 7, 15, 16, 20, 23, 32, 34, 35), 72 and 74% similarity with genotype II (9a), and 63 and 61% with genotype III (4), respectively. The sequence similarity between the two African isolates was 86%. A high degree of sequence conservation in some regions of the HDV genome, corresponding to the genomic and antigenomic cleavage domains as well as the HDV RNA binding domain of HDAg (14, 19, 25, 26), was observed in alignment analysis. Figure 1 shows full-length sequence alignment of the two African isolates with the HDV prototype strain (34, 35).

The open reading frame presumed to code for HDAg is

TABLE 2. Sequences, polarities, and positions of HDV primers used for amplification and sequencing analysis[a]

Designation	Sequence	Polarity	Position (nt)
Segment A			
1216 OU	5′ TCG GTC AAC CTC CTG AGT TC 3′	Outer and sense	1195–1214
1694 OR	5′ GGG GAG TCC AGC AGT CTC 3′	Outer and antisense	1670–1653
Delta UP	5′ CTT GTT CTC GAG GGC CTT CC 3′	Inner and sense	1266–1285
1673 R	5′ CGA GTC CAG CAG TCT CCT CT 3′	Inner and antisense	1668–1649
Segment B			
1554 OU	5′ GGC CAC CCA CTG CTC GAG 3′	Outer and sense	1533–1550
337 OR	5′ TGT TCG CTG AAG GGG TCC T 3′	Outer and antisense	311–329
1603 IU	5′ CTC GAC TCG GAC CGG CTC AT 3′	Inner and sense	1579–1598
333 ID	5′ CGC TGA AGG GGT CCT CTG GA 3′	Inner and antisense	325–306
Segment C			
299 OU	5′ AGC AGA CAA ATC ACC TCC AGA GGA 3′	Outer and sense	291–314
746 OR	5′ CGG TCC CCT CGG AAT GTT G 3′	Outer and antisense	744–726
310 IU	5′ CAC CTC CAG AGG ACC CCT TC 3′	Inner and sense	302–321
DW	5′ AGC GAG GAG GCT GGG ACC AT 3′	Inner and antisense	693–712
Segment D			
454 OU	5′ CCG AGA GGG GAC GAG TGA GG 3′	Outer and sense	454–473
890 OD	5′ TTC CTC TTC GGG TCG GCA TG 3′	Outer and antisense	909–890
464 IU	5′ ACG AGT GAG GCT TAT CCC GG 3′	Inner and sense	464–483
880 ID	5′ GTC GGC ATG GCA TCT CCA C 3′	Inner and antisense	898–880
Segment E			
847 OU	5′ CTC CTT CGG ATG CCC AGG T 3′	Outer and sense	847–865
1302 OD	5′ GGA TTC ACC GAC AAG GAG AG 3′	Outer and antisense	1322–1303
853 IU	5′ CGG ATG CCC AGG TCG GAC C 3′	Inner and sense	853–871
1297 ID	5′ GGC AGG ATC ACC GAC GAA GG 3′	Inner and antisense	1303–1284
Segement F			
150 OU	5′ TGA AGC TCT AGG AAG CGG AA 3′	Outer and sense	150–168
487 OD	5′ TTC CCC GGG ATA AGC CTC A 3′	Outer and antisense	487–469
152 IU	5′ AGC TCT AGG AAG CGG AAG AA 3′	Inner and sense	152–171
471 ID	5′ TCA CTC TTC CCC TCT CGG 3′	Inner and antisense	471–454

[a] Numbering is based on that in references 34 and 35.

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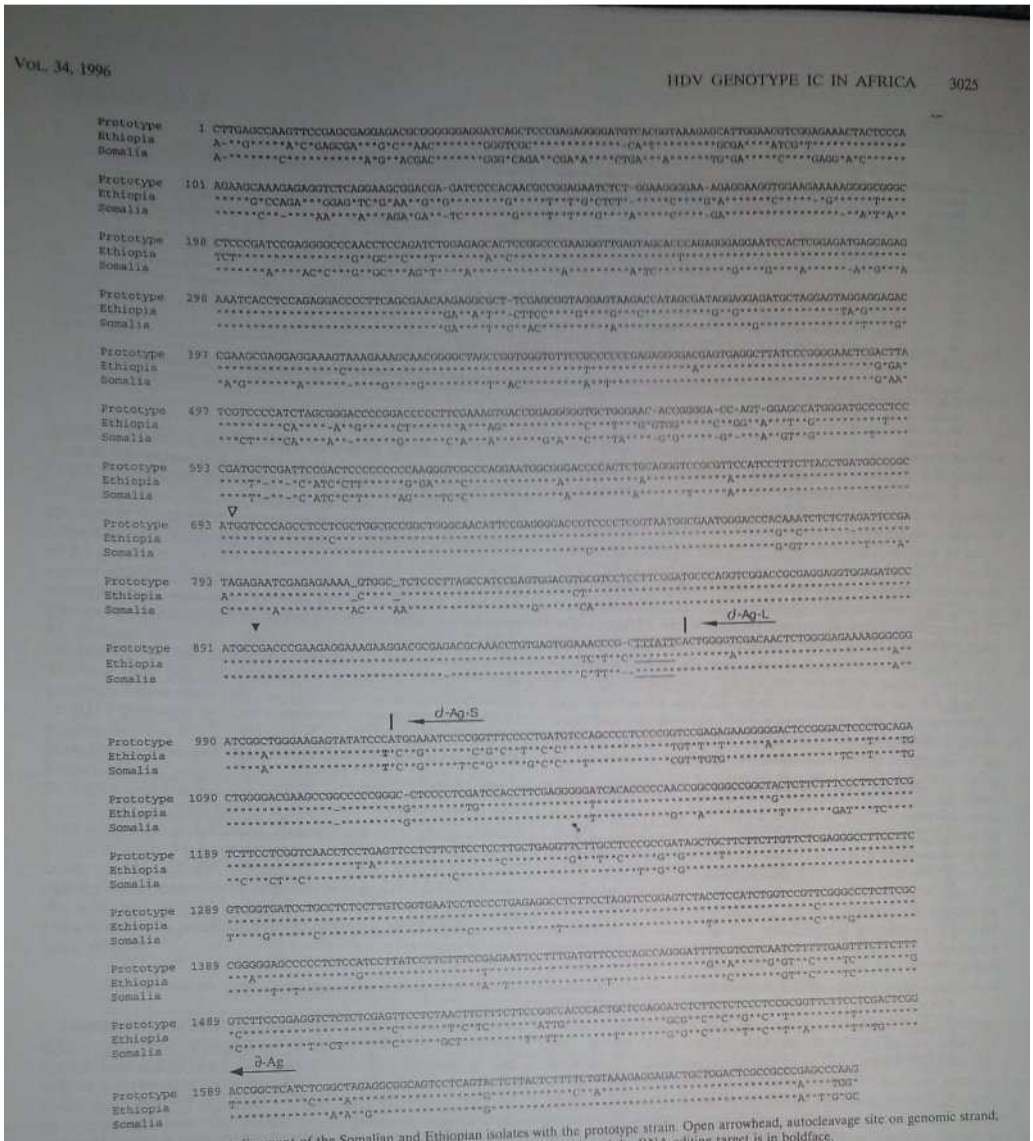


FIG. 1. Full-length-sequence alignment of the Somalian and Ethiopian isolates with the prototype strain. Open arrowhead, autocleavage site on genomic strand; filled arrowhead, autocleavage site on antigenomic strand. The poly(A) signal is underlined, and the RNA editing target is in boldface.

present in the antigenomic strand of the two isolates, with initiation and termination codon positions identical to those shown in Fig. 1, encoding the 195-amino-acid short form of HDAg (∂Ag-S). When RNA editing occurs (3, 5, 22, 37), a new frame is created; this frame has the same start codon but its stop codon is 57 bases further downstream, resulting in synthesis of the large form of HDAg (∂Ag-L), with 214 amino acids. Both the Somalian and the Ethiopian isolates contain presumed RNA editing sites (Fig. 1). The editing structure surrounding the RNA editing target required for maximum editing efficiency (3) as predicted by the program FoldRNA is similar to that described earlier, but with a 1-bp difference from AU to GC corresponding to positions 578 and 1014. The poly(A) signals and the autocleavage sites on the genomic and antigenomic strands are indicated in Fig. 1.

Nucleotide and amino acid sequence comparisons. Figure 2 shows an alignment of a 645-nt stretch encoding the HDAg from the six Ethiopian, the Somalian, the two Moldavian, and the Jordanian, Bulgarian, Kuwaiti, and Swedish isolates with the prototype strain. The sequence similarity between the pro-

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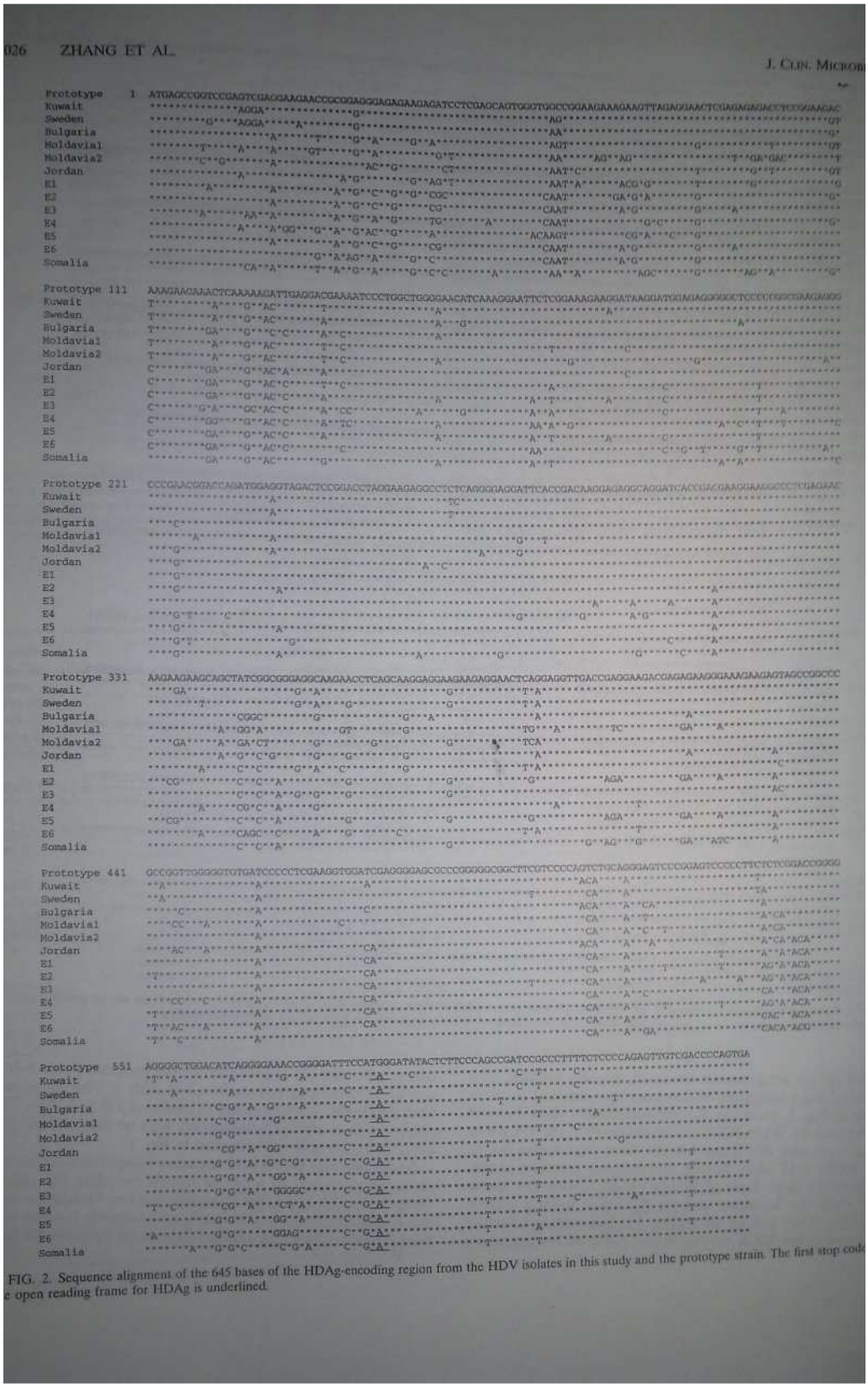


FIG. 2. Sequence alignment of the 645 bases of the HDAg-encoding region from the HDV isolates in this study and the prototype strain. The first stop cod
e open reading frame for HDAg is underlined.

TABLE 3. Mean evolutionary distances between HDV isolates on both the nucleotide and amino acid levels

HDV (strain[a])	No. of isolates	Mean evolutionary distance calculated from 3 data sets[b]											
		I			Ethiopia			Somalia			II (Japan 1)		
		358 nt	645 nt	214 aa	358 nt	645 nt	214 aa	358 nt	645 nt	214 aa	358 nt	645 nt	214 aa
I	15	0.10	0.10	0.13									
Ethiopia	6	0.13	0.14	0.15	0.10	0.10	0.13						
Somalia	1	0.15	0.15	0.20	0.12	0.13	0.19						
II (Japan 1)	1	0.36	0.45	0.39	0.38	0.42	0.41	0.42	0.40	0.37			
III (Peru 1)	1	0.57	0.31	0.52	0.60	0.30	0.52	0.58	0.31	0.51	0.53	0.45	0.48

[a] I, II, and III represent genotypes I, II, and III, respectively.
[b] 358 nt, fragment between nt 908 and 1256; 645 nt, whole HDAg-coding region; 214 aa, large form of HDAg (aa, amino acids). Intragroup distances are underlined.

greater calculated evolutionary distances at the nucleotide than at the amino acid level.

(ii) Phylogenetic groupings. Phylogenetic analysis was performed on the 358- and 645-nt and the 214-amino-acid sequences of the 13 HDV isolates of this study plus 11 previously known isolates, including 9 genotype I, 1 genotype II (Japan 1), and 1 genotype III (Peru 1) samples, and the results are presented as unrooted trees. The trees showed the African isolates emerging as a new phylogenetic group or branch from the previously characterized strains of genotypes I, II, and III (Fig. 4), although some of the isolates were given somewhat different positions in the different trees. In the one built from the 358-base data set, three distinctly separated clusters within genotype I were seen (Fig. 4a); the first cluster corresponded to HDV genotype IA and consisted of samples from Italy, France, Sweden, the United States, and Kuwait (the "western" branch); the second cluster corresponded to HDV genotype IB and consisted of isolates from mainland China, Taiwan, Nauru, Moldavia, and Bulgaria (the "eastern" branch); and the third cluster contained the six Ethiopian HDV isolates and the isolates from Jordan, Lebanon, and Somalia (the "African-Middle East" branch), now designated HDV genotype IC. The mean genetic distance between the African-Middle East and eastern branches was 0.14, and that between the African-Middle East and western branches was 0.15. The western and eastern branches were calculated to have a mean genetic distance of 0.10.

DISCUSSION

Identification of HDV variants. It has been proposed that differences in the pathogenesis of hepatitis D may depend on certain features of the HDV genome (4, 36). Because of the lack of a serotyping assay for HDV, classification is currently based upon genetic sequence comparisons. However, there is no consensus about the choice of subgenomic regions and methods for such comparisons. Either of two methods, measurement of pairwise sequence similarity or phylogenetic analysis of sequences, could be used to measure genetic distances. The two methods may yield similar results. However, pairwise sequence similarity determination does not allow for multiple substitutions and might underestimate the extent of divergence between sequences. Therefore, we favor the phylogenetic analysis as a tool for classification. An ideal region should be sufficiently variable to distinguish differences between isolates. The 358-base stretch between nt 908 and 1256 has previously been used in the designation of HDV genotypes (4). As noticed in this study as well as others (9, 17), the amino acid sequence divergences could be greater than those of the nucleotide sequences, and the evolutionary distances calculated from the amino acid sequence data set were also greater than those from comparison of the nucleotide sequences. Analysis of amino acid sequences could, therefore, provide information complementary to nucleotide sequence comparisons.

Classification of HDV isolates. In a recent sequence analysis of more than 100 HDV strains collected from different parts of the world, most of the HDV isolates were classified as genotype I, which was suggested to be divided into the subgroups IA and IB. IA included HDV isolates from western Europe and North America, and IB contained isolates from Asia (1a, 4). When our 13 HDV isolates were analyzed together with 11 previously described HDV strains, the Somalian and Ethiopian isolates could be phylogenetically classified as a new genotype subgroup, tentatively designated IC, while those previously classified as IA and IB isolates remained in the IA and IB branches, respectively. It appeared that the samples within each of the groups corresponded to certain geographic regions. In the present study the western (IA) group consisted of six HDV isolates from western Europe and the United States plus one sample from Kuwait. The eastern (IB) group contained two isolates from Moldavia and one each from Bulgaria, Nauru, mainland China, and Taiwan, and the new distinctly separated African-Middle East (IC) group consisted of the six HDV isolates from Ethiopia and those from Somalia, Jordan, and Lebanon. The HDV genome of the Somalian isolate was more distantly related to that of the genotype I prototype strain than the other African isolates; only genotypes II and III were more distant at the full-length-sequence level. The mean genetic distances to the Ethiopian isolates and to the other genotype I strains were 0.19 and 0.20, respectively, calculated at the amino acid level.

Our data indicate that at least one unique HDV phylogenetic group exists on the African continent. Severe cases of hepatitis D have been reported from the Central African Republic (18) with histological features similar to those seen in cases of fulminant hepatitis in South America. Investigation of more HDV isolates from different parts of the African continent will disclose whether further HDV genotypes or subtypes exist and to what extent clinical features could be linked to genetic variants.

Genetic features of the two African full-length HDV sequences. It has been considered that the delta antigen is the only HDV-encoded protein expressed, although a number of other open reading frames can be identified on both the genomic and antigenomic strands. However, the number of reading frames as well as their lengths and the positions of start and stop codons vary among isolates, strongly indicating that they do not code for any viral proteins. It was therefore noteworthy when, in 1993, Khudyakov et al. (11) reported antibodies against a synthetic peptide corresponding to a region designated ORF-K positioned in a −1 reading frame relative to that of ∂Ag-L and ∂Ag-S. This reading frame was predicted to

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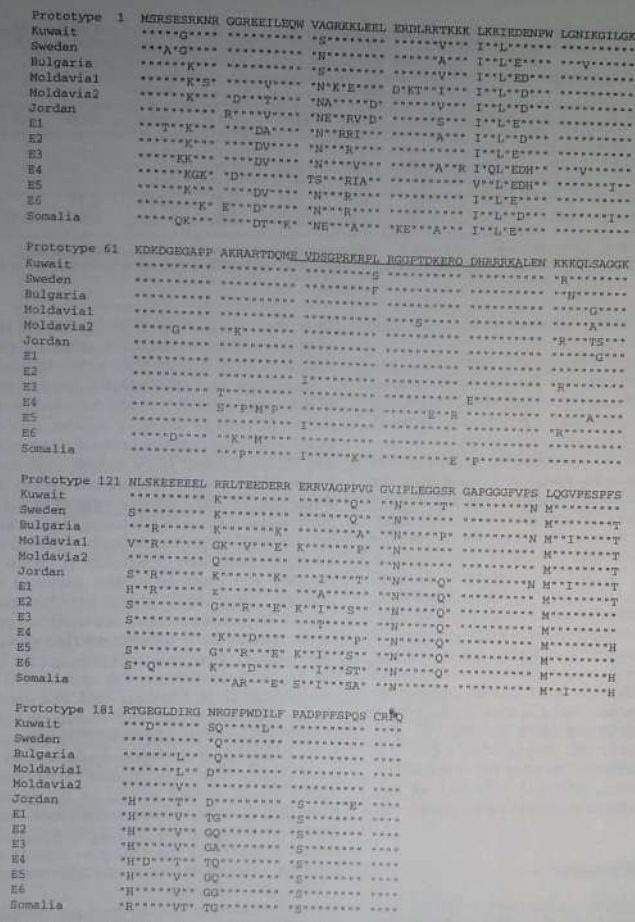


FIG. 3. Amino acid sequence alignment of the HDV isolates in this study and the prototype strain. The presumed HDV RNA binding domain is underlined.

totype strain and the African isolates was 85 to 89%, and that between the prototype strain and the other isolates was 90 to 93%.

About 56 and 37% of the nucleotide differences in the HDAg-encoding region of the two fully sequenced African isolates, compared with the HDV prototype strain, occurred in the first and/or second base of the codons, respectively. Therefore, the differences between the deduced 214-amino-acid sequences were greater than those between the corresponding nucleotide sequences. The Somalian and Ethiopian isolates had 81 to 85% and 78 to 90% amino acid sequence similarity with genotype I strains versus 86 to 88% and 85 to 90% nucleotide sequence similarity, respectively. Figure 3 shows an amino acid sequence alignment of the 13 HDV isolates investigated in this study with the prototype strain.

Phylogenetic analysis. (i) Evolutionary distances between new and previously described HDV isolates. The mean genetic distances between 24 HDV isolates at both the nucleotide and amino acid sequence levels were calculated from three data sets (the 358 bases between nt 908 and 1256 [4], the 645 bases of the whole HDAg-encoding region, and the 214 amino acids of the large form of HDAg) (Table 3).

The genetic distances varied depending upon whether the nucleotide or amino acid sequence data sets were used. Generally, the amino acid sequences indicated evolutionary distances between the HDV isolates greater than those obtained from the two nucleotide data sets. One of the exceptions was the distances between the Peru 1 and the other HDV isolates obtained with the 358-base stretch, where the high sequence divergence found in the 5′ part of the Peru 1 isolate resulted in

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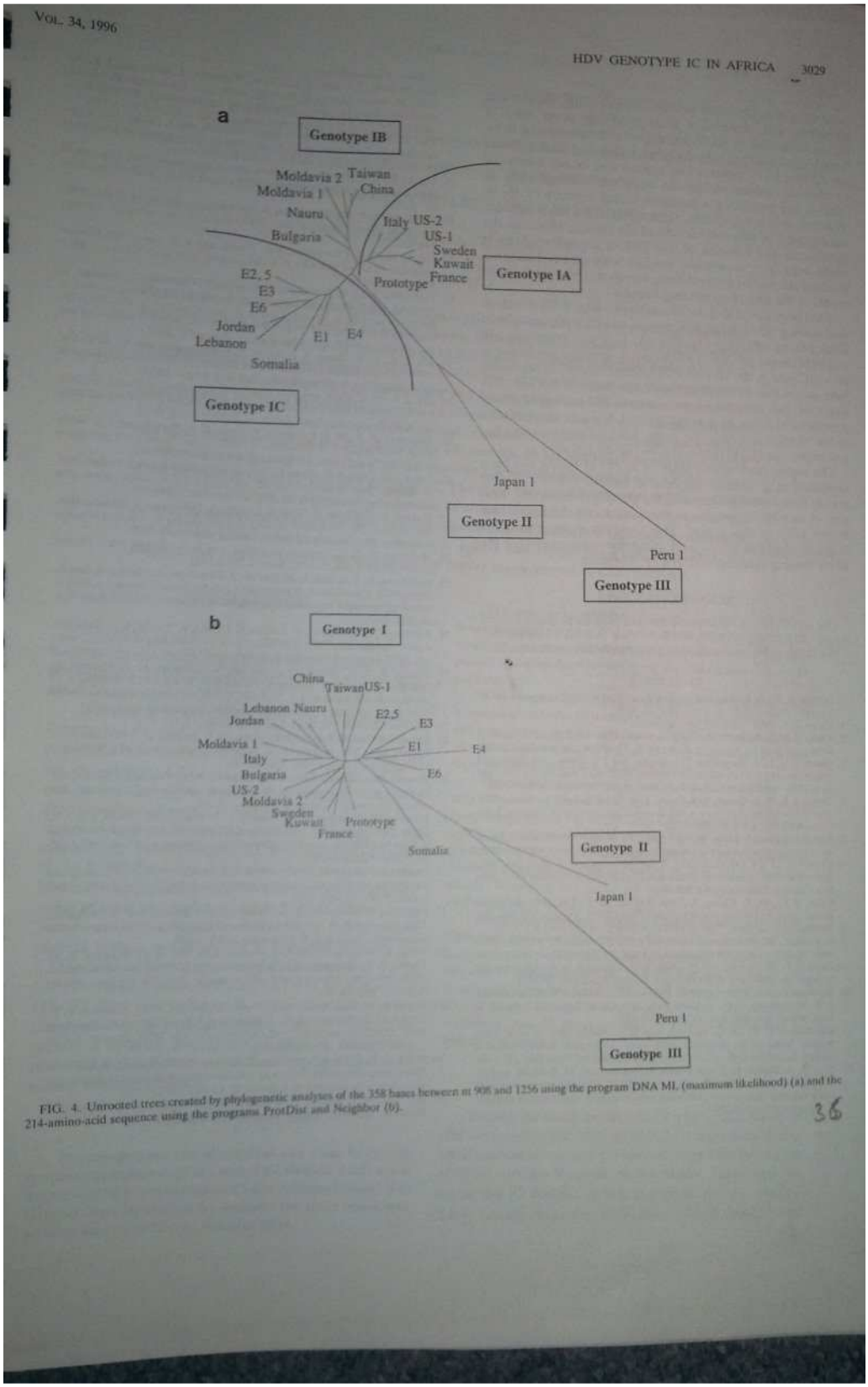


FIG. 4. Unrooted trees created by phylogenetic analyses of the 358 bases between nt 908 and 1256 using the program DNA ML (maximum likelihood) (a) and the 214-amino-acid sequence using the programs ProtDist and Neighbor (b).

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code for a 63-amino-acid protein. Subsequently, Bichko and coworkers also reported a correspondingly novel form of HDAg detectable in HDV-transfected cell cultures and in infected woodchucks (1). In the same region and reading frame the African HDV isolates also contain one or two additional termination codons within the sequence corresponding to ORF-K and predicting a potential coding capacity of 35- or 30-amino-acid polypeptides, respectively. This could indicate the existence of different variants of the protein. However, the significance and function of this protein are still unclear. It might even be a translation product without any biological function created by frameshifting due to insertion or deletion events in the ∂Ag open reading frame.

It has been stated that the characteristic 8-bp RNA structure surrounding the editing site pair is required for maximum RNA editing efficiency (3). The AU base pair (positions 578 and 1014) in the predicted RNA editing structure was found to be a GC pair in the two African isolates. Earlier experiments have shown that a change of the AU at positions 578 and 1014 into a GC pair resulted in a reduction to 75% editing efficiency compared with that of the wild type (3). It would be interesting to see whether this variation in the RNA editing structure has any impact on HDV replication in vivo and in vitro.

The region from amino acids 79 to 107 of HDAg has been identified as a domain responsible for HDV RNA binding mainly from investigations with the prototype strain (26). Several of our HDV isolates showed variations of one to four amino acids in this region. This naturally existing variation indicates that the amino acid sequence requirement for the RNA binding function may not be that strict.

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REFERENCES

1. **Bichko, V. V., Y. E. Khudyakov, and J. M. Taylor.** 1996. A novel form of hepatitis delta antigen. J. Virol. **70:**3248–3251.
1a. **Casey, J. L.** 1996. Personal communication.
2. **Casey, J. L., B. M. Baroudy, and J. L. Gerin.** 1993. US-2 isolate of hepatitis delta virus from an American male patient. GenBank accession no. L22066.
3. **Casey, J. L., K. F. Bergmann, T. L. Brown, and J. L. Gerin.** 1992. Structural requirements for RNA editing in hepatitis delta virus: evidence for a uridine to cytidine editing mechanism. Proc. Natl. Acad. Sci. USA **89:**7149–7153.
4. **Casey, J. L., T. L. Brown, E. J. Colan, F. S. Wignall, and J. L. Gerin.** 1993. A genotype of hepatitis D virus that occurs in northern South America. Proc. Natl. Acad. Sci. USA **90:**9016–9020.
5. **Casey, J. L., and J. L. Gerin.** 1995. Hepatitis D virus RNA editing: specific modification of adenosine in the antigenomic RNA. J. Virol. **69:**7593–7600.
6. **Chao, Y.-C., C.-M. Lee, H. S. Tang, S. Govindarajan, and M. M. C. Lai.** 1991. Molecular cloning and characterization of an isolate of hepatitis delta virus from Taiwan. Hepatology **13:**345–352.
7. **Chao, Y.-C., M. F. Chang, I. Gust, and M. M. C. Lai.** 1990. Sequence conservation and divergence of hepatitis ∂ virus RNA. Virology **178:**384–392.
8. **Hoofnagle, J. H.** 1989. Type D (delta) hepatitis. JAMA **261:**1321–1325.
9. **Imazeki, F., M. Omata, and M. Ohto.** 1990. Heterogenicity and evolution rates of delta virus RNA sequences. J. Virol. **64:**5594–5599.
9a. **Imazeki, F., M. Omata, and M. Ohto.** 1991. Complete nucleotide sequence of hepatitis delta virus RNA in Japan. Nucleic Acids Res. **19:**5439.
10. **Jacobson, I., J. L. Dienstag, B. G. Werner, D. B. Brettler, P. H. Levine, and I. K. Mushahwar.** 1985. Epidemiology and clinical impact of hepatitis D virus (delta) infection. Hepatology **5:**188–191.
11. **Khudyakov, Y. E., M. O. Favorov, and H. A. Fields.** 1993. A small open reading frame of the hepatitis delta virus antigenomic RNA encodes a protein that elicits antibodies in some infected patients. Virus Res. **27:**13–24.
12. **Kos, T., A. Molijn, L. J. van Doorn, A. van Belkum, M. Dubbeld, and H. Schellekens.** 1991. Hepatitis delta virus cDNA sequence from an acutely HBV-infected chimpanzee: sequence conservation in experimental animals. J. Med. Virol. **34:**268–279.
13. **Kuo, M. Y. P., J. Goldberg, L. Coates, W. Mason, J. Gerin, and J. Taylor.** 1988. Molecular cloning of hepatitis delta virus RNA from an infected woodchuck liver: sequence, structure, and applications. J. Virol. **62:**1855–1861.
14. **Kuo, M. Y. P., L. Sharmeen, G. Dinter-Gottlieb, and J. Taylor.** 1988. Characterization of self-cleaving RNA sequences on the genome and antigenome of human hepatitis delta virus. J. Virol. **62:**4439–4444.
15. **Lai, M. E., A. P. Mazzoleni, A. Porru, and A. Balestrieri.** 1995. Hepatitis D virus complete genome containing delta antigen ORF. GenBank accession no. X85253.
16. **Lee, C.-M., F.-Y. Bih, Y.-C. Chao, S. Govindarajan, and M. M. C. Lai.** 1992. Evolution of hepatitis delta virus RNA during chronic infection. Virology **188:**265–273.
17. **Lee, C.-M., C. S. Changchien, J. C. Chung, and Y. F. Liaw.** 1996. Characterization of a new genotype II hepatitis delta virus from Taiwan. J. Med. Virol. **49:**145–154.
18. **Lesbordes, J. L., P. Ravisse, A. J. Georges, P. Chevallier, C. Pichoud, F. Vitvitski, and C. Trepo.** 1987. Studies of the role of HDV in an outbreak of fulminant hepatitis in Bangui (Central African Republic), p. 451–459. *In* M. Rizzetto, J. L. Gerin, and R. H. Purcell (ed.), The hepatitis delta virus and its infection. Alan R. Liss, New York.
19. **Lin, J.-H., M.-F. Chang, S. C. Baker, S. Govindarajan, and M. M. C. Lai.** 1990. Characterization of hepatitis delta antigen: specific binding to hepatitis delta virus RNA. J. Virol. **64:**4051–4058.
20. **Liu, S. L., M. Y. Zhan, and W. J. Tan.** 1994. Complete nucleotide sequence of hepatitis delta virus RNA isolated from central China. GenBank accession no. X77627.
21. **Ljunggren, K. E., M. E. Patarroyo, R. Engle, R. H. Purcell, and J. L. Gerin.** 1985. Viral hepatitis in Colombia: a study of the "hepatitis of the Sierra Nevada de Santa Marta." Hepatology **5:**299–304.
22. **Luo, G., M. Chao, S.-Y. Hsieh, C. Sureau, K. Nishikura, and J. Taylor.** 1990. A specific base transition occurs on replicating hepatitis delta virus RNA. J. Virol. **64:**1021–1027.
23. **Makino, S., M. Chang, C. Shieh, T. Kamahora, D. Vannier, S. Govindarajan, and M. Lai.** 1987. Molecular cloning and sequencing of a human hepatitis delta virus RNA. Nature (London) **329:**343–346.
24. **Moestrup, T., B. G. Hansson, A. Widell, and E. Nordenfelt.** 1983. Clinical aspects of delta infection. Br. Med. J. **286:**87–90.
25. **Nishikawa, S., Y.-A. Suh, P. K. Kumar, J. Kawakami, F. Nishikawa, and K. Taira.** 1992. Identification of important bases for the self-cleavage activity at two single-stranded regions of genomic HDV ribozyme. Nucleic Acids Res. **20:**3959–3965.
26. **Poisson, F., P. Roingeard, A. Baillou, F. Dubois, F. Bonelli, R. A. Calogero, and A. Goudeau.** 1993. Characterization of RNA-binding domains of hepatitis delta antigen. J. Gen. Virol. **74:**2473–2477.
27. **Popper, H., S. N. Thung, M. A. Gerber, S. C. Hadler, M. de Monzon, A. Ponzetto, E. Anzola, D. Rivera, A. Mondolfi, A. Bracho, D. P. Francis, J. L. Gerin, J. E. Maynard, and R. H. Purcell.** 1983. Histologic studies of severe delta agent infection in Venezuelan Indians. Hepatology **3:**906–912.
28. **Rizzetto, M.** 1983. The delta agent. Hepatology **3:**729–737.
29. **Rizzetto, M., M. G. Canese, J. L. Gerin, W. T. London, D. L. Sly, and R. H. Purcell.** 1980. Transmission of the hepatitis B virus-associated delta antigen to chipanzees. J. Infect. Dis. **141:**590–602.
30. **Rizzetto, M., S. Hadziyannis, B. G. Hansson, A. Toukan, and I. Gust.** 1992. Hepatitis delta virus infection in the world: epidemiological patterns and clinical expression. Gastroenterol. Int. **5:**18–32.
31. **Rizzetto, M., B. Hoyer, M. G. Canese, J. W. Shih, R. H. Purcell, and J. L. Gerin.** 1980. Delta agent: association of delta antigen with hepatitis B surface antigen and RNA in serum of delta-infected chimpanzees. Proc. Natl. Acad. Sci. USA **77:**6124–6128.
32. **Saldanha, J. A., H. C. Thomas, and J. P. Monjardino.** 1990. Cloning and sequencing of RNA of hepatitis delta virus isolated from human serum. J. Gen. Virol. **71:**1603–1606.
33. **Tsega, E., B. Mengesha, B. G. Hansson, J. Lindberg, and E. Nordenfelt.** 1986. Hepatitis A, B, and delta infection in Ethiopia: a serologic survey with demographic data. Am. J. Epidemiol. **123:**344–351.
34. **Wang, K. S., Q.-L. Choo, A. J. Weiner, J.-H. Ou, R. C. Najarian, R. M. Thayer, G. T. Mullenbach, K. J. Denniston, J. L. Gerin, and M. Houghton.** 1986. Structure, sequence and expression of the HDV genome. Nature (London) **323:**508–514.
35. **Wang, K. S., Q.-L. Choo, A. J. Weiner, J.-H. Ou, R. C. Najarian, R. M. Thayer, G. T. Mullenbach, K. J. Denniston, J. L. Gerin, and M. Houghton.** 1986. Structure, sequence and expression of the HDV genome. Nature (London) **328:**456. (Corrigendum.)
36. **Wu, J. C., K. B. Choo, C. M. Chen, T. Z. Chen, T. I. Huo, and S. D. Lee.** 1995. Genotyping of hepatitis D virus by restriction fragment length polymorphism and relation to outcome of hepatitis D. Lancet **346:**939–941.
37. **Wu, T.-T., V. V. Bichko, W.-S. Ryu, S. M. Lemon, and J. M. Taylor.** 1995. Hepatitis delta virus mutant: effect on RNA editing. J. Virol. **69:**7226–7231.

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CHRONIC LYMPHOCYTIC LEUKAEMIA IN ETHIOPIANS

M. SHAMEBO and A. GEBREMEDHIN

SUMMARY

One hundred and two cases of chronic lymphocytic leukaemia (CLL) were seen at the Tikur Anbessa (Black Lion) Hospital, in Addis Ababa, Ethiopia, from January 1982 to December 1994. The age range was 35-91 (mean 55.6±11.08) years. The male to female ratio was 3.6:1. The commonest symptoms were weakness, weight loss, fever and sweating. The commonest signs were lymphadenopathy, splenomegaly and hepatomegaly. Fifty six per cent had Rai stage III and IV, only three patients were in stage 0. Of those treated with chemotherapy, 22.0% and 48.8% achieved complete and partial remissions respectively. Twelve patients are still alive and on follow up for 2-138 (median 18) months, 69 are lost to follow up after 0-132 (median 3) months and 21 are dead 0.5-84 (median 2.8) months after diagnosis. Of those that died, 13 were in stage IV and five in stage III. The main causes of death were septicaemia of undetermined origin in eight and pneumonia in seven. Thus CLL is not a rare disease in this centre. Its presentations are similar to cases reported in the literature. Optimal treatment is not possible due to lack of chemotherapeutic agents and supportive care. Therefore, we suggest that referral centres be equipped for better management of CLL patients.

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is a haematological malignancy characterised by monoclonal proliferation and accumulation of relatively mature looking small lymphocytes(1-5).

It is said to be the commonest type of leukaemia in Europe and North America but is rare in the Orient(1-6) and Papau New Guinea(7). Reports from Africa show that the prevalence of CLL is variable. In Nigeria(8,9), it accounted for 40% of all leukaemias, while in Zimbabwe(10), Kenya(11) and Sudan(12), it is the least common type of leukaemia at 20%, 19% and 13.0% respectively. The median age in Caucasians is 60-65 years (1-5,13) while in Africans it occurs about ten years earlier (8-12,14). In Caucasians and Africans, males are affected twice as often as females except in Nigeria where young females are affected more frequently(8,9). The dominant clinical feature in Caucasians is lymphadenopathy (1-5,13) while in Africans splenomegaly dominates (8-9,14). In Ethiopia, CLL accounted for 12-21% of all leukaemias (15-17); these studies, however, were not detailed. In this study, which includes the previously reported 38 cases, we present a detailed description of clinical, laboratory, treatment and follow up results and compare these with similar studies elsewhere.

MATERIALS AND METHODS

One hundred and two consecutive new cases of chronic lymphocytic leukaemia (CLL) seen in the medical wards and at the haematology referral clinics of Tikur Anbessa (Black Lion) Hospital, were included in the analysis. The study period was between January 1982 and December 1994.

The age, sex, address, date of diagnosis, clinical, haematological, biochemical, bone marrow aspiration results, treatment, outcome and follow up status were recorded for each patient. Antiglobulin (Coomb's) test was performed only on 31 patients.

The diagnosis of CLL was based on clinical and haematological findings: persistent lymphocytosis ≥10,000/mm^3 and bone marrow lymphocyte count of ≥30% of marrow cells (1-6) stained with the Wright's stain. Immunological cell markers were not performed due to lack of facilities.

The clinical staging was according to Rai *et al*(18). The indications for treatment were; Rai stages III, IV or bulky and symptomatic stages I or II (3-6,21). Chlorambucil 0.1-0.2 mg/kg/day or cyclophosphamide 2-3 mg/kg/day with or without prednisolone 40-60 mg/m^2/day were used (3-6,19). Four patients received radiotherapy over the spleen as used by Bayhardt *et al*(20). Treatment was given for at least six months before evaluating response(5).

Response criteria were as those used by Sawitsky *et al*(19). Complete remission (CR)=normalisation of all clinical and haematological parameters; Partial remission (PR)=reduction in the size of organomegaly to 50% of that at diagnosis and lymphocyte count less than 15,000/mm^3; No response (NR)=No change in the clinical or lymphocyte count; Progression= worsening over 28 days; increase in size of organs and in lymphocyte count.

RESULTS

During the study period of 12 years, 102 cases of chronic lymphocytic leukaemia (CLL) were seen in the adult medical wards and the haematology referral clinics of Tikur Anbessa Hospital, Addis Ababa. There were 80 males and 22 females giving a male to female ratio of 3.6:1. The age range was 35-91 (mean 55.6) years. Table

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1 shows the age and sex distribution of CLL in adults. In 36.3%, CLL occurred below the age of 51 years of age.

Table 1

Age and sex distribution of 102 chronic lymphocytic leukaemia patients in Tikur Anbessa Hospital

Age group (years)	Sex		Total	
	Male	Female	No	%
30-40	8	3	11	10.8
41-50	18	8	26	25.5
51-60	30	6	36	35.3
61-70	15	5	20	19.6
71-80	8	-	8	7.8
≥80	1	-	1	0.98
Total	80	22	102	100

Table 2

Presenting symptoms in 102 chronic lymphocytic leukaemia patient

Symptom	No.	%
Weakness	48	47.6
Weight loss	26	25.5
Fever and/or sweating	16	15.7
Cough	15	14.7
Abdominal mass	13	12.7
Incidental	5	4.9
Pruritis/skin lesions	5	4.9
Bleeding tendencies	3	2.9
Other	5	4.9

Table 3

Presenting signs in 102 chronic lymphocytic leukaemia patients

Sign No.	%	
Lymphadenopathy	94	92.2
Splenomegaly	57	55.9
Hepatomegaly	33	32.5
Pallor	38	37.3
Sternal tenderness	3	2.9
Skin lesions	2	1.9
Others	3	2.9

The clinical presentation at diagnosis are shown in Tables 2 and 3. Lumps in the neck and constitutional symptoms accounted for 89.2% and 88.8% respectively. In five patients (4.9%) the diagnosis was incidental, ie. patients were undergoing investigations for unrelated symptoms or for employment purposes. Lymphadenopathy was the predominant sign seen in 92.2% and was usually generalized. Splenomegaly and hepatomegaly occurred in 55.9% and 32.4% respectively. Sternal tenderness was noted in only three patients.

Table 4

Haematological parameters at diagnosis in chronic lymphocytic leukaemia patients

Parameter	Mean±2SD
Haemoglobin (g/dl)	10.77±3.78
Lymphocyte count (x10 mm^3)	150±183
Platelet count (x 10mm^3)	137.5±112

Table 4 shows the haematological parameters. The mean haemoglobin (Hgb) was 10.77 g/dl, range 3.1-16.4 g/dl. 37.3% of the patients had a Hgb of less than 10.0 g/dl. The lymphocyte count ranged between 16,340-864,000/mm^3, with a mean of 150,187/mm^3. 65.8% had lymphocyte count greater than 50,000/mm^3. The platelet count ranged from 10,000 to 550,000/mm^3 with a mean of 137,574/mm^3; 37.4% had a platelet count of less than 100,000/mm^3.

The antiglobulin (Coomb's) test was negative in the 31 patients it was tested. Serum protein electrophoresis was performed in only 23 patients; gamma globulins were depressed in 7(30.4%).

Table 5 shows the clinical stages of CLL patients at diagnosis according to Rai *et al*(18). The majority (56%) presented with stages III and IV. Only 12.7% were at stages 0 and 1.

Table 5

Rai clinical states in 102 patients with chronic lymphocytic leukaemia, Tikur Anbessa Hospital

Stage	No.	%
0	3	2.8
I	10	9.8
II	32	31.4
III	19	18.6
IV	38	37.4

Fifty five patients were treated with what was available at the time of diagnosis. Seventeen received chloroambucil and 34 cyclophosphamide, with or without prednisolone. Four received local radiotherapy over the spleen. Forty seven patients were not treated; 15 had early disease, 24 were lost to follow up and eight died shortly after the diagnosis of CLL.

Chloroambucil produced complete or partial responses in 26.7% and 46.7% respectively. Clinical improvement was seen in 26.7%. Cyclophosphamide produced complete or partial responses in 19.2% and 50% respectively. Clinical improvement was noted in 30.8%. Two patients in chlorambucil group and eight in cyclosphosphamide group were not evaluable as they were lost to follow up soon after intiation of therapy. Of the four who were treated with radiotherapy over the spleen, one attained partial remission, two had clinical improvement and one was lost to follow up.

As of December 1994, twelve patients were still alive

and on follow up 1-138 (median 18) months since diagnosis. Sixty nine were lost to follow up 0-132 (median 3.0) months and 21 were known to have died 0.5-84 (median 2.8) months after diagnosis. Of those that died 1,2,5 and 13 were at stages I, II, III and IV, with mean survival of 65, 31, 33.4 and 13.5 months respectively. The causes of death were; septicaemia in eight, pneumonia in seven, pulmonary tuberculosis in one, head injury in one, perforated peptic ulcer in one and unknown in three patients.

DISCUSSION

Chronic lymphocytic leukaemia (CLL) is said to be the most common form of leukaemia in the Western hemisphere accounting for 30% of all leukaemias in humans, but is rare in Orientals (1-6) and Papau New Guinea(7). In Africa, rates ranging from 12-40% were reported (8-12,15-17). The median age at presentation in Caucasians is 60-65 years (1-5), only 10-15% are below 50 years of age(6). For our patients the mean age was 55.6 years, 36.3% are below 51 years. This is also noted in other African series. The male to female ratio of 3.6:1 in our patients is higher than that observed in Caucasians, but unlike that in Nigerians where young females predominate(8,9).

The clinical features in our patients are similar to those reported in Caucasians, where lymphadenopathy is the dominant feature(1-5,13). Splenomegaly and hepatomegaly accounted for 55.9% and 32.4% unlike in Kenyans where these accounted for 80.9% and 76.5% respectively(14). Significant pallor was noted only in 37.3% of our patients, Oloo *et al*(14) reported it in 59.5% in their series. Stenal tenderness was observed in 2.9% of our patients in contrast to 27% reported by Boggs *et al*(13). Two patients had skin infiltration. The mean haemoglobin (Hgb) was 10.77 g/dl with 62.7% having less than 12.0 g/dl and 37.3% less than 10.0 g/dl. In Caucasians Hgb less than 10.0 g/dl was noted in only seven percent(13). In Kenyans anaemia was even more marked, Hgb less than 8.0 g/dl was found in 66.7%.

Like in Kenyans, no patient in our series had evidence of autoimmune haemolysis; this occurs in 10-15% in Caucasians(1,3,5). Lymphocytosis greater than 100,000/mm^3 was seen in 42.5% of our patients compared to 32% in Boggs *et al*(13) series. Very high lymphocyte counts are said to be associated with poor prognosis(3-6). Platelet counts less than 100,000/mm^3 and 50,000/mm^3 were seen in 37.8% and 20.5% respectively. Three patients presented with bleeding tendencies. Platelet count less than 50,000/mm^3 was noted in only one of 130 patients by Boggs *et al*(13).

In western hemisphere, 25% of patients were diagnosed incidentally and 40-60% were asymptomatic(1-5). In our patients, only 5 were diagnosed incidentally. Only three were in stage 0. Stages I and II, III and IV accounted for 41.2% and 56% respectively. Gale *et al*(3) reported stage 0=25%, I+II=50%, III+IV=25%. This shows that most patients in our series present at late stages like in the Kenyan series(14).

In patients that could be evaluated for outcome of treatment, overall responses of 73.4% and 69.2% for chlorambucil and cyclophosphamide were similar to those reported in the literature(3-6,19). As sixty nine patients were lost to follow up, it is not possible to determine the median survival for our patients. The median survival in reported series ranges from 6-9 years (1-6,13,19). Twenty one patients in our series are known to have died. The commonest causes of death were septicaemia and pneumonia. This is similar to the reported series in the literature(1-6,13,19).

Although it is not possible to cure CLL with the currently available chemotherapeutic agents, good palliation is possible. In Africa in general and in our centre in particular, these agents are not readily available, therefore, we suggest that referral centres be equipped for better management of CLL patients.

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REFERENCES

1. Wintrobe, M.M. Chronic lymphocytic leukaemia. *In: Clinical haematology* 8th edn. , (eds). M.M. Wintrobe, G.R. Lee, D.R. Boggs et al), 1981, Chapter 67, pp. 1631-1647. Lea and Febiger, Philadelphia, PA. USA.
2. Gale, R.P. and Foon, K.A. Recent advances in the tiology and treatment of chronic lymphocytic leukaemia. *Ann. intern. Med.* 1985; 103:101-120.
3. International Workshop on Chronic lymphocytic Leukaemia. Chronic Lymphocytic Leukaemia: Recommendations for diagnosis, staging and response criteria. *Ann. intern. Med.* 1989; 110:236-238.
4. Kamitirs, T.D., Lister, T.A. and Rohatiner, A25. Chronic lymphocytic leukaemia. *Brit. J. Hosp. Med.* 1991; 46:379-385.
5. Rozman, C. and Montserrat, E. Chronic lymphocytic leukaemia. *N. Engl. J. Med.* 1995; 333:1052-1057.
6. Damshek, W. and Gunz, F. Epidemiology: geographic, racial and social distribution. *In: Leukaemia* 4th edn., Ed. F. Gunz, Chapter 3, pp. 18-34. Grune and Stratton, New York. 1983.
7. Booth, K. and Amato, D. Leukaemia in Papua New Guinea. *Trop. geogr. Med.* 1978; 30:343-349.
8. Essien, E.M. Leukaemia in Nigerians: The chronic leukaemias. *East. Afr. Med. J.* 1976; 53:96-110.
9. Kulkarni, A.D. Leukaemias in Guinea Savana area of North of Nigeria. *East. Afr. Med. J.* 1986; 63:660-665.
10. Levy, L.M. The pattern of leukaemia in Zimbabweans. *Central Afr. J. Med.* 1984; 30:57-63.
11. Kasili, E.G. and Taylor, J.R. Leukaemia in Kenya. *East Afr. Med. J.* 1970; 47:461-468.
12. Ahmed, M.A.M., Kordofani, A.A.y, Hidaytalla, A. and Omer, A. Leukaemia in the democratic republic of Sudan. *East. Afr. Med. J.* 1982; 59:533-538.
13. Boggs, D.R. Sofferman, S.A., Wintrobe, M.M. and Cartwright, G.E. Factors influencing the duration of survival of patients with chronic lymphocytic leukaemia. *Amer. J. Med.* 1966; 40:243-254.
14. Oloo, A.J. and Ogada, T.A. Chronic lymphocytic leukaemia (CLL): Clinical study at Kenyatta National Hospital (KNH). Nairobi. *East Afr. Med. J.* 1984; 61:797-801.
15. Lester, F.T. Haematological malignancies in adults in an Addis Ababa hospital. *East Afr. Med. J.* 1981; 58:278-283
16. Milkias Shamebo. Pattern of haematological diseases among adult hospitalized Ethiopians. *Ethiop. Med. J.* 1987; 25:113-118.

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OCULAR MANIFESTATIONS OF LEUKAEMIA IN ETHIOPIANS

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ABSTRACT: *A prospective ophthalmic evaluation of 74 newly diagnosed and 34 old (on follow-up) leukaemic patients, carried out from March 1990 to December 1995 is described. Primary ocular involvement, that is leukaemic retinal infiltrates, were detected in 32% of the newly diagnosed. In contrast, none of the old leukaemic patients had this lesion. In 69% of the new and 21% of the old cases, secondary ocular manifestations of leukaemia were observed. The major secondary ocular manifestation of leukaemia in both groups was intra-retinal haemorrhage. A variety of miscellaneous ocular findings, such as cataract, pterygium, pingeculae, etc. were detected in 36% of all leukemics. These findings indicate the importance of a complete ophthalmologic evaluation in the diagnosis, follow-up and management of leukaemic patients.*

INTRODUCTION

Virtually all ocular structures have been found to be involved both in the adult and childhood leukaemias (1,2). Most of the involvement can be related to direct infiltration by neoplastic cells while some are a result of haematological abnormalities associated with the leukaemia such as anaemia and hyperviscosity which is a result of greatly increased circulating leucocytes or platelets. Nodular retinal infiltrates (primary leukaemic manifestation) have been described (3-6). Leukaemic retinopathy is observed in both the acute and chronic forms of leukaemia. In addition to these, presence of retinal microaneurysms, capillary closure and neovascularization have also been documented (5). As sited by Rosenthal in his review of ocular manifestations of leukaemia, optic nerve, orbital and lid involvement may occur. Infiltration

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manifestations include, conjunctival swelling, haemorrhages in the conjunctiva, episclera and sclera, corneal ring ulcer, lacrimal gland enlargement with Sjogren's syndrome, open-angle glaucoma, and vitreous involvement (6).

Varying estimates of prevalence of ophthalmic manifestations of leukaemia ranging from as high as 90% to as low as 8% have been reported (7,8). The true prevalence and incidence is not yet known. In Ethiopia, the prevalence of Leukaemia was estimated at 3.5% in the adult and 0.2% in the paediatric hospital population (9-11). However, there is a total lack of data on ocular manifestations in Ethiopian leukemics. This information is essential because of the fact that it may be the initial mode of presentation of the leukaemic process. Moreover, in combination with other systemic and laboratory findings, these findings will make a valuable contribution in the overall management of patients and may also aid in determining prognosis. The purpose of this study, therefore, is to determine the prevalence of ocular manifestations of leukaemia in Ethiopians.

PATIENTS AND METHODS

Newly diagnosed and old (on follow-up) leukaemic patients of all age groups and cell types (chronic as well as acute) seen at the Tikur Anbessa and Ethio-Swedish Children's Hospitals during the period of March 1990 to February 1994 were prospectively enrolled in the study. Diagnosis of the leukaemia and typing was done using standard techniques (12).

Ophthalmic evaluation included determination of near and distant vision, examination using slit lamp biomicroscopy and funduscopy with a dilated pupil. The ophthalmic evaluation was performed within three days of diagnosis and prior to initiation of therapy in the newly diagnosed. Examination was done at the bedside on inpatients or in the ophthalmic centre at Menelik II Hospital on ambulatory patients. Data was then entered in a pre-prepared form.

For the purpose of this study, the following classification of the ocular manifestations of leukaemia devised by Schachat et al (4) was adopted.

Primary: Ophthalmic signs related to leukaemic infiltration of ocular structures like preretinal white clumps or masses.

Secondary: Ophthalmic signs related to anaemia or thrombocytopenia or hyperviscosity states like subconjunctival haemorrhage, retinal haemorrhages or cotton-wool spots.

Miscellaneous: Findings unrelated to the leukaemia diagnosis like cataract and amblyopia.

RESULTS

A total of 108 Ethiopian leukaemic patients, 74 newly diagnosed and 34 who were under follow-up were studied. The male to female ratio was 2:1. Children 15 years of age or below accounted for 27% of all the leukemics (Table 1).

As seen in Table 2, chronic myeloid leukaemia (CML) was the leading type of leukaemia (57%) in the newly diagnosed. However, the leading type of leukaemia in the paediatric population in this study is

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acute lymphoid leukaemia (ALL).

Table 3 shows age distribution by type of leukaemia in the old cases. CML, as expected, was the leading type of leukaemia and there were no cases of acute myeloid leukaemia (AML) in this group.

The rate of primary ocular manifestation of leukaemia (32%) in the newly diagnosed is as shown in Table 4. Primary ocular manifestation was detected in 3/7 (43%); 14/42 (33%); 4/14 (29%); and 3/11 (27%) of the cases of chronic lymphoid leukaemia (CLL), CML, ALL and AML, respectively, amongst the newly diagnosed. Furthermore, leukostatic symptoms (priapism, deafness) were seen in three of the 10 patients with newly diagnosed CML who had primary lesions, while none of the 23 CML's with secondary lesions had these symptoms. Primary manifestation was not observed in those under follow-up.

Table 1. Age and sex distribution of the newly diagnosed and old leukaemic patients (n = 108).

Age group (in years)	Newly diagnosed		Old			
	M	F	M	F	Total	%
0-15	13	6	7	3	29	26.9
16-30	19	8	3	2	32	29.6
31-45	7	5	4	4	20	18.5
46-60	7	5	5	2	19	17.6
61+	4	0	4	0	8	7.4
Total	50	24	23	11	108	100

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Table 2. Age distribution of the newly diagnosed by types of leukaemia (n=74)

Age group (in years)	Diagnosis					
	CML*	ALL**	CLL***	AML****	Total	%
0-15	6	8	0	5	19	25.7
16-30	17	5	1	4	27	36.5
31-45	10	1	1	0	12	16.2
46-60	7	0	3	2	12	16.2
61+	2	0	2	0	4	5.4
Total	42	14	7	11	74	100
%	56.7	18.9	9.5	14.9	100	

* Chronic myeloid leukaemia
** Acute lymphoid leukaemia
*** Chronic lymphoid leukaemia
**** Acute myeloid leukaemia

Table 3. Age distribution of the old patients by type of leukaemia (n=34).

Age group (in years)	CML	ALL	CLL	AML	Total	%
0-15	2	8	0	0	10	29.4
16-30	4	1	0	0	5	14.7
31-45	6	0	2	0	8	23.5
46-60	4	0	3	0	7	20.6
61+	2	0	2	0	4	11.8
Total	18	9	7	0	34	100
%	52.9	26.5	20.6	0	100	

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Table 4. Primary ocular manifestations in the newly diagnosed by types of leukaemia (n = 24).

Age group (in years)	CML	ALL	CLL	AML	Total	%
0-15	3	1	0	2	6	25.0
16-30	4	2	1	1	8	33.3
31-45	5	1	0	0	6	25.0
46-60	2	0	1	0	3	12.5
61+	0	0	1	0	1	4.2
Total	14	4	3	3	24	100
%	58.3	16.7	12.5	12.5	100	

Table 5 shows the frequency and type of secondary ocular manifestations in all leukaemics. The overall prevalence of secondary manifestation is 54% occurring in 52/74 (70%) of the newly diagnosed and 6/34 (18%) of those on follow-up. As seen in this table, some patients had more than one secondary manifestations. The commonest secondary manifestation was intraretinal haemorrhage. In contrast to the occurrence of primary manifestations in the newly diagnosed, the highest rate of secondaries were observed in CML rather than in CLL cases in whom none were encountered. Conjunctival pallor was observed in 33% of all patients (not included in table 5). Miscellaneous findings with pterygium and cataract being the leading observations were found in 36% of our patients (Table 6).

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Table 5. Secondary ocular manifestations by type of leukaemia in the newly diagnosed and follow-up cases (n = 58)

Secondary manifestations	Newly diagnosed					Old cases				
	CML	ALL	CLL	AML	Total	CML	ALL	CLL	AML	Total
Intra-retinal Haemorrhage	23	9	0	4	36	7	1	1	0	9
Roths's spot	10	1	0	1	12	2	1	1	0	4
Congested venules	9	1	0	0	10	2	0	0	0	2
Exudates	4	1	0	0	5	2	0	0	0	2
Disc oedema	3	0	0	0	3	0	1	0	0	1
Sub-hyaloid haemorrhage	0	0	0	1	1	0	1	0	0	1
Disc haemorrhage	0	0	0	0	0	0	1	0	0	1

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Table 6. Miscellaneous ocular findings in all leukaemics (n=39).

Finding	No.	Per cent
Pterygium	10	25.6
Cataract	6	15.4
Refractive error	3	7.7
Conjunctivitis	3	7.7
Strabismus	3	7.7
Others	3	7.7

DISCUSSION

Despite a conservative definition of primary ocular manifestation of leukaemia, we found a strikingly high rate of 32% among newly diagnosed Ethiopian leukaemics. This is most probably attributable to late diagnosis, severity of disease on admission, and the highly selective nature of the patient population coming to these referral and teaching centers. A similar study (4) revealed a much lower prevalence (3%), but the authors have expressed their opinion that this rate could well be an under-estimate. However, the prevalence of the overall ocular manifestations (primary and secondary) in the two studies is very similar, being 76% in our series and 70% in theirs.

The fact that no primary change was detected in the old cases might have been the result of therapeutic effect or a mortality rate difference in the different cell types of leukaemias with or without intraocular involvement at diagnosis. This is also reflected in the total absence of cases of AML, in whom the mortality rate is high, amongst those on follow-up.

A difference in the rate of occurrence of intraocular leukaemias with different cell types as seen in our study has been reported. It has also been demonstrated that leukaemic retinal infiltration combined with high leucocyte count is an ominous prognostic sign (5,6,13).

Secondary (conjunctival pallor was not included as it was not part of the definition used (4)) and miscellaneous ocular manifestations can be related to the disease process itself but may also be associated to toxicities of chemotherapeutic agents (4-6). Ocular nerve palsies and orbital involvement was not observed in our series as these patients are more likely to present to ophthalmic or neurologic rather than haematology clinics from which our patients were recruited. The rate of secondary ocular manifestation in the newly diagnosed is in agreement with other studies (4-6). The apparent decline in the rate of secondaries observed in the old cases could be the combined effect of mortality and therapy. However, because of the design of this study, valid conclusion cannot be drawn in this regard. Therefore, a future study designed to look into outcome, correlation of ocular, clinical, haematologic as well as pathologic findings will be highly relevant.

This study has demonstrated that ocular

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involvement in leukaemia is common. Clinicians and Ophthalmologists should be aware of this fact and positively contribute in the overall management of the leukaemic patient.

REFERENCES

1. Allen RA, Straatsma BR. Ocular involvement in leukaemia and allied disorders. *Arch Ophthalmol* 1961;66:490-508.
2. Soylu M, Tanyeli A, Ozdemir N, Eroglu A, Ersoz T R. Ocular involvement in childhood leukaemias. *Turk J Pediatr* 1994;36:35-41.
3. Kuwabara T, Aiello L. Leukaemic milliary nodules in the retina. *Arch Ophthalmol* 1964;72:494-7.
4. Schachat AP, Markowitz JA, Guyer DR et al. Ocular Manifestations of leukaemia. *Arch Ophthalmol* 1989;107:697-700.
5. Mahneke A, Videbaeh A. On changes in the Optic fundus in leukaemia: aetiology, diagnostic and prognostic role. *Acta Ophthalmol* 1964;42:201-10.
6. Rosenthal AR. Ocular Manifestations of leukaemia: a review. *Ophthalmology* 1983;90:901-5.
7. Duke-Elders. System of Ophthalmology. St. Louis, Mo: CV Mosby Co. 1967;10: 393-400.
8. Regway EW, Jaffe N, Walton DS. Leukemic ophthalmopathy in children. *Cancer* 1976;38:1744-9.
9. Milkias S. Leukemia in adult Ethiopians. *Ethiop Med J* 1990;28:31-7.
10. Milkias S. Pattern of haematological diseases among adult hospitalised Ethiopians. *Ethiop Med J* 1987;25:113-8.
11. Tilahun T, Abubaker B. Lukaemia in Children's Hospital, Addis Ababa. *Ethiop Med J* 1988;26:29-32.
12. Dacie JV, Lewis SM. Basic haematological techniques in Practical Hematology(eds JV Dacie and SM Lewis), 6th edit., Churchill Livingstone, Edinburgh, London, Melborne and New York. 1984; pp 20-60.
13. Robb RM, Ervin LD, Sollan SE. A pathological study of eye involvement in acute leukemia of childhood. *Trans Am Ophthalmol Soc* 1978;76:90-101.

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Perceptions of blindness

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A study in a mainly rural area of Ethiopia has revealed that sighted, unilaterally blind and totally blind people have many misconceptions about blindness. The work of cure and prevention demands that widespread ignorance about the causes and implications of blindness be overcome.

In Ethiopia the causes of blindness are, in the large majority of cases, preventable or curable. However, if preventive and therapeutic measures are to have the greatest possible effect it is necessary to understand the attitudes of the public towards blindness. A survey has therefore been conducted in the mainly rural subdistrict of Meskan and Mareko, which has a population of 250 000; the participants were 1751 randomly selected heads of household, of whom 1401 were sighted, 246 were unilaterally blind, and 104 were blind in both eyes. After informed consent had been obtained at both the village and household levels, trained lay health workers administered questionnaires translated into Amharic, the official language of Ethiopia, and, where necessary, into local dialects. The community was largely illiterate and had had little or no exposure to modern medicine.

Knowledge and attitudes of the fully sighted

Most of the interviewees in this category were aged 20-60 years. Whereas 83% of fully sighted people were married, this was true of only 41% of totally blind people and 67% of people who were unilaterally blind.

Virtually all the fully sighted people interviewed had heard or read about blindness or had seen a blind person; 73% thought they knew the causes of blindness, 94% said it was not inherited and 98% that the causes were not contagious. It was claimed by 70% that they knew how to prevent blindness, while 82% believed that the condition could not be cured. Blindness was a problem of the elderly according to 85% of the respondents. Among the same interviewees, 77% said they would object to a member of their family marrying a blind person, while 80% said they themselves would not marry a blind person. It was thought by 85% that blind people could not attend school because of their disability, and 87% were unaware that there were schools and scripts for the blind. When asked what society should do for blind people, 83% suggested giving alms. Only 14% said they would be willing to employ a blind person were they in a position to do so.

Knowledge and attitudes of the totally and unilaterally blind

The highest levels of widowhood and divorce were found among the totally blind, at 47% and 8% respectively. Only 1% of the totally blind could read (in Braille). Of the totally blind people who were married, 71% had married before the onset of blindness; all who were single and 25% of divorcees considered

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that blindness accounted for their marital status. Among the unilaterally blind, however, only 11% of those who were single and none who were divorced considered this to be the case.

Of the totally blind, 86% thought they knew the causes of blindness. The three main groups of causes were said to be febrile illnesses (45%), supernatural factors including punishment by God and curses (33%), and accidents (15%). Hardly anyone believed blindness to be inherited or contagious.

Only 33% of the totally blind and 48% of the unilaterally blind considered blindness to be incurable. With regard to the prevention of blindness, 53% of the totally blind said they knew how to achieve this, and 93% of these people considered that the solution was to obtain immediate treatment when illness occurred; the responses of the unilaterally blind on this matter were similar.

Willingness to work was expressed by 53% of the totally blind, but only 12% were working and the same percentage said they would have employed a blind person had this possibility arisen. In contrast, 95% of the unilaterally blind were willing to work and almost the same percentage were actually working; however, only 19% would have been prepared to employ a blind person. Both groups of

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respondents said they would allow their own children to play with blind children. Only 5% of the totally blind and 1% of the unilaterally blind wished to mix socially with fully sighted people.

Almost all totally and unilaterally blind people thought blindness prevented education and that educational opportunities should not, therefore, be given to the blind. Large majorities were unaware that there were special schools and scripts for blind people. Virtually all interviewees considered that society should give special care and help to the blind, around three-quarters saying that alms were the best form of assistance. About a fifth said that medication should be provided, while 3% of the totally blind and 6% of the unilaterally blind said the government should provide help.

There was little knowledge in the community about the major causes of blindness, which, contrary to what many interviewees suggested, is by no means a problem limited to the elderly. It is a significant public health problem, having a negative effect on productivity and requiring heavy expenditure on rehabilitation, education and care. Moreover, it has a major adverse impact on rates of morbidity and mortality. Endemic trachoma and associated infections affect a substantial proportion of the people. Other important causes of blindness are cataract, which is remediable, and glaucoma, which is preventable.

A dearth of information appears to have led many in the community to mistaken beliefs and attitudes, resulting in a lack of concern for visually impaired and blind people. In general, negative attitudes prevailed in respect of marriage, other social interactions, job opportunities, rehabilitation and education. Of course, these observations have to be seen in the context of the level of development of the society in question.

Clearly, prevention programmes should be in place. Hygiene is particularly important; in Mexico, for example, it has been shown that the prevalence of trachoma was reduced among children who frequently washed their faces *(1)*.

Heightened awareness and treatment-seeking behaviour are important factors in the fight

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against curable blindness. The people in the subdistrict of Meskan and Mareko should be informed of the causes of blindness and of the possibilities of prevention and cure. The nature of cataract, trachoma and other infectious conditions should be explained to them. Blindness caused by glaucoma can only be prevented by raising the community's awareness of the problem.

The treatment that society has meted out to blind people is reflected in their very negative attitudes towards both the sighted and the blind. The community, the medical profession and the state should jointly endeavour to understand their problems and work towards achieving not only the acceptance of the blind but also their ability to be self-supporting and self-respecting members of society.

By distributing information about blindness to the community and blind people's families it should be possible to encourage and enable them to participate in prevention activities and rehabilitation. The ability of health care providers to cope with cases of blindness should be enhanced through education.

Much more is needed than the giving of alms. Eliminating blindness requires large measures of understanding and dedicated work. It is necessary to improve socioeconomic conditions and strengthen the health infrastructure. Technical assistance and co-operation from international and nongovernmental organizations would also be of tremendous value. ■

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Reference

1. **Taylor HR, Velasco FM, Sommer A.** The ecology of trachoma: an epidemiological study in southern Mexico. *Bulletin of the World Health Organization*, 1985, **63**: 559–567.

Pleural tuberculosis in patients infected with HIV in Addis Abäba.

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Objective: To determine the prevalence of HIV-1 infection in patients with pleural tuberculosis and compare the clinical and radiological characteristics, effects and side effects of drugs, compliance to treatment and outcome by HIV status.
Design: A hospital based, prospective study.
Subjects: 90 adult patients with pleural tuberculosis. 120 surgical patients admitted for non-HIV related conditions served as controls.
Setting: Black Lion Hospital, a tertiary care referral hospital in Addis Ababa, Ethiopia.
Results: 22% of the patients with pleural tuberculosis versus 6.7% for the controls ($p<0.01$) were seropositive for HIV-1. Amongst patients with pleural tuberculosis, HIV seropositive patients were more likely to be male, and had longer duration of illness prior to coming to hospital ($p<0.05$). They had more disseminated disease ($p<0.01$), bilateral pleural effusion ($p<0.05$), adverse reaction to anti-tuberculosis drugs ($p<0.05$) and increased mortality ($p<0.05$). Hepatitis was the most common side effect observed. There was no difference in size of the effusion, clinical improvement, radiographic resolution or defaulter rate.
Conclusion: HIV associated pleural tuberculosis commonly occurs with other forms of extra pulmonary tuberculosis and responds well to treatment. Hepatitis is a frequent side effect and entails poor prognosis. We therefore recommend careful documentation of a previous history of liver disease and baseline investigation of liver function tests before initiation of treatment. The finding of bilateral pleural effusion may indicate the presence of underlying HIV infection.

Introduction

Both pulmonary and extra-pulmonary tuberculosis are increasing at an alarming rate in developing countries such as Ethiopia. The reasons for this are:

1. The HIV/AIDS epidemic.
2. Protracted starvation and subsequent malnutrition.
3. Poor tuberculosis control programmes.

Earlier on, extra-pulmonary tuberculosis was reported as the most common form of HIV-associated tuberculosis in North America.[1] However, as the AIDS epidemic started to unfold in Africa, despite the fact that HIV seroprevalence was higher in patients with extra pulmonary tuberculosis alone,[2,3] it became evident that the most frequent presentation was pulmonary disease.[4,5]

In Ethiopia, both pulmonary and extrapulmonary tuberculosis were diagnosed in 30% of 100 HIV infected patients between 1986 and 1989 and 35% of 636 AIDS patients in November 1990[6,7]. In both reports, there is no mention of pleural tuberculosis as a major manifestation of extrapulmonary tuberculosis. Even before the era of AIDS, the prevalence of pleural tuberculosis was reportedly low, between 3.8% to 9%.[8,9]

Over the past two years however, we have noted an increase in the number of patients with pleural tuberculosis referred to the chest unit of our hospital. We, therefore, prospectively studied the prevalence of HIV infection and compared the clinical and radiological presentation, efficacy and side effects of anti-tuberculosis drugs, compliance to treatment and outcome in HIV seropositive and HIV seronegative patients.

Materials and Methods

Between August 1990 and July 1991, 90 consecutive adult patients with pleural effusion referred to the chest unit of Black Lion Hospital and subsequently diagnosed as having pleural tuberculosis were included in the study. Black Lion Hospital is a tertiary case referral hospital in Addis Ababa, Ethiopia. One hundred and twenty age and sex matched surgical patients admitted for non-HIV related conditions served as controls to compare the HIV status. Serum samples were analyzed for HIV using the ELISA test and those positive were later confirmed by the Western Blot method.

All patients with pleural tuberculosis had a detailed clinical examination including data for involvement of other extrapulmonary sites, other AIDS related signs and symptoms and a postero-anterior and lateral chest X-ray before the start of the treatment. A diagnosis of tuberculosis was made on one or more characteristic findings on cytology, histopathology and bacteriology of the involvement organs and biological fluids. Patients were then randomly assigned to one of the

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following treatment regimens:
(i) 2SHRZ + 6HR;
(ii) 2SHE + 1OHE;
(iii) 2SHT + 1OHT.

They were then followed for clinical and radiographic response, adverse reaction to drugs, compliance to treatment and outcome at two, six and 12 months.

Significant differences in findings between HIV positive and HIV negative patients were analyzed using the chi-square test for proportions and the t test to compare the means. The Fisher exact test was employed wherever appropriate.

Results

Twenty patients (22%) with pleural tuberculosis versus eight patients (6.7%) for the controls were seropositive for HIV-1 ($p<0.01$).

Age and sex distribution.

Of the 90 patients, 46 were male and the male to female ratio was 2:1 for HIV seropositive patients and 1:1 for seronegative patients. The mean (SD) age for HIV seropositive and HIV seronegative patients was 29 (9.86) and 25.1 (8.84) years respectively. Among HIV seropositive patients the mean age for males was 33 years and for females 22 years ($p<0.01$). The mean age for HIV seronegative male and female patients was 24.8 and 25.4 years respectively.

Clinical and chest X-ray findings.

The clinical and chest X-ray presentation in HIV seropositive and seronegative patients is compared in Table I. The mean duration of illness prior to coming to hospital was longer in the seropositive patients than in the seronegative patients ($p<0.05$). In nine of the 20 seropositive patients, the disease has already disseminated to several other sites including the lymphnodes in eight, liver and spleen in five, peritoneum in four, pericardium in two and the meninges in one. Disseminated tuberculosis was diagnosed in only nine of the 70 seronegative patients ($p<0.01$).

Fifty five percent of the seropositive patients fulfilled a revised version of the WHO clinical case definition which excluded cough as a minor criteria. Four patients had chronic diarrhoea and profound weight loss at the time of presentation. Three patients each had oral thrush and HIV encephalopathy and a fourth patient had peripheral neuropathy. A tuberculin and energy skin test were not performed in our patients.

Twelve of the 20 seropositive patients had a moderate to large among of pleural effusion on a chest X-ray. Three time patients had massive pleural effusion with mediastinal shift and two patients had a mild amount of pleural fluid. These rates were not different for the seronegative patients, 60%, 10% and 18% respectively. Three of the 20 seropositive patients compared to only one of the 70 seronegative patients (1.4%) had bilateral pleural effusion ($p<0.05$). There was no other difference in the clinical presentation of the two groups of patients.

Adverse reactions and outcome.

The results of drug side effects and treatment outcome are shown in Table II. Of the 90 patients, 32 were started on 2SHRZ + 6HR, 30 on 2SHE + 1OHE and 28 patients on 2SHT + 1OHT. The corresponding number of HIV positive patients put on these regimen were 7,8 and five. Five patients in the seropositive group versus two patients (3%) in the seronegative group ($p<0.05$) developed adverse

Table I: Clinical and chest X-ray findings.

Clinical/C-X-ray characteristics	HIV +VE n=20	HIV -ve n=70 (%)	CI	p value
Duration of illness in days — mean (SD)	100.6	68.4 (59.2)		<0.05
Type of tuberculosis:				
Pleural alone	8	54 (77)	0.3 (0.14, 0.65)	<0.01
Pleuropulmonary	3	7 (10)	1.41 (0.5, 3.98)	0.68
Disseminated	9	9 (13)	3.27 (1.6, 6.68)	<0.01
Chest X-ray findings:				
Massive effusion	3	7 (10)	1.41 (0.5, 3.98)	0.68
Moderate to large	12	49 (59.6)	0.71 (0.33, 1.55)	0.56
Mild	2	13 (18)	0.56 (0.14, 2.18)	0.5
Bilateral	3	1 (1.4)	6.79 (1.87, 7.2)	<0.05

Table II: Drug side effects and outcome.

Side effects and outcome	HIV +ve patients: Total n of pts	HIV +ve patients: Pts. with n	HIV -ve patients (n=70): Total n of pts	HIV -ve patients (n=70): Pts. with n (%)	CI	p value
Side effects:	18		48			
Hepatitis		4		2 (4)		
Dermatitis		2		0 (0)		
Total		5		2 (4)	3.24 (1.66, 6.34)	p<0.05
Defaulter rate	20	5	70	22 (31)	0.78 (0.31, 1.92)	p=0.78
Mortality	15	3	48	1 (2)	3.69 (1.73, 7.87)	p<0.05

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reactions to the anti-tuberculosis drugs. Four of the five patients had hepatitis while two had dermatitis. Of the side effects, 60% occurred in the thiazina group. One patient each in the ethambutol and rifampicin group developed hepatitis. The two patients on the rifampicine and ethambutol containing regimen who developed hepatitis also expired. Fatal Steven Johnson syndrome was not observed in our patients.

Five of the 20 seropositive patients and 22 of the 70 seronegative patients (31%) defaulted their treatment and therefore could not be followed up to 12 months. Amongst the patients who completed their treatment, three patients in the seropositive group versus one patient (2%) in the seronegative group expired ($p<0.05$). Two of the three patients who expired had disseminated tuberculosis and were on a standard regimen that included isoniazid and ethambutol. The third patient had chronic diarrhoea, oral thrush and HIV encephalopathy. He was on short course chemotherapy and developed hepatitis during the course of the treatment.

Discussion

The seroprevalence of HIV infection in our patients (22%) was lower than what has been reported for extrapulmonary tuberculosis from several African countries, which ranged from 17% to 77%.[3,10] In Abidjan, Cote d'Ivoire, amongst 751 patients with extrapulmonary tuberculosis 60% were seropositive for HIV-1.[3] In Lusaka, Zambia, 81% of patients with pleural tuberculosis were positive for HIV-1.[3] The seroprevalence for HIV infection among all types of tuberculosis both in a sanatorium and an urban general hospital in Ethiopia was 40% (Kassahun Temesgen, unpublished data and Bert Lindtjorn, personal communication).

Most of our seropositive patients were young (range 17 to 48 years) and 80% were below 40 years of age. The average for both sexes was 29 years. This indicates that sexual acts, as in most countries in Africa, are the most frequent mode of HIV transmission in Ethiopia.[3,11] Among HIV seropositive patients, female patients tended to be younger than males, a pattern also observed in several other places including Lusaka, Zambia.[3]

In 12 of the 20 patients, pleural tuberculosis occurred as either pleuropulmonary or disseminated disease. Most patients with disseminated tuberculosis had very advanced disease and two died while on treatment. The most common extrapulmonary manifestation was lymphadenopathy (40%). Lymphadenopathy is the most frequent form of extrapulmonary tuberculosis as reported from Central African Republic,[12] Zambia[3] and Kenya.[13] Hepatosplenomegaly, though not proved by biopsy, occurreed in five patients.

The amount of pleural fluid did not differ in the two groups of patients. However, more patients in the seropositive group had bilateral pleural effusion than in the seronegative group. In South African Blacks, amongst a total of 23 seropositive patients with pleural tuberculosis, the effusion was evenly distributed on th eleft and right.[14] Our finding suggests that the presence of bilateral pleural effusion may indicate the presence of underlying HIV infection. The clinical and radiological improvement was comparable in the two groups of patients. Complete resolution of the effusion was attained in 67% and 80% of the seropositive and seronegative patients respectively.

The most common addverse reaction observed in our patients was hepatitis. Previous reports have emphasized that fatal exfoliative dermatitis and milder forms of generalized rash are the most frequent side effects related to thiacetazone in HIV-associated tuberculosis. Very little has been reported about the frequent occurrence of hepatitis. In our clinical experience and in this study, hepatitis probably related isoniazid was the most frequent side effect and occurred early in the course of treatment. Two patients with hepatitis in our series expired. The presence of hepatitis appeared to entail poor prognosis. We, therefore, recommend that careful documentation of a previous history of liver disease and baseline investigation of liver function tests be done before initiation of treatment. Besides, future studies should look into this and the possible relationship of drug induced hepatitis with the acetylation status of patients.

The case fatality from tuberculosis in HIV infected African patients is higher than non-HIV infected individuals.[15-18] Besides, mortality and relapse rates have been shown to be higher in patients treated with standard chemotherapy than short course chemotherapy.[16,18] In our series, two patients on standard chemotherapy and one patient on short course chemotherapy expired. It will be difficult, however, to draw any reasonable association between mortality and drug regimen because the number of patients who died was too small. All the three patients who died had advanced immunosuppression and two developed hepatitis. Considering the fact that 11 of our patients fulfilled the clinical case definition for AIDS and 9 had disseminated tuberculosis, the case fatality (3/15) was probably lower than expected.

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References

1. Tuberculosis and acquired immunodecifiency syndrome. *MMWR* 1987;36:785-90, 795.
2. Kelly P, Burnham G, Radford C. HIV seropositivity in a rural Malawi hospital. *Trans R Soc Trop Med Hyg* 1990;84:725-7.
3. Elliot AM, Luo N, Tembo G, *et al.* Impact of HIV tuberculosis in Zambia: a cross sectional study *Br Med J* 1990;301:412-5.
4. DeCock KM, Gnaaore E, Adjorlolo G, *et al.* Risk of tuberculosis in patients with HIV-1 and HIV-II infection in Abidjan, Ivory Coast. *Br Med J* 1991;302:496-9.
5. DeCock KM, Selik RM, Soro B, Gayle H, Colebunders RL. AIDS surveillance in Africa: a reappraisal of case definition. *Br Med J* 1991;303:1185-8.
6. Tsega E. The demographic, social and clinical presentation of one hundred Ethiopian patients with Human Immunodeficiency Virus (HIV) infection. *Ethiop Med J* 1990;28:81-8.

7. Hailu N, Hailu K, Khodakovich L, Debrework Z, Bekele S. Profile of AIDS cases in Ethiopia. *Ethiop J Health Dev* 1990;4(2):213–7.
8. Getachew F, Bayu T. Analysis of adult tuberculosis admission to St Peter sanatorium, Addis Ababa. *Ethiop Med J* 1983;21(3):143–7.
9. Eyassu HG, Befekadu G, Nengistu H, *et al*. Analysis of admission to Gondar Hospital in northwestern Ethiopia. *Ethiop Med J* 1976;14(2):49–59.
10. Cathebras P, Vohito JA, Yete ML, *et al*. Tuberculose et infection par le virus de l immunodeficience humaine en Republique Centrafricaine. *Med Trop* 1988;48:401–7.
11. Howlett WP, Nkya WM, Mmuni KA, Missalck WR. AIDS in northern Tanzania. III International Conference on AIDS and associated cancers in Africa Arusha, Tanzania. September 14–16, 1988; abstract TP21.
12. Lesbordes JL, Baquillon G, Georges MC, *et al*. La tuberculase au cours de l' infection par le virus de l' immunodeficience humanine (HIV) à Bangui (Republique Centrafricaine). *Medicine Tropicale* 1988;48:21–5.
13. Gilks CF, Brindle RJ, Otieno LS, *et al*. Extrapulmonary and disseminated tuberculosis in HIV-1 seropositive patients. AIDS 1889;4:981–5.
14. Saks AM, Poser R. Tuberculosis in HIV positive patients in South Africa: a comparative radiological study with HIV negative patients. *Clin Radiol* 1992;46:387–90.
15. Raviglione MC, Narain JP, Koch A. HIV associated tuberculosis in developing countries: Clinical features, diagnosis and treatment. *Bull WHO* 1992;70(4):515–26.
16. Perriens JH, Colebunders RL, Karahunges C, *et al*. Increased mortality and tuberculosis treatment failure rate among human immunodeficiency virus (HIV) seropositive compared with HIV seronegative patients with pulmonary tuberculosis treated with "standard" Chemotherapy in Kinshasa, Zaire. *Am Rev Respir Dis* 1991;144:750–5.
17. Murray CJL, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. *Bull Int Union Tuberc Lung Dis* 1990;65:624.
18. Colebunders RL, Ryder RW, Nzilambi N, *et al*. HIV infection in patients with tuberculosis in Kinshasa, Zaire. *Am Rev Respir dis* 1989;139:1082–5.
19. Nunn P, Wasunna K, Galtha S, *et al*. Cohort study of HIV among tuberculosis patients in Nairobi, Kenya: Baseline features. VI International Conference on AIDS, San Francisco, California, 20–24 June 1990. Abstract Th. B486:243.

Fractures in children in south eastern Nigeria

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Objectives: To evaluate the pattern of fractures in children in the south eastern region of Nigeria, seeking to highlight the planning and designs of buildings, roads and playgrounds to prevent paediatric fractures.
Design: Retrospective review of paediatric patients with fractures who presented at the only tertiary referral centre in the region.
Setting: A university teaching hospital situated in the south eastern corner of Nigeria.
Subjects: 102 paediatric patients treated for fractures between January 1993 and December 1995.
Main outcome measures: Causes of fractures and their anatomical distribution.
Results: 71 boys and 31 girls were treated for fracturs. The commonest cause of fractures was road traffic accidents accounting for 50% of the patients, followed by falls and collapsing mud walls. Most of the fractures seen (58 patients) were of the green stick type. The femur was the commonly fractured bone (25 patients) follwed by the radius (22 patients) and humerus (20 patients). Road traffic accident victims presented earlier at the hospital than victims of other causes. Also lower limb fracture patients presented earlier than patients with upper limb fractures.
Conclusion: Paediatric fractures are amenable to conventional conservative therapy and open reduction became necessary in long neglected cases with associated malunion. Since the causes of fractures are largely preventable, public enlightenment campaigns, provision of pedestrian pathways, care on the part of school authorities and proper structural construction of the traditional houses may help reduce the number of cases of fracture in our environment.

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BILATERAL PULMONARY ASPERGILLOMA: CASE REPORT
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BILATERAL PULMONARY ASPERGILLLOMA: CASE REPORT

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SUMMARY

Bilateral pulmonary aspergilloma colonising an old tuberculous cavity and a pyogenic lung abscess in a 25-year old Ethiopian lady is reported. The diagnosis was based on the findings of a rounded density with typical air crescent sign on a chest x-ray and isolation of Aspergillus species from a bronchoscopic aspirate. Despite the high prevalence of cavity pulmonary tuberculosis, pulmonary aspergilloma is rarely reported from this and other African countries and the likely reasons for this are discussed.

INTRODUCTION

Aspergillus is a fungus that is ubiquitous in nature and found mainly in soil water decaying materials and in the air. Allegedly, the fungus is of world-wide distribution although most reports have come from Europe and North America(1). Aspergillus infection in man ranges from saprophytic colonisation of an air space cavity in the lungs (aspergilloma), non-invasive bronchial allergic form of the disease to the invasive disseminated aspergillosis in a severely neutropenic and immunodeficient patient. Pulmonary aspergillomas frequently occur with chronic cavity lung disease such as tuberculosis. Despite the high prevalence of cavitary tuberculosis in Ethiopia and other African countries, the disease appears to be rare. To our knowledge, this is the first report of pulmonary aspergilloma from Ethiopia.

CASE REPORT

EG was a 25-year old female student from Sidamo, who had been treated for pulmonary tuberculosis twice in the past, nine and five years ago, and claimed to have been well until three months ago when she developed cough productive of purulent sputum, fever, sweating and progressive weight loss, and was admitted to St. Peter TB Sanatorium. She was suspected to have developed drug resistant tuberculosis and was started on retreatment regimen with five drugs (streptomycin, isoniazid, rifampicine, pyrazinamide and ethambutol). After two months of treatment, sputum culture for *Mycobacterium tuberculosis* was negative and her symptoms got worse. Her general condition progressively deteriorated and was therefore referred to the Chest Unit of Black Lion Hospital for further work up.

On physical examination, she looked sick and wasted and was constantly coughing and expectorating foul smelling sputum. She was moderately pale and had advanced finger clubbing. There were scattered coarse crepitations in the lower third of the lung fields. Examination of the other system was unremarkable. Investigations revealed Hgb = 10gm%, WBC = 4600/mm^3 ESR = 110mm 1st hr. Urine analysis, stool examination, liver enzymes, renal function tests and serum electrolyte were all within normal limits. Sputum for AFB was negative respectedly. Initial chest x-ray showed a cavity with a rounded mass in the left apical lung zone and an ill-defined capacity with a central opacity in the right mid-lung zone. Subsequent chest x-rays following treatment showed that the ill-defined capacity has evolved to form a well-defined mass with an air crescent on top (Figure 1). Chest x-rays taken at different positions showed the mass to be changing position within the cavity. Bronchoscopy

Figure 1

A well formed ball in the LUL zone and a new one in RLL zone with an air crescent sign.



showed copius amount of pus coming from the right main stem bronchus and the aspirate grew Aspergillus species and *Klebsiella pneumoniae*. The patient was treated with high dose parenteral penicillin and gentamicin as a case of lung abscess and showed remarkable improvement and was discharged in good condition.

DISCUSSION

Pulmonary aspergillomas frequently occur in association with chronic cavitary disease more often caused by tuberculosis. It is estimated up to 17% of all aspergillomas occur on the background of previous cavitary TB(2). Other predisposing conditions include stage III cystic sarcoidosis(3), cystic bronchiectasis, cavitary fungal diseases, chronic bacterial abscesses, cavitary tumours.

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etc. Occasionally aspergillomas can evolve as saprophytic colonisers of a dead tissue following acute necrotising bacterial infections or infarctions. Conversely, invasive pulmonary aspergillosis can rapidly cavitate and develop an intracavitary mass(4).

Pulmonary aspergillomas commonly present with cough and haemoptysis. Haemoptysis has been reported in 50-95% of cases and can result in fatal exsanguination(5,6). Aspergillomas tend to be single and unilateral, usually involving the apical lung zones. Our patient developed the fungus ball in the left upper lobe cavity long before her present illness started. The present complaint started as a necrotizing bacterial infection with an ill-defined alveolar opacity in the right mid-lung zone which soon progressed to form a well-defined mass with atypical air crescent sign (Figure 1). The fact that the respiratory symptoms and the chest x-ray findings improved with penicillin treatment alone suggests that the aspergillus was probably playing the innocent by-stander role and subsequently colonised the cavity formed by the necrousing klebsiella infection.

The diagnosis of pulmonary aspergilloma can usually be made on the basis of its characteristic appearance on a chest x-ray, a rounded density surrounded particularly by a crescent of air. Changes in the position of the patient cause the density move within the cavity. The diagnosis is confirmed by demonstration of serum precipitin for aspergillus or culture grown from bronchoscopic aspirate.

The management of aspergillomas and its influence on progosis is highly controversial. Medical treatment has no value and the indications for surgery are limited. Because of the unacceptably high post-operative complications and mortality, resectional surgery is indicated for patients with massive haemoptysis to prevent fatal exsanguination. Fortunately, our patient did not have haemoptysis. She also had a bilateral disease and very poor functional pulmonary reserve which will have made surgery an unlikely choice of treatment.

Pulmonary aspergilloma is rarely reported from Ethiopia and other African countries despite the fact that upto 17% of patients with cavitary tuberculosis develop the disease(2). Review of the African literature shows that the largest series reported were from Djibouti and Cote d'Ivore(7,8). Aspergilloma of the paranasal sinuses and pulmonary aspergillosis caused by *Aspergillus flavus* have been reported from the Sudan(9,10). To our knowledge, this is the first report from Ethiopia. Why then the disease so rarely reported from Africa? This is unlikely to be due to difficulties in diagniosis because aspergilloma is easily suspected from a chest x-ray. It is also unlikely to be due to under-reporting. It has been reported that aspergillus is an ubliquitous organism of world-wide distribution although most reports to date have come from Europe and North America(1). This statement casts whether the distribution of the organism is uniform worldwide or even amongst regions in Africa. Could there be some regions in Africa where the fungus is more prevalent than others? Could there be an acquired or genetic factor that makes the African more resistant to the disease? These and other possible factors for the rarity of the disease need to be addressed both in an epidemiologic and large prospective studies in the future.

REFERENCES

1. Infectious disease of the lung. In. Fraser R.G., Peter Pare J.A., Pare P.D., Fraser R.S. and Generaux G.P. eds. Diagnosis of Disease of the chest 3rd. ed. Philadelphia. Saunders Company. 1989; 989.
2. Research Committee of the British Thoracic and Tuberculosis Associations: Aspergilloma and residual tuberculosis cavities the results of a resurvey. *Tubercle*. 1970; 51:227.
3. Wallschlager, C. and Khan, F.. Aspergilloma complicating sarcoidosis a prospective study in 100 patients. 1984; 86:585.
4. Przyjemski, C. and Matut, R. The formation of pulmonary mycetomata. *Cancer*. 1980; 46:1701.
5. Fraukner, S.L., Vernon, R. Brown, pp et al. Haemoptysis and pulmonary aspergilloma operative versus non-operative treatment. *Ann. thorac. Surg*. 1978; 25:289.
6. Pennington J.E. Aspergillus lung disease. *Med. Clin. N. Amer*. 1980; 64:475.
7. Auregan G., Chakis S., Etienne H. Ibrahim M. and A.M Lex Aspergillomes Du Poumon A Djibouti. *Ann. Soc. Med. Trop*. 1988; 68:357-364.
8. Tiendrebeogo, H., Sangare, S.I., Roudaut, M., Schimidt, D. and Assale, N. cent un cas d' aspergillose pulmonaire en Cote d' Ivoire. *Med. Trop*. 1982; 452:47-52.
9. Milosev, B., Mahgoub, E.I. S., Abdel Aal, O. and El. Hassen, A.M. Primary aspergilloma of paranasal sinuses in the Sudan. A review of seventeen cases. *Brit. J. Surg*. 1969; 56:132-137.
10. Mahgoub, E.S. and EL Hassen, A.M. Pulmonary aspergillosis caused by *Aspergillus flavus*. *Thorax* 1972; 27:33.

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Getachew Aderaye, 1996. *Ethiop Med J* 34,

CASE REPORT

HYPERTROPHIC PULMONARY OSTEOARTHROPATHY IN PULMONARY METASTASIS - A CASE REPORT

Getachew Aderaye, MD[1].

ABSTRACT: *Hypertrophic pulmonary Osteoarthropathy is most commonly encountered in association with bronchogenic carcinoma and tumours of the pleura. Its association with pulmonary metastasis from extrathoracic neoplasms is rare. We report a 33 years old male Ethiopian who presented with Cannon ball lesions, clubbing of the digits, periostitis, gynaecomastia, acromegalic features and bilateral knee arthritis. Related literature is briefly reviewed.*

INTRODUCTION

Hypertrophic Osteoarthropathy (HOA) is a clinical condition that occurs in association with a diverse number of diseases involving the lungs, pleura, mediastinum and digestive systems. The most common underlying causes are intrathoracic tumours, notably bronchogenic carcinoma and tumours of the pleura (1,2). It is said to occur in 0.7 to 12% of patients with bronchogenic carcinoma (3,4). This frequency is in sharp contrast to the rarity of the association of HOA with haematogenous metastasis to the lungs from extrathoracic neoplasms. We report this rare entity for the first time in an Ethiopian who presented with a primary soft tissue tumour with metastasis to the lungs.

CASE REPORT

A.K. was a 34 year old man residing in Butajira, 180 km outside Addis Ababa, when he first presented to the nearby Atat Hospital because of an easily bleeding and non-healing ulcer over the dorsum of the left foot. The lesion was suspected to be a malignant ulcer and below ankle amputation was performed. Biopsy of the lesion was not taken neither before nor after the surgery. He remained symptom-free for over a year until he developed an ulcer on the amputation stump. This was considered to be a recurrence of the tumour and above the ankle amputation was performed. Tissue was not sent for pathological study this time, too. He remained asymptomatic until one year later when he noted a lump in the left inguinal region. This was considered to be a metastatic node and was removed surgically. Eight months prior to his referral to our hospital, he developed a bleeding ulcer on the amputation stump and below knee amputation was performed for the third time. Following this, he noted club-

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bing of the digits, progressive enlargement of the distal extremities and bilateral gynaecomastia. He also noted painful swelling of the ankle and knee joints. Four months prior to presentation, he developed dry cough. Chest x-ray at this point showed Cannon ball lesions in both lung fields. He was subsequently referred to our hospital for bronchoscopy.

Investigations at the time of referral revealed Hgb = 10 gm%, WBC = 9000/^{3}, Hct. 32%, platelet = 681,000/mm^{3}, ESR = 55mm 1st hr., VDRL = non-reactive, Rose Waaler = negative. Serum uric acid = 3.6 mg%. Liver function tests including transaminases were within normal limits. X-ray of the distal extremities and small joints of the hands showed typical periosteal elevation and new bone formation. Febreoptic bronchoscopy showed no endobronchial lesion and bronchoscopic aspirate was negative for malignant cells. The patient was put on indomethacin 75 mg daily and showed remarkable improvement in the reduction of bone pain and joint swelling, but tended to relapse with the discontinuation of the treatment. At present, he is under follow up in our chest clinic.

DISCUSSION

Hypertrophic pulmonary osteoarthropathy is a clinical syndrome that consists of digital clubbing, painful swelling and tenderness of the distal third of the arms and legs, periosteal elevation and new bone formation of the long bones and polyarthritis. These findings tend to be symmetrical and the most common joints affected are that of the elbows, knees and ankles. Gynaecomastia and acromegalic features occur in some patients but are rare. HOA occurs as a primary heredofamilial disorder (pachydermopersiostosis) or more commonly secondary to intrathoracic tumours with bronchogenic carcinoma accounting for 80% of the cases (1). Prior to the antibiotic era, it was commonly encountered with suppurative lung diseases, cyanotic heart diseases and diseases of the digestive and biliary system (5-7). Suppurative lung diseases may play a major role in the causation of HOA in Africa. In a study from Nigeria, amongst 15 cases of HOA studied over 15 years, bronchogenic carcinoma accounted for only 33% while 53% of the cases were due to bronchiectasis, lung abscess, empyema thoracics or tuberculosis (8). In the only case report from Ethiopia, HOA was associated with teratocarcinoma of the mediastinum (9). The prevalence of HOA in suppurative lung diseases in Ethiopia is not known but appears to be rare.

HOA from extrathoracic tumour with haematogenous metastasis to the lungs is very rare (10). The most common extrathoracic tumour are those arising from mesenchymal tissue having a predominantly fibrous tissue stroma such as fibromas, fibrosarcomas, osteosarcomas, mesotheliomas, etc. These tumours account for nearly half of all the reported cases in the literature (11). The factor responsible for this frequent tissue, non-healing ulcer which required multiple amputations, is likely to be malignant squamous cell carcinoma or tumour of fibrous tissue origin. In none of the surgical procedures was biopsy taken for histopathological study and our diagnosis therefore remains presumptive.

Our patient presented with both the common and rare features of HOA. Clubbing and joint pain occur in more than 90% of patients with HOA. Clubbing alone, however, does not constitute a diagnosis of HOA. The diagnosis of HOA requires the demonstration of periosteal elevation and

new bone formation radiography. In the Nigerian study, for example, only 18 out of 116 (16%) patients with clubbing had HOA, too. The mechanism that underlies the relationship between clubbing and HOA is not yet clear.

Gynaecomastia and acromegalic features are rare accompaniments of HOA (12,13). Increased oestrogen secretion has been postulated to be the underlying mechanism for gynaecomastia. However, several studies have shown that oestrogen level is increased in most patients with HOA, but only few develop gynaecomastia.

Both primary and secondary HOA are superficially similar to acromegaly. Acromegalic features tend to occur in most cases of pachydermoperiostosis and some cases of secondary HOA. Increased production of the human growth hormone from either the pituitary or the tumour has been demonstrated in most patients with HOA. However, only few patients develop acromegalic features. The underlying mechanisms responsible for the development of acromegalic features are not yet clear. Our patient had a disproportionate enlargement of the distal extremities without involvement of the mandible. The absence of enlargement of the mandible distinguishes HOA from acromegaly.

One of the objectives of management in HOA is to bring about relief of the bone and joint pain. In intrathoracic tumours, this can be achieved by either resecting the tumour or by vagotomy. Unfortunately, this cannot be done in patients with pulmonary metastasis. For patients with non-operable tumours, non-steroidal anti-inflammatory drugs have been shown to induce transient but effective control of joint and bone pain. Our patient responded very well to indomethacin capsules at a modest dose. The long term prognosis in both intrathoracic tumours and pulmonary metastasis, however, is guarded.

REFERENCES

1. Coury C. Hippocratic fingers and hypertrophic osteoarthropathy: study of 350 cases. *Br J Dis Chest* 1960;54:202-9.
2. Shapiro M. Hypertrophic osteoarthropathy. *Arch Intern Med* 1956;98:700-11.
3. Semple T, McCuskie RA. Generalized hypertrophic osteoarthropathy in association with bronchial carcinoma: A review based on 24 cases. *Br Med J* 1955;1:754-9.
4. Wierman WH, Clagett OT, McDonald JR. Articular manifestation in pulmonary diseases: an analysis of their occurrence in 1024 cases in which pulmonary resection was performed. *JAMA*. 1954;155:1459-63.
5. Barelays N. Ogbeide M, Grielo I. A Gross hypertrophic pulmonary osteoarthropathy in a 7 year old child. *Thorax* 1970;25:484-9.
6. Buchan DJ, Mitchell DM. Hypertrophic osteoarthropathy in partial cirrhosis. *Ann Intern Med* 1967;66:130-5.
7. Han SY, Collins LC. Hypertrophic osteoarthropathy in cirrhosis of the liver: report of two cases. *Radiology* 1968;91:795-6.
8. Onadeko BO, Kolawole TM. The clinical and etiological pattern of finger clubbing and hypertrophic Oteoarthropathy in Nigerians. *Trop Geogr Med* 1979;31:191-9.

Amir sultan (MD), 2014

9. Yacob Fikreyesus, Yigeremu Abebe. Hypertrophic osteoarthropathy in an Ethiopian. *Ethiop Med J* 1987;25:49-52.:2
10. Firooznia H, Seliger G, Genieser NB, Braasch E. Hypertophic pulmonary osteoarthropathy in pulmonary metastasis. *Radiology* 1975;115(2):269-74.
11. Yacoub MH, Simon G, Ohnsarge J. Hypertrophic pulmonary osteoarthropathy in association with pulmonary metastasis from extrathoracic tumours. *Thorax* 1967;22:226-31.
12. Craig JW. Hypertrophic pulmonary osteoarthropathy as the first symptom of pulmonary neoplasm. *Br Med J* 1937;1:750-2.
13. Hammarstein JF, O'Leary J. The features and significance of hypertrophic osteoarthropathy. *Arch Intern Med* 1957;99:431-41.

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Indeed, previous reports suggest that it is difficult to discriminate cerebral malaria from meningitis without analysis of the CSF[3,4]. In cases where there is doubt or in a situation in which partial antibiotic treatment might modify the clinical form of meningococcal disease, empirical institution of antibiotic therapy should be considered. On the other hand, in patients with meningitis, the possibility of malaria should be considered and a blood smear examination should be done.

Severe falciparum malaria can present in association with meningitis and prompt diagnosis and treatment with intravenous quinine and antibiotics is urgent. Failure to do this may lead to significant increase in morbidity or mortality.

REFERENCES

1 World Health Organization. Severe and complicated malaria. *Trans R Soc Trop Med Hyg* 1990;**84** (suppl 2): 1–65

2 World Health Organization. *Practical Chemotherapy Of Malaria*. Technical Report Series, No. 805: Geneva: WHO, 1990

3 Wright PW, Avery WG, Ardill WD, McLarity JW. Initial clinical assessment of comatose patient: cerebral malaria vs. meningitis. *Pediatr Infect Dis J* 1993;**12**:37–41

4 Manson-Bahr PEC, Apted FIC. *Manson's Tropical Diseases*, 18th edn. London: Baillière Tindall, 1982:51

5 World Health Organization. *Advances In Malaria Chemotherapy*. Technical Report Series, No. 711. Geneva: WHO, 1984

Systematic inspection of insulin injection sites for local complications related to incorrect injection technique

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SUMMARY

We systematically inspected insulin injection sites in 100 insulin-requiring patients attending the Diabetic Clinic of the Tikur Anbassa Hospital (TAH) in order to identify local complications related to incorrect injection technique: local complications were found in 53 cases: skin hyperpigmentation and/or indurations in 30 patients; and fat atrophy or hypertrophy in 31 patients. Illiteracy was significantly more common among those with local complications (18/53 versus 6/47, χ^2 5.03, $P<0.05$). Mean fasting blood glucose on the day of the inspection was significantly higher (14.9 + 6.3 mmol/l versus 10.5 + 6.1 mmol/l, $P<0.001$) and a fasting blood glucose >10 mmol/l more common (41/53 versus 20/47, χ^2 14.1, $P<0.0005$) in those with than in those without local complications. There was no significant difference between the two groups in mean duration of diabetes (6.9 + 5.6 years versus 6.6 + 5.8 years), frequency of hypoglycaemic episodes (12/53 versus 5/47, χ^2 1.76, $P>0.05$) or mean daily insulin dose (44 + 18 units versus 44 + 22 units per day).

Therefore, we concluded that local complications resulting from incorrect injection technique, a common finding in the group of patients studied, may be common among insulin requiring diabetic patients in general. Incorrect insulin injection causes local complications and disfigurement which may compromise compliance. Furthermore, insulin absorption tends to be erratic from intradermal and fat hypertrophy sites thus interfering with effective diabetic control. Insulin injection sites should be inspected routinely to detect and correct faulty technique promptly.

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INTRODUCTION

Insulin injection may be associated with a variety of complications at the injection site if incorrectly performed. Intradermal deposition of insulin may cause local reaction leading to dyspigmentation, induration, scarring and formation of fibrous nodules while failure to rotate the injection site regularly may result in local fat atrophy, or fat hypertrophy which in the course of time becomes fibrous and relatively avascular. These complications are disfiguring, but more importantly, injection of insulin intradermally or into the site of fat hypertrophy may be associated with inadequate or erratic absorption into the circulation which can contribute to problems in diabetic control[1,2]. Intramuscular deposition of insulin has also been described[3] and this can lead to similar difficulties in control but not to local complications. However, this type of problem has not been commonly encountered at our clinic possibly because, for insulin injection, we advise lifting a skin fold and penetration with the needle at an angle of 45° rather than vertically.

We were already aware of the local complications of intradermal insulin injection, but a systematic inspection of injection sites in a representative sample of insulin-requiring patients had not been conducted. In this study we examined injection sites systematically in 100 of our insulin-requiring patients in order to determine the frequency and pattern of local complications.

PATIENTS AND METHODS

A systematic examination of injection sites was conducted on the first 10 insulin-requiring patients on each clinic day from the starting date of the study until a total

G2

of 100 patients had been seen. Patients in the initial phase of instruction in insulin injection technique were excluded from the study. The examination consisted of inspection and palpation of the injection sites on the upper arms, abdomen and thighs for dyspigmentation, indurations, nodules, depressions (indicative of fat atrophy) and fat hypertrophy.

The Student's t test and the χ^2 test were applied for statistical analysis.

RESULTS

Local complications related to insulin injection were found in 53 of the 100 patients. The pattern and frequency of the different types of local complications is shown in Table 1. There was no significant difference in the mean duration of diabetes, mean insulin dose or frequency of symptoms of hypoglycaemia between those with local complications and those without local complications (Table 2). However, a blood glucose level of >10 mmol/l on the day of examination was significantly more frequent ($P<0.001$) and the mean fasting blood glucose was significantly higher ($P<0.0001$) among those with local complications. Illiteracy was also more common (χ^2 5.03, $P<0.05$) among those with local complications.

DISCUSSION

The finding of 53 patients (53%) with local complications related to wrong injection technique with deposition of part or all of the injection insulin intradermally or failure to rotate the injection site was unexpected. Most of the patients had been trained in injection techniques at our clinic by our diabetes nurse practitioners. Since illiteracy was significantly more common among patients with local complications than those without local complications it is possible that a low educational level is an impediment to effective transference of the simple information and skills required to master correct insulin injection technique. However, Wright *et al.*[4] at their diabetic centre in UK found abnormalities at injection sites in 40% of their insulin taking patients. Therefore, a low educational level does not appear to be the only factor responsible for a high frequency of local complications related to wrong injection technique.

The significantly higher frequency of fasting blood glucose (FBG) >10 mol/l and a significantly higher mean FBG (Table 2) among patients with local complications is not surprising because injection of insulin intradermally or into an area of fat hypertrophy is associated with erratic and incomplete absorption into the circulation. HbA1c measurements would have provided a more reliable means for comparing diabetic control between the two groups but could not be performed because of lack of the necessary reagent. There was no difference in the frequency of hypoglycaemic attacks between those with local complications and those without local complications. However, hypoglycaemia developed in several patients with local complications after correction of their injection technique until the insulin dose was readjusted. This finding is in agreement with the observation of other investigators[5].

As described earlier these local complications are evidence of wrongly injected insulin. However, fat atrophy, which was found in seven patients in this study, is thought to be due to the immunogenicity of heterologous insulin or impurities in insulin preparations[6,7]. Wright *et al.*[4] found lipoatrophy in 49 of 511 patients (9.6%) taking unchromatographed mixed insulin but in none of those treated with purified pork insulin. We believe, however, that lipoatrophy can be prevented by regular rotation of the injection site regardless of the type of insulin used. Since deposition

Table 1. Types of local complications in 53 patients

Abnormality	*No.*	*%*
Hyperpigmentation	1	1.9
Hyperpigmentation and induration	13	24.5
Hyperpigmentation and nodules	13	24.5
Fat hypertrophy	17	32.1
Fat atrophy*	7	13.2

*This is not by itself an indication of incorrect injection technique but is thought to be related to impurities in the insulin injected

Table 2. Comparison of patients with local complications and without local complications in selected parameters

	With complications	*Without complications*	χ^2	P
No. of patients	53	47		
Mean duration of diabetes	6.9±5.6 years	6.6±5.8 years		
Mean insulin dose	44±18 units	44±22 units		
Illiteracy	18	6	5.03	<0.05
Frequent hypoglycaemia	12	5	1.76	NS
FBG* <10 mmol/l	12	27	14.1	<0.005
Mean FBG	14.9±6.3 mmol/l	10.5±6.1 mmol/l	–	<0.0001†

*FBG = fasting blood glucose
†Student's t test

of insulin in the skin or in an area of fat hypertrophy interferes with its steady absorption into the circulation, it results in inadequate diabetic control. In addition, the complications are disfiguring and may thus contribute to impairment of compliance. It is important to search for local complications related to insulin injection regularly in all insulin requiring patients and to correct faulty injection technique promptly.

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REFERENCES

1 Paley RG, Scott MH. Severe insulin lipodystrophy as a possible cause of instability in diabetes. *BMJ* 1958; **ii**:1331–4

2 Oakley WG, Pyke DA, Taylor KW. Complications of treatment and hypoglycaemia. In: *Diabetes and Its Management*. London: Blackwell Scientific Publications, 1987:103–7

3 Thow J, Home P. Insulin injection technique. *BMJ* 1990;**301**:3–4

4 Wright AD, Walsh CH, Fitzgerald MG, Malines JM. Very pure porcine insulin in clinical practice. *BMJ* 1979;**i**:25–7

5 Lamar CP. Insulinogenic lipodystrophies. Their relationship to "brittle" diabetes and to insulin resistance. *JAMA* 1950;**142**:1350–3

6 Galloway JA. Insulin treatment for the early 80's. Facts and questions about old and new insulins and their usage. *Diabetes Care* 1980;**3**:615–22

7 Jones GR, Statham B, Owens DR, Jones MK, Hayes TM. Lipoatrophy and monocomponent porcine insulin. *BMJ* 1981;**282**:190

Conducting a descriptive survey: 1. planning the survey

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INTRODUCTION

Your first step is to ask: is it actually appropriate to conduct a descriptive survey? The answer will depend on the reasons for undertaking the study, its objectives and the circumstances in which it is to be conducted. This series of articles has been written on the assumption that this decision has already been taken, but make sure you have considered carefully whether a descriptive survey is the right approach to the question you wish to address.

The design of descriptive surveys varies with the type of population and the nature of the observation to be described. Some examples recently reported in *Tropical Doctor* provide descriptions of: the traditional beliefs of women towards onchocerciasis in rural Nigeria[1]; the obstetric skills of medical doctors working in district and sub-district hospitals in Bangladesh[2] the prescribing patterns of outpatient dispensaries in Tanzania[3]; the nature of blast injuries of the eye amongst inpatients in Ethiopia[4]; the prevalence of dental caries and gingivities in a rural community in India[5]. A glance at these and other articles will demonstrate the range and relevance of descriptive surveys in the investigation of health problems in developing countries.

OBJECTIVES OF THE STUDY

The overall objectives of a descriptive survey are to *describe* selected characteristics of a defined population, for example: the prevalence of ill health, such as intrauterine infection amongst teenage women, disability amongst adult men; the distribution of measurements, such as haemoglobin amongst pregnant women, birth weight of babies, nutritional indices of young children; the range of attitudes towards health interventions, such as the choice of method of contraceptive amongst couples eligible for family planning, the reasons for women preferring to give birth at home. Overall objectives may be lofty but to plan the study properly it is necessary to be quite specific. Questions you must answer in planning a survey are:

What is the target population to be described?

A target population consists of all members of a defined group. Most commonly, it consists of people but a population may comprise, for example, villages, households, hospitals, dispensaries or even vaccines or refridgerators! Eligibility for membership might include address, race, age, sex, admission to hospital, diagnosis, religion, socio-economic status, education or in the case of refridgerators, the source of power, location in a health facility, etc. Membership is closed, if the investigator is describing the population at a fixed point in time, or open when describing, for example, a population of babies to be born or patients to be admitted to hospital over a specified period of time.

When a population is very large, it may be impossible to obtain a complete membership list. Instead its geographic/administrative structure needs to be examined, e.g. by obtaining lists of districts, villages and households. This will provide the basis for subsequent sampling of the population.

You must decide the eligibility criteria very carefully before the start of the study. It is this population which will be sampled and described.

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Blood pressure variation among Ethiopians on the Simien Plateau

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Summary. This report presents information on determinants of blood pressure variation in a rural sample of 263 Ethiopian highlanders 14–86 years of age, resident at 3530 m on the Simien Plateau. Mean systolic and diastolic blood pressures for males and females were 109/75 and 106/73, respectively, and there were no age differences. These findings confirmed that men and women can have low normal blood pressure throughout adulthood. Blood pressures increased with increasing body mass index (BMI) among adult males, although the mean BMI of 19·1 kg/m^2 was low compared with US values. This illustrates that BMI variation may be associated with blood pressure variation in men even at low mean values of both. Blood pressure did not vary with adult haemoglobin concentration.

1. Introduction

An age-related increase in blood pressure is so widespread in Western industrial populations that it is considered a part of the usual ageing process and considered remarkable when a few individuals escape (Finch 1990). However, data from over 36 populations in which blood pressure does not change with age illustrate that it is not an inevitable concomitant of the passage of time (James and Baker 1995). These populations also offer a natural experimental situation in which to evaluate the independent influence of other sources of variation such as body mass index (BMI). Furthermore, many of these populations reside in tropical or high-altitude environments, and their physiological adaptations may influence blood pressure variation. The purpose of this report is to present information on determinants of blood pressure variation in a sample of 263 Ethiopian rural agropastoralists 14–86 years of age and resident at 3530 m on the Simien Plateau. Mean systolic and diastolic blood pressures for males and females were low, and did not increase with age or haemoglobin concentration. Blood pressure increased with BMI among adult males.

2. Materials and methods

2.1. *Population and sample*

Data were collected in December 1995 from 314 males and females 14 years of age and older, native to, and residing around, 3530 m in the Ambaras Region of the Simien Mountains National Park, North Gondar, Ethiopia. The rural population farms barley and herds sheep, goats and cattle for sale and consumption. The sample is about 18% of the resident population in the relevant age range in the two Peasant Associations from which the study sample was drawn.

2.2. *Measurements*

An Ethiopian physician (A.G.) collected from each participant a brief health history that focused on cardiopulmonary signs and symptoms. Blood pressure was taken with a sphygmomanometer and stethoscope by an American member of the

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research team (M.D.) after the study participant had been seated for a few minutes while other measurements were obtained. Systolic and diastolic blood pressures were recorded as the appearance (phase 1) and disappearance (phase V) of sounds. Three measurements were taken and averaged for analysis. If one measurement were more than 100 mmHg different from another, then a fourth measurement was made immediately, and the outlier was discarded. This was usually the individual's first experience with blood pressure measurement. Measurements were taken in an open-air field laboratory in the presence of other villagers. Measurements of height, weight and a venepuncture blood sample for immediate determination of haemoglobin concentration were also obtained.

2.3. *Analysis*

The analysis was limited to 263 healthy, non-pregnant villagers. Forty-two people were excluded because of acute or chronic illness, and seven were excluded because of pregnancy. The sample was homogeneous for many lifestyle characteristics. All drank locally brewed barley beer daily and were non-smokers, 260 drank coffee, 252 were full-time agropastoralists, 249 had no education. Table 1A describes the morphological characteristics of the study sample. Because height, weight and BMI increased during the teens, mean values of these variables are given separately for those under 21 years of age and for adults. Reported ages were approximate: 54% gave ages that ended in zero or five. However, reported ages were consistent with life cycle stage revealed by genealogies collected from study participants. For example, only those claiming ages of 50 years or older reported adult children and grandchildren, while those claiming ages in their 20s and 30s reported only young children.

BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure was classified as normal (systolic < 130 and diastolic < 85 mmHg), high normal (systolic 130–139 and diastolic 85–89), stage 1 hypertension (systolic 140–159 and diastolic 90–99), stage 2 hypertension (systolic 160–179 and diastolic 100–109), stage 3 hypertension (systolic 180–209 and diastolic 110–119) or stage 4 hypertension (systolic ≥ 210 and diastolic ≥ 120) (Joint National Committee V 1993). When systolic and diastolic pressures fell in different categories the higher category was used. Means and standard errors are reported. Correlation, partial correlation and multiple regression analyses describe the relationship between blood pressure and other variables. A significance level of 0·05 is used.

3. Results

Mean systolic and diastolic blood pressures for males and females were 109/75 and 106/73 mmHg, respectively (Table 1B). Figure 1 demonstrates that systolic and diastolic blood pressures remained stable across adulthood in this sample ($r_{\text{systolic-age}} = -0{\cdot}08$ and $r_{\text{diastolic-age}} = -0{\cdot}16$ for men; $r_{\text{systolic-age}} = +0{\cdot}08$ and $r_{\text{diastolic-age}} = 0$ for women, all $p > 0{\cdot}05$). The mean male systolic blood pressure was significantly higher than female ($t = 2{\cdot}1$, $p < 0{\cdot}05$). There were no sex differences in mean diastolic blood pressure ($t = 1{\cdot}5$, $p > 0{\cdot}05$); 95·7% of males and 97·6% of females had normal or high normal blood pressure (table 2). Figure 2 demonstrates that adult male blood pressures increased slightly with increasing BMI ($r_{\text{systolic-BMI}} = r_{\text{diastolic-BMI}} = +0{\cdot}25$, $p < 0{\cdot}05$ for men; $r_{\text{systolic-BMI}} = +0{\cdot}07$ and $r_{\text{diastolic-BMI}} = +0{\cdot}17$ for women, $p > 0{\cdot}05$). Figure 3 demonstrates that adult blood pressures did not vary with haemoglobin concentration in this high-altitude population ($r_{\text{systolic-hb}} = +0{\cdot}15$ and $r_{\text{diastolic-hb}} = +0{\cdot}12$ for men and $r_{\text{systolic-hb}} =$

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Table 1. Characteristics of Ambaras, Ethiopia blood pressure sample.

	All males ($n = 139$), $\bar{X} \pm$ SEM	Males under 21 ($n = 23$)		Males 21+ ($n = 113$), $\bar{X} \pm$ SEM	All females ($n = 124$), $\bar{X} \pm$ SEM	Females under 21 ($n = 28$)		Females 21+ ($n = 96$) $\bar{X} \pm$ SEM
		Range	$\bar{X} \pm$ SEM			Range	$\bar{X} \pm$ SEM	
A: General								
Age (years)	38 ± 1·5	14–86	17 ± 0·4	42 ± 1·5	35 ± 1·3	14–80	17 ± 0·4	40 ± 1·3
Height (cm)†	162 ± 0·7	138–176	151 ± 1·9	164 ± 0·5	151 ± 0·5	136–165	148 ± 1·3	152 ± 0·5
Weight (kg)	49 ± 0·7	29–67	38 ± 1·7	51 ± 0·5	43 ± 0·5	30–66	39 ± 1·3	45 ± 0·6
BMI (kg/m^2)	18·6 ± 0·2	13·3–26·1	16·6 ± 0·4	19·1 ± 0·15	18·8 ± 0·2	14·0–26·4	17·6 ± 0·4	19·2 ± 0·2
Haemoglobin concentration (g/dl)†	15·9 ± 0·1	12·9–19·1	–	–	15·0 ± 0·1	12–18·4	–	–
Percentage oxygen saturation of arterial haemoglobin	95·7 ± 0·2	84–99	–	–	94·8 ± 0·2	83–99	–	–
B: Blood pressure								
Systolic (mmHg)	109 ± 1·1	79–134	–	–	106 ± 1·1	82–141	–	–
Diastolic (mmHg)	75 ± 0·8	55–101	–	–	73 ± 0·7	56–91	–	–
Pulse (f/min)	70 ± 0·9	44–104	–	–	83 ± 1·0	60–124	–	–

† Female height sample is 123 because height was not measurable in one woman. Haemoglobin concentration sample sizes (limited to iron-sufficient individuals) are 136 for males and 117 for females.

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$+0{\cdot}06$ and $r_{\text{diastolic-hb}} = +0{\cdot}14$ for women; all $p > 0{\cdot}05$). BMI did not vary with age among adults ($r = -0{\cdot}14$ for men and $r = -0{\cdot}18$ for women, $p > 0{\cdot}05$). Haemoglobin concentration decreased with age among men ($r = -0{\cdot}36$, $p < 0{\cdot}05$), but not women ($r = +0{\cdot}05$, $p > 0{\cdot}05$). Multiple-regression analyses confirmed that BMI is the only one of the three variables that explains significant variation in systolic and diastolic blood pressures (table 3). There were no significant interactions among the three variables (analyses not shown).

4. Discussion

Mean blood pressure was low, and there were no age differences in systolic or diastolic blood pressure in this cross-sectional sample of Ethiopian highlanders 14–86 years of age. Longitudinal studies will be required to determine whether blood pressure remains stable throughout adulthood in this population. These studies would yield accurate information on the passage of time (important in the absence of birth certificates verifying age), and consider possible alternative explanations to the apparent blood pressure stability. The possibility that an adult decrease in BMI accounts for the apparent adult blood pressure stability is discounted by the lack of association between adult age and BMI. The possibility that a secular trend towards higher blood pressure among young adults accounts for the apparent stability seems unlikely, in light of reported mean blood pressure of 121/80 for a sample of 22 males of unreported age studied in a nearby area during the mid-1960s (Harrison, Kuchemann, Moore, Boyce, Baju, Mourant, Godber, Glasgow, Kopec, Tills and Clegg 1969). A re-analysis of those data reported that blood pressure was negatively correlated with age, and suggested that this was due to selective elimination of blood pressures above average (Clegg, Jeffries and Harrison 1976). It is not possible to

Table 2. Blood pressure classification of Ambaras sample.

	Males		Females	
	n	Percentage	*n*	Percentage
Normal	116	83·5	115	92·0
High normal	17	12·1	7	5·6
Hypertension, stage 1	5	3·6	3	2·4
Hypertension, stage 2	1	0·7	0	0
Hypertension, stage 3	0	–	0	–
Hypertension, stage 4	0	–	0	–

Table 3. Sets of beta weights and multiple correlation coefficients of adult systolic and diastolic blood pressure.

	Multiple *R*	Age	BMI	Haemoglobin concentration
Male				
Systolic	+0·257*	−0·06	+0·20	+0·09
Diastolic	+0·286**	−0·14	+0·21	+0·02
Female				
Systolic	+0·126	+0·07	+0·10	+0·06
Diastolic	+0·234	−0·09	+0·15	+0·15

* $p = 0{\cdot}06$; ** $p < 0{\cdot}05$.

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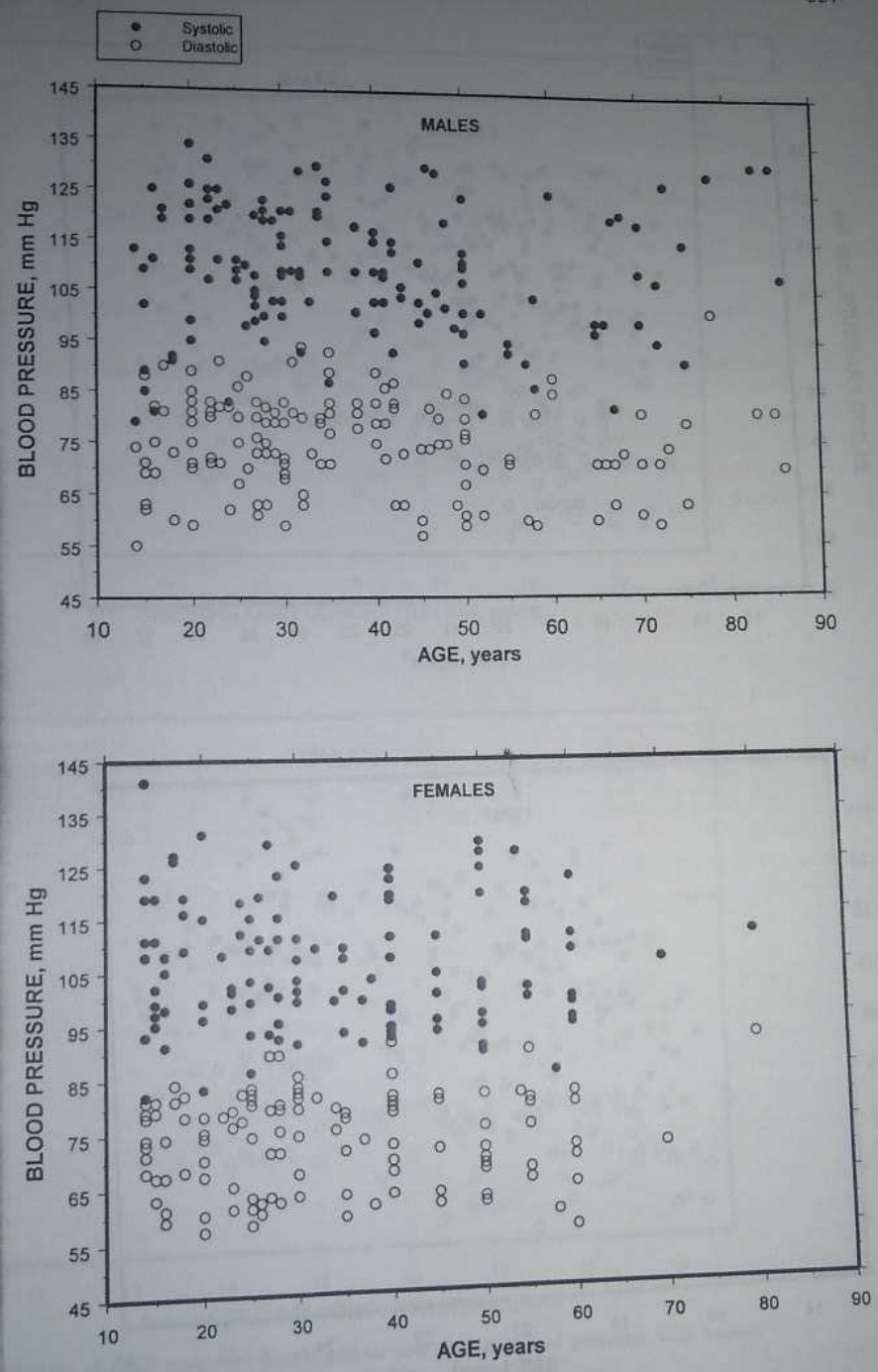
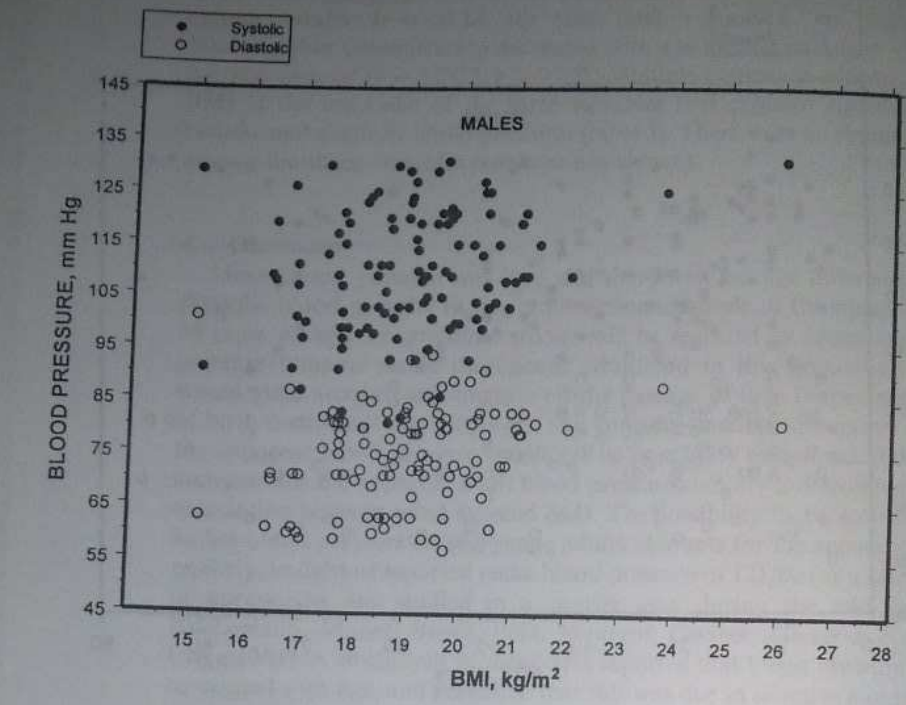


Figure 1. Scatterplot of male and female systolic and diastolic blood pressures with age.



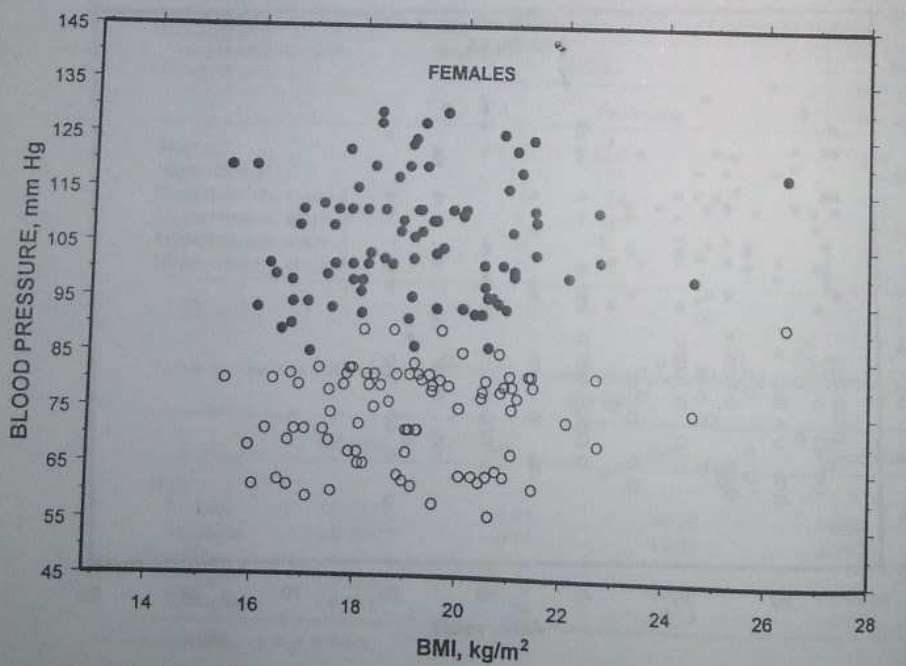
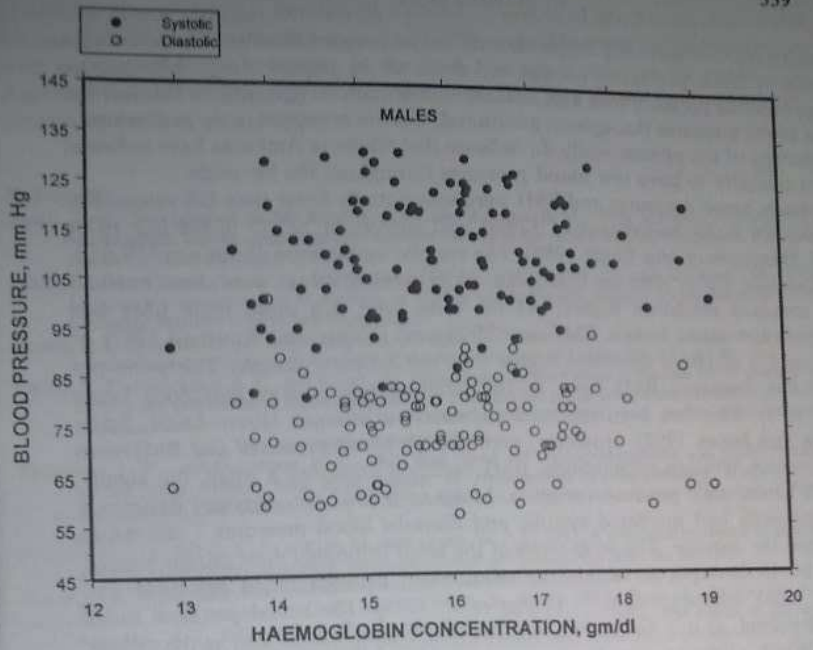


Figure 2. Scatterplot of adult male and female systolic and diastolic blood pressures with BMI.



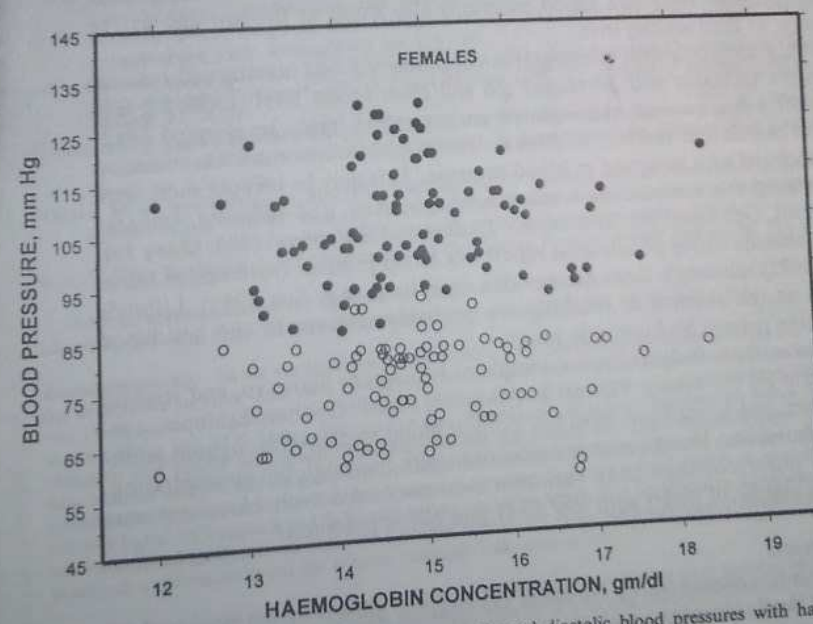


Figure 3. Scatterplot of adult male and female systolic and diastolic blood pressures with haemoglobin concentration.

determine whether there is a substantive difference between those results indicating a decrease in blood pressure with age and those of the present study indicating no change in blood pressure with age, because information on age, and on the distribution of blood pressures throughout adulthood, was not presented in the earlier study. The findings of the present study do indicate that adults in Ambaras have sufficient arterial elasticity to have low blood pressures throughout the life cycle.

Ambaras blood pressures and BMI were substantially lower than US values. For example, US males had means of 129/81 and females of 123/77 in the late 1970s (Drizd, Dannenberg and Engel 1986). The systolic means were about one US standard deviation higher than the Ethiopian, while diastolic means were about one-half a US standard deviation higher. At the same time, US mean male BMI was 25·3 kg/m^2 and mean female BMI was 25·0 kg/m^2 (Najjar and Rowland 1987) or about one US standard deviation higher than the Ambaras sample. Thirty-five per cent of the Ambaras BMI were in the adult chronic energy deficiency range (BMI < 18·5). This has been reported elsewhere in Ethiopia (Ferro-Luzzi, Sette, Franklin and James 1992). However, even though blood pressures and BMI were low relative to Western populations, BMI variation explained a small, but significant, 6% of the male pressure variation. Males with BMI two standard deviations above the mean had predicted systolic and diastolic blood pressures 7 and 5 mm higher than the average. The re-analysis of the small Ethiopian male sample studied during the 1960s reported a positive relationship between blood pressures and ponderal index (stature/weight$^{1/3}$) (Clegg *et al.* 1976). The actual ponderal index was not reported, so it is not known whether that sample was as lean as the present sample. While attention usually focuses on overweight and hypertension (e.g. Krieger and Landsberg 1995), the present Ethiopian findings suggest that the general relationship between BMI and blood pressures also exists at the low end of the normal range, at least among men.

A possible association with haemoglobin concentration was investigated because blood pressure increases with increased red cell mass at sea level (Eschbach and Davidson 1995) and because haemoglobin concentration might be elevated adaptively in this population resident at 3530 m. Haemoglobin concentration variation was not associated with variation in blood pressure. This may be because there were few high haemoglobin concentration values among healthy, iron-sufficient Ambaras residents (Beall, Gebremedhin, Brittenham, Decker and Shamebo 1996). Many studies of high-altitude native populations report low blood pressure (reviewed in James and Baker 1995), although there are notable exceptions (e.g. Sun 1986). Lifestyle factors such as the absence of smoking are probably relevant to the low blood pressures of the present high-altitude sample.

In conclusion, these findings from a sample of Ethiopians native to, and resident around, 3530 m on the Simien Plateau in Ambaras, North Gondar, Ethiopia, confirmed that men and women can have low normal blood pressure throughout adulthood. They showed no blood pressure association with haemoglobin concentration. Finally, they illustrated that BMI variation was associated with blood pressure variation in a sample of males with low BMI and blood pressures.

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References

BEALL, C. M., BRITTENHAM, G. M., MACUAGA, F., and BARRAGAN, M., 1990, Variation in hemoglobin concentration among samples of high-altitude natives in the Andes and the Himalayas. *American Journal of Human Biology*, **2**, 639–651.

BEALL, C. M., GEBREMEDHIN, A., BRITTENHAM, G. M., DECKER, M. J., and SHAMEBO, M., 1996, Hemoglobin concentration of Amhara at 3530 m on the Simien Plateau. Paper delivered at the Ethiopian Medical Society Meetings, May.

CLEGG, E. J., JEFFRIES, D. L., and HARRISON, G. A., 1976, Determinants of blood pressure at high and low altitudes in Ethiopia. *Proceedings of the Royal Society of London* (B), **194**, 63–81.

DRIZD, T., DANNENBERG, A. L., and ENGEL, A., 1986, Blood pressure levels in persons 18–74 years of age in 1976–80, and trends in blood pressure from 1960 to 1980 in the United States. Vital and Health Statistics, series 11, no. 234. DHHS Publication No. (PHS)86-1684, Public Health Service (Washington, DC: US Government Printing Office).

ESCHBACH, J. W., and DAVIDSON, R. C., 1995, Red blood cell mass/erythropoetin and blood pressure: lessons from patients with renal disease. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 390–398.

FERRO-LUZZI, A., SETTE, S., FRANKLIN, M., and JAMES, W. P. T., 1992, A simplified approach of assessing adult chronic energy deficiency. *European Journal of Clinical Nutrition*, **46**, 173–186.

FINCH, C. E., 1990, *Longevity, Senescence, and the Genome* (Chicago: University of Chicago Press.

HARRISON, G. A., KUCHEMANN, C. F., MOORE, M. A. S., BOYCE, A. J., BAJU, T., MOURANT, A. E., GODBER, M. J., GLASGOW, B. G., KOPEC, A. C., and TILLS, D., 1969, The effects of altitudinal variation in Ethiopian populations. *Philosophical Transactions of the Royal Society of London* (B), **256**, 147–182

JAMES, G. D., and BAKER, P. T., 1995, Human population biology and blood pressure: Evolutionary and ecological considerations and interpretations of population studies. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 115–126.

JOINT NATIONAL COMMITTEE V, 1993, The Fifth Report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC V), *Archives of Internal Medicine*, **153**, 154–208.

KRIEGER, D. R., and LANDSBERG, L., 1995, Obesity and hypertension. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 2367–2388.

NAJJAR, M. F., and ROWLAND, M., 1987, Anthropometric reference data and prevalence of overweight, United States, 1976–80. Vital and Health Statistics, series 11, no. 238. DHHS Pub. No. (PHS) 87–1688. Public Health Service (Washington, DC: US Government Printing Office).

SUN, S., 1986, Epidemiology of hypertension on the Tibetan Platean. *Human Biology*, **58**, 507–515.

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Zusammenfassung. In der vorliegenden Studie werden Informationen über Determinanten der Variation des Blutdrucks bei einer ländlichen Stichprobe von 263 Bewohnern des äthiopischen Hochlandes im Alter von 14–86 Jahren vorgestellt. Die Probanden leben auf dem Simien Plateau des äthiopischen Hochlandes in einer Höhe von 3530 Meter. Systolischer und diastolischer Blutdruck betrugen im Mittel 109/75 bei Männern und 106/73 bei Frauen, Altersunterschiede lagen nicht vor. Diese Beobachtungen bestätigen, dass Männer und Frauen im gesamten Erwachsenenalter geringe Blutdruckwerte aufweisen können. Bei erwachsenen Männern stieg der Blutdruck mit ansteigendem Body Mass Index (BMI) an, obwohl der BMI mit 19·1 kg/m^2 im Vergleich zu US-Werten gering war. Dies belegt, dass bei Männern Variationen im BMI mit Variationen im Blutdruck sogar bei geringen Werten für beide Merkmale assoziiert sein können. Der Blutdruck variierte nicht mit der adulten Hämoglobinkonzentration.

Résumé. On présente des informations sur les déterminants de la variation de la pression sanguine dans un échantillon rural de 263 montagnards éthiopiens âgés de 14 à 86 ans, résidant à 3530 m d'altitude sur le plateau Simien. Les pressions systolique et diastolique moyennes pour les hommes et pour les femmes,

étaient respectivement 109/75 et 106/73 et dépourvues de différences d'âge. Ces résultats confirment que ces hommes et ces femmes ont une pression sanguine normale basse tout au long de la vie adulte. Les pressions sanguines augmentent avec l'accroissement de l'indice de masse corporelle (IMC) chez les adultes masculins, bien que l'IMC moyen de 19·1 kg/m^2 soit faible comparé aux valeurs des Etats-Unis. Ceci montre que la variation d'IMC peut être associée à celle de la pression sanguine chez les hommes, même à valeur moyenne basse des deux caractéristiques. La pression sanguine ne variait pas avec la concentration en hémoglobine chez l'adulte.

Syndrome cognitif et moteur associé au VIH-1

M. Mussa, P.M. Preux, C. Debrock, M. Chazot-Balcon, Z. Melaku, M. Druet-Cabanac, K. Kayembe, M. Dumas

atteinte du système nerveux central est fréquente au cours de l'infection par le virus de l'immunodéficience humaine type 1 (VIH-1). Plus de 40 % des cas sida avérés présentent, au cours de r évolution, une symptomatologie rologique patente. Le système ner- ix central peut être le siège d'une einte directe par le virus ou d'une nologie infectieuse et/ou tumorale ésécutive à l'immunodéficience indui- (infections opportunistes) [1-3]. Les des neuropathologiques ont montré sstence de lésions du système nerveux tral dans 80 à 90 % des cas [4-6]. ncéphalopathie du VIH-1, dénommée uellement « syndrome cognitif et teur associé au VIH-1 », fait l'objet ette revue qui insistera sur les aspects iologiques et cliniques.

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s à part : M. Mussa

Historique

En 1983, Snider *et al.* ont décrit une encéphalite subaiguë observée chez des patients atteints de sida [4]. En 1986, Navia *et al.* [5, 6] ont précisé que cette encéphalite subaiguë pouvait se présenter sous forme d'un syndrome démentiel observé dans la phase tardive de l'infection par le VIH-1, ou *acquired immunodeficiency syndrom dementia complex* (ADC). Aux États-Unis [6], le complexe démentiel du sida a été considéré comme une des complications neurologiques les plus fréquentes du sida (65 %).
Depuis 1987, les patients présentant une encéphalite subaiguë liée au VIH-1 sont classés dans la catégorie sida avéré selon la définition révisée du Center for Disease Control (CDC) d'Atlanta [7]. En 1991, l'encéphalite subaiguë a été rebaptisée « syndrome moteur et cognitif associé au VIH-1 » par l'Académie américaine de neurologie [8]. La dernière révision de la définition clinique du sida par le CDC, qui a eu lieu en 1993, permet de classer les patients à l'intérieur d'une nosographie en fonction de leur statut sérologique et des manifestations cliniques. Elle situe le syndrome cognitif et moteur associé au VIH-1 dans la catégorie C *(encadrés 1* et *2)*. Dans les publications, les dénominations encéphalopathie du VIH-1, démence du sida, démence associée au VIH-1 et encéphalite subaiguë du VIH-1 sont diversement utilisées.

Épidémiologie

« L'encéphalopathie du VIH-1 est probablement devenue la première cause d'infection du système nerveux central dans l'histoire de la médecine » a déclaré Epstein à Seattle, au dernier congrès de l'Académie américaine de neurologie de mai 1995.
La probabilité de développer un syndrome cognitif et moteur associé au VIH-1 pour un sujet séropositif n'est pas encore connue. Les résultats des différentes études ne sont toujours pas comparables. En effet, certaines d'entre elles sont essentiellement cliniques alors que d'autres se sont appuyées sur l'autopsie

Encadré 1

Classification clinique de l'infection par le VIH-1 (CDC 1993)

Catégorie A : asymptomatique ou primo-infection ou lymphadénopathie persistante généralisée.
Catégorie B : symptomatique, n'ayant pas d'infection indicatrice du sida.
Catégorie C : sida.

Clinical symptoms classification of HIV-1 infections

Encadré 2

Classification sérologique de l'infection par le VIH-1 (CDC 1993)

Il existe trois catégories en fonction du nombre de lymphocytes CD4 :
– pour A1, B1 et C1,
CD4 > 500/mm³ ;
– pour A2, B2 et C2,
200 < CD4 ≤ 500/mm³ ;
– pour A3, B3 et C3,
CD4 ≤ 200/mm³.

Serological classification of HIV-1 infections

avec des corrélations cliniques *a posteriori*. Malgré toutes ces difficultés, le syndrome cognitif et moteur associé au VIH-1 est de mieux en mieux individualisé et caractérisé [8].

Cette affection intéresse les patients de tous âges et survient à n'importe quel stade de la maladie, bien qu'elle soit plus fréquente à un stade avancé de l'immunodépression [9].

La prévalence est très variable selon les types d'études, les critères diagnostiques, les techniques d'investigations employées ainsi que les particularités géographiques *(tableau 1)*.

Aux États-Unis, dans une étude clinique et neuropathologique, la prévalence de cette affection a été estimée par Snider *et al.* [4] à 35 %, en 1983, pour atteindre, en 1986, 60 à 90 % dans une étude identique réalisée par Navia *et al.* [5, 6].

En 1993, Janssen *et al.* [16] trouvaient, dans une cohorte de 144 184 patients atteints par le virus et suivis entre 1987 et 1993 par le CDC, une prévalence de 7,3 %.

Une étude effectuée par McArthur *et al.* [19] en 1993 aux États-Unis, sur une cohorte de 1 790 homosexuels ou bisexuels suivis depuis 1984, a montré une incidence annuelle du syndrome cognitif et moteur associé au VIH-1 de 7 % à partir du stade de sida.

Certains facteurs de risque de développer un syndrome cognitif et moteur associé au VIH-1 ont été identifiés. McArthur *et al.* [19] ont prouvé que l'anémie, l'âge avancé et un faible indice de masse corporelle étaient des signes prédictifs du développement rapide de l'affection après le diagnostic du sida. En revanche, les patients hémophiles infectés par le VIH-1, chez qui l'évolution de la maladie est souvent accélérée par les complications propres à l'hémophilie (hémorragie cérébrale et cirrhose post-hépatite), présentaient plus rarement des infections opportunistes ou un syndrome cognitif et moteur associé au VIH-1 [3].

En Europe, plusieurs études ont été menées. En France, Hénin *et al.* [10] ont trouvé, en 1987, une prévalence de 54,8 % tandis que, à Londres, Catalan *et al.* [14], en suivant prospectivement 200 patients, signalaient que 11 % d'entre eux avaient des troubles cognitifs mais qu'ils étaient seulement 4 % à présenter une démence liée au sida. Cependant, le chiffre qui a été avancé par Portegies *et al.* [17] aux Pays-Bas en 1993 (7,5 %) rejoint celui de Janssen *et al.* [16] aux États-Unis pendant la même période.

Sur le continent asiatique, dans la province de Kérala en Inde, Laly *et al.* [18], en 1994, trouvaient dans une étude prospective portant sur 50 patients atteints de sida une prévalence de 6 % du syndrome cognitif et moteur lié au VIH-1.

En Afrique, les rares études effectuées montrent également la même variabilité. Deux hypothèses sont susceptibles d'expliquer cette variabilité sur ce continent : les manifestations chroniques du sida tropical n'entraînent pas toujours une hospitalisation et les sujets infectés peuvent décéder d'une infection opportuniste aiguë avant de développer une symptomatologie de syndrome cognitif et moteur lié au VIH-1. En 1989, Belec *et al.* [12] ont toutefois signalé 3,3 % de syndrome démentiel en République centrafricaine tandis que, à la même période, Howlett *et al.* [11] trouvaient une prévalence de 54 % d'encéphalite subaiguë dans une population de 200 patients atteints de sida en Tanzanie, le diagnostic s'appuyant sur des critères cliniques élargis. Au Burundi en 1991, Nzisabira *et al.* [13] décrivaient 7 cas de démence associée au VIH-1 sur 15, soit 46,6 %, dans une étude clinique et neuropsychologique. Au Zaïre en 1992, dans une étude clinique et neuropsychologique d'un échantillon de 104 patients séropositifs symptomatiques suivis dans un service de médecine interne, Perriens *et al.* [15] signalaient 8,7 % de cas présentant un syndrome démentiel associé au VIH-1.

Par contre, à Abidjan en Côte d'Ivoire, Kouassi *et al.* [20] n'ont trouvé aucun cas de démence dans une série de 193 patients VIH-1 séropositifs symptomatiques suivis en hospitalisation, bien que 71 % d'entre eux aient eu des problèmes neurologiques.

Physiopathologie

Au moment de la séroconversion, le virus est déjà présent dans le liquide céphalo-rachidien et dans le système nerveux central. Il infecte les monocytes circulants après fixation sur le récepteur membranaire des CD4 [3]. Les cellules ainsi infectées pénètrent dans le système nerveux central en traversant la barrière hémato-encéphalique et contaminent secondairement les macrophages résidents du système nerveux central : les cellules microgliales, principal réservoir du VIH dans le cerveau [9].

Tableau 1

Prévalence de la démence associée au VIH-1

Auteurs (pays) [réf.]	%	Année	Effectifs (nombre)
Snider (États-Unis) [4]	36,0	1983	50
Navia (États-Unis) [5]	65,0	1986	70
Hénin (France) [10]	54,8	1987	31
Howlett (Tanzanie) [11]	54,0	1989	200
Belec (République centrafricaine) [12]	3,3	1989	93
Nzisabira (Burundi) [13]	46,6	1991	15
Catalan (Grande-Bretagne) [14]	4,0	1991	700
Perriens (République démocratique du Congo) [15]	8,7	1992	104
Janssen (États-Unis) [16]	7,3	1992	144 184
Portegies (Pays-Bas) [17]	7,5	1993	536
Laly (Inde)[18]	6,0	1994	50

HIV-1 associated dementia prevalence

Amir sultan (MD), 2014

Summary

HIV-1 associated cognitive and motor syndrome

M. Musta, P.M. Preux, C. Debrock, M. Chazot-Balcon, Z. Melaku, M. Druet-Cabanac, K. Kavembe, M. Dumas

The central nervous system (CNS) is often affected by HIV-1 infection. Over 40% of AIDS cases present with neurological symptoms and CNS lesion are detected by anatomical and pathological studies in 80 to 90% of AIDS cases. There may be infections and tumors secondary to the immunodeficiency and pathologies may occur directly due to the neurotropism of the virus. Neurological problems associated with HIV-infection include encephalopathies, myelopathies, neuropathies and myopathies.

HIV-1-induced encephalopathy may develop at any stages of HIV-1 infection and affects all risk groups equally. Its frequency worldwide is between 4 and 65% among individuals seropositive for HIV-1. The frequencies reported differ between studies due to differences in sampling methods, geographical factors, diagnostic criteria and investigative methods used. The pathogenesis of HIV-1-associated encephalopathy is not understood, but there are several hypotheses. The involvement of HIV-1 infected macrophages and microglial cells has been demonstrated. Indirect mechanisms such as release of lymphokines (tumor necrosis factor – TNFα – and interleukin-1) and neurotoxicity of the HIV envelope protein, gp 120, have also been suggested.

This disorder is known as HIV-1-associated cognitive and motor syndrome. It presents clinically as a form of sub-cortical dementia with cognitive problems, motor deficits and behavioral disorders depending on the type and stage of HIV infection. The diagnosis can only be made after all other infections and tumors common in HIV-1 patients have been ruled out by appropriate investigations such as cerebrospinal fluid analysis, cerebral scan and magnetic resonance imaging. Electrophysiological studies, such as evoked responses and electroencephalograms, are particularly useful in its diagnosis. Anatomical examination shows diffuse paleness of the white matter, multi-nucleated giant cells and microglial nodes. Neuropsychological studies could be of value in diagnosis and in assessing the response to anti-retroviral treatment. There is currently no specific therapy for HIV-1-associated cognitive and motor syndrome. The use of new nucleoside analogue drugs in combination with existing drugs may provide new approaches to managing these patients.

Cahiers Santé 1997; 7: 187-93.

Cette dissémination va entraîner une activation cellulaire avec transformation morphologique. En effet, les cellules microgliales vont fusionner et former des cellules géantes multinucléées qui sont les stigmates neuropathologiques non seulement du syndrome moteur et cognitif associé au VIH-1 mais aussi de la leucoencéphalopathie due au VIH-1 [9].

En dehors de ces anomalies histologiques, on s'est aperçu très rapidement que les neurones pouvaient être atteints. Plusieurs équipes de chercheurs ont tenté de comprendre comment l'infection des cellules microgliales du cerveau peut entraîner une atteinte de la substance blanche et une destruction des cellules neuronales provoquant cliniquement des troubles cognitifs. Cette neurotoxicité fait intervenir plusieurs facteurs. La cellule microgliale infectée sécrète des produits inflammatoires comme les cytokines (*tumor necrosis factor*-α, ou TNF-α), l'interleukine 1-β ou d'autres (tel le *platelet activating factor*, ou PAF) et la neurotoxine (acide arachidonique, peroxide...). Certaines protéines virales, comme la gp 120, pourraient aussi être délétères. Ces substances peuvent entraîner la mort neuronale par des mécanismes divers : activation d'une sous-classe de récepteur glutamatergique, le N-méthyl D-aspartate (NMDA), et irruption de calcium dans la cellule, ou déclenchement d'un programme de mort cellulaire (apoptose) [21, 22].

Neuropathologie

Macroscopiquement, une atrophie plus ou moins marquée de l'encéphale est observée lors de l'infection du système nerveux central par le VIH-1.

Microscopiquement, l'infection est définie par la présence de multiples foyers comportant des cellules microgliales, des macrophages et des cellules géantes multinucléées ou, à défaut, de grandes quantités de virus disséminés dans la substance blanche, les noyaux gris centraux et le tronc cérébral. Le cortex est plus rarement affecté, une atteinte médullaire n'est pas exceptionnelle [3, 9]. Les cellules blanches multinucléées ont les caractéristiques de la lignée des monohistiocytes/macrophages. Elles contiennent le VIH-1 dans leur cytoplasme et résultent de la fusion, sous l'influence du virus, de cellules macrophagiques/microgliales mononucléées. Leur présence témoigne à la fois d'une infection productive par le VIH-1 et d'un effet cytopathogène de ce dernier. Le syndrome cognitif et moteur associé au VIH-1 et la leucoencéphalite liée au VIH-1 représentent les deux aspects extrêmes d'un même processus. La seconde est définie par des lésions diffuses de la substance blanche comportant une perte myélinique, une gliose astrocytaire réactive, la présence de macrophages et de cellules géantes multinucléées, mais peu ou pas d'infiltrats inflammatoires. Les lésions habituellement symétriques intéressent la substance blanche hémisphérique et peuvent s'étendre au cervelet [9]. Pour Wiley *et al.* [23], la présence des cellules géantes multinucléées n'est pas un signe pathognomonique du syndrome moteur et cognitif associé au VIH-1. Dans une étude autopsique, ces auteurs ont remarqué que les cellules géantes multinucléées n'étaient observées que dans la moitié des cas. Par contre, ils ont noté chez tous les patients la présence d'une quantité abondante de virus dans le système nerveux central associée à une perte neuronale importante.

Clinique

Diagnostic

Le diagnostic clinique du syndrome cognitif et moteur associé au VIH-1 est un diagnostic d'élimination. Les infec-

tions opportunistes et les tumeurs du système nerveux central doivent être éliminées par des examens d'imagerie cérébrale (tomodensitométrie ou imagerie par résonance magnétique) et par d'autres investigations paracliniques (ponction lombaire). Les critères diagnostiques du CDC pour le syndrome cognitif et moteur associé au VIH-1 sont :

– la présence de troubles cognitifs et/ou moteurs évoluant depuis plusieurs semaines ou mois ;

– l'absence de pathologie concomitante ou de maladie autre que l'infection au VIH-1 susceptible d'expliquer les troubles cognitifs ;

– l'absence d'infection opportuniste d'après les résultats des examens d'imagerie cérébrale et de la ponction lombaire [22].

Actuellement, deux nomenclatures regroupant l'ensemble des manifestations cognitives, motrices et comportementales permettent, associées aux tests de laboratoire (neuropsychométriques), de poser le diagnostic [24]. La première, reprise par Mayer et Sevino [24], évalue la gravité des atteintes cognitives et motrices ainsi que leur répercussion sur la vie quotidienne *(encadré 3)*, en utilisant l'échelle d'évaluation des activités quotidiennes. La seconde, élaborée conjointement par l'Organisation mondiale de la santé (OMS) et l'Académie américaine de neurologie (AAN), a un objectif plus général : elle vise à standardiser le diagnostic clinique et à rendre toutes les recherches comparables *(encadré 4)*.

Sémiologie neuropsychologique

Le syndrome moteur et cognitif associé au VIH-1 se présente sous forme d'une démence sous-corticale [24].

Troubles des fonctions cognitives

Les anomalies neuropsychologiques les plus caractéristiques qui apparaissent au début de la maladie concernent les performances dans la réalisation de tâches séquentielles complexes et de tâches en temps limité, contrôlé ou impliquant l'exécution de mouvements précis et rapides. Les troubles de mémoire portent principalement sur le processus de rappel électif. Les capacités préservées sont les capacités verbales comme la dénomination d'images et les épreuves de vocabulaire. De même, on ne relève pas de trouble praxique ou gnosique. La lenteur dans la réalisation de ces épreuves semble l'élément dominant plus que les erreurs ou l'inadéquation des réponses, qui évoquent plutôt des troubles au niveau des capacités de raisonnement mais qui peuvent aussi être observés. Le concept de démence sous-corticale [24], même s'il peut être discuté, permet de souligner la différence des troubles cognitifs observés, par rapport à ceux des démences corticales comme la maladie d'Alzheimer se caractérisant par un syndrome aphaso-apraxo-agnosique.

Encadré 3

Stades d'évolution du syndrome cognitif et moteur associé au VIH-1 (d'après Mayer et Sevino [24])

– Stade 0 : normal.
– Stade 0,5 : équivoque/subclinique.
– Stade 1 : démence légère.
– Stade 2 : démence modérée.
– Stade 3 : démence sévère.
– Stade 4 : stade final.

Stages in the development of HIV-1-associated cognitive and motor syndrome

Troubles moteurs

Ils surviennent plus tardivement que les troubles des fonctions cognitives et sont constitués par des difficultés à la marche, des troubles de l'équilibre mal systématisé, une faiblesse des membres inférieurs ainsi que des signes de maladresse qui se manifestent, notamment, par une détérioration de l'écriture et, surtout, une difficulté progressive d'exécution de gestes demandant de la précision [24].

Troubles du comportement

Ils sont fréquents mais inconstants. Ils sont constitués par une apathie, une aboulie, un désintérêt à l'égard de la vie sociale et professionnelle, une tendance à l'isolement et à l'apragmatisme, une dysphorie avec labilité émotionnelle, une irritabilité et, parfois, des manifestations agressives et anxieuses. Il a été également signalé des épisodes psychotiques de type délires, hallucinations, accès maniaques et dépressions majeures [24].

En fin d'évolution, la plupart des patients présentent un dysfonctionnement cognitif global et des troubles moteurs majeurs (akinésie, trémor, myoclonie). Par ailleurs, certains patients présentent des signes d'atteinte frontale (réflexes de préhension et de succion) à l'examen neurologique. Quant à la vigilance, elle est normalement maintenue, même lors de la phase terminale.

Diagnostic différentiel

Il fait appel aux autres causes possibles de syndrome démentiel chez des sujets séropositifs pour le VIH-1.

Les formes encéphalitiques diffuses non nécrotiques de la toxoplasmose cérébrale diffuse ont été décrites chez les patients atteints par le VIH. Cependant, leur diagnostic clinique est très difficile, surtout lorsqu'il n'existe aucune lésion focale détectable à l'examen tomodensitométrique. L'institution d'un traitement antitoxoplasmique d'épreuve chez tout sidéen ayant une encéphalopathie fébrile permet d'éliminer cette hypothèse [9].

Le diagnostic de neurosyphilis repose sur la ponction lombaire systématique en cas de sérologie syphilitique positive et doit être évoqué devant la constatation d'au moins deux des anomalies suivantes : hyperprotéinorachie, hypercytorachie et réaction d'agglutination syphilitique positive dans le liquide céphalo-rachidien [9].

L'encéphalite à cytomégalovirus, pour sa part, n'est pas rare (un quart des cas dans la plupart des séries autopsiques), mais elle ne se manifeste cliniquement que de façon exceptionnelle. En cas de suspicion, il faut chercher des signes d'atteintes rétiniennes et viscérales.

La leucoencéphalopathie multifocale progressive à virus JC (polyomavirus humain) se traduit, cliniquement, par un syndrome démentiel progressif avec une association de signes déficitaires focaux (cécité corticale, aphasie, hémiparésie).

Les lymphomes cérébraux primitifs, ou lymphomes malins non hodgkiniens, s'expriment souvent sous forme d'un tableau tumoral d'évolution subaiguë avec des signes de localisation et d'hypertension intracrânienne.

Les encéphalopathies métaboliques telles que les anoxies et autres troubles métaboliques peuvent donner un tableau démentiel au stade terminal des patients atteints par le VIH. Il s'agit d'une encéphalopathie de Wernicke, qui peut survenir chez les malades ayant des carences en vitamines B, d'une encéphalopathie hépatique chez les toxicomanes ou les hémophiles ou d'une myélinolyse centropontique [9].

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Amir sultan (MD), 2014

Encadré 4

Manifestations cliniques du syndrome cognitif et moteur associé au VIH-1* (d'après Mayer et Sevino [24])

Syndrome cognitif et moteur associé au VIH-1
Complexe démentiel du sida

I – Manifestations sévères
A – Complexe démentiel associé au VIH-1 :
– encéphalite subaiguë ;
– encéphalopathie du VIH ;
– démence liée au sida.
B – Myélopathie associée au VIH-1 :
– encéphalopathie du VIH.

II – Manifestations légères
Trouble cognitivo-moteur associé au VIH-1 :
– désordre neurocognitif associé au VIH ;
– anomalies neurocomportementales associées au VIH.

* Nouvelle nomenclature proposée par l'Organisation mondiale de la santé et l'American Academy of Neurology. En gras : nouvelle nomenclature ; en italique : terminologie en usage jusqu'en 1990.

Clinical symptoms of HIV-1-associated cognitive and motor syndrome. New nomenclature suggested by the WHO and the American Academy of Neurology

Examens paracliniques

Le diagnostic formel de la démence du VIH-1 reposerait en fait sur l'examen neuropathologique, le plus souvent à l'autopsie. La démence du VIH-1 n'est pas une indication de biopsie cérébrale. Seront examinés successivement les marqueurs biologiques, l'imagerie cérébrale, l'examen neuropsychologique et l'électrophysiologie.

Marqueurs biologiques

• Lymphocytes CD4

Le nombre des CD4 circulants (normalement entre 500 et 1 500/mm³) a une valeur d'orientation *(encadré 2)*. Un nombre de CD4 effondré (inférieur ou égal à 200/mm³) devra aussi orienter vers les infections opportunistes sévères (toxoplasmose généralisée et encéphalite à cytomégalovirus) ou vers un lymphome cérébral primitif.

• Liquide céphalo-rachidien

Chez les patients séropositifs pour le VIH-1 dépistés par Elisa et confirmés par Western blot, l'examen du liquide céphalo-rachidien en cas de suspicion de syndrome cognitif et moteur associé au VIH-1 est indispensable pour tenter d'écarter les autres pathologies pouvant expliquer les signes cliniques.
Une méningite aseptique non spécifique avec hypercytose modérée (de 4 à 50 cellules à prédominance lymphocytaire) est retrouvée dans un quart des cas. Il a été signalé, dans deux tiers des cas, une hyperprotéinorachie modérée (autour de 0,60 g/l).
L'antigène p24 a été détecté dans 40 à 50 % des cas avec des immunoglobulines G (IgG) présentant un aspect oligoclonal [10]. D'autres marqueurs biologiques plus ou moins spécifiques sont également augmentés dans le liquide céphalo-rachidien des patients présentant un syndrome cognitif et moteur associé au VIH-1. Il s'agit de la β_2-microglobuline, de la néoptérine, de l'interféron-α et de certaines monokines comme le TNF-α et les interleukines 1 et 6 [25, 26]. Chez les sujets séropositifs, le rapport entre la β_2-microglobuline du liquide céphalo-rachidien et celle du sérum est normal. En revanche, une élévation intrathécale aurait une valeur prédictive pour le diagnostic du syndrome cognitif et moteur associé au VIH-1 en l'absence d'atteinte infectieuse opportuniste et néoplasique du système nerveux central. De la même façon, l'augmentation de la synthèse intrathécale et sérique de néoptérine et d'interféron-α semble être associée aux complications neurologiques dues au VIH-1 [25, 26].
L'étude virologique du liquide céphalo-rachidien n'apparaît pas non plus spécifique du diagnostic du syndrome cognitif et moteur associé au VIH-1. Une sécrétion intrathécale d'anticorps spécifiques est présente chez 80 à 90 % des sujets infectés par le VIH-1 [6].

Imagerie cérébrale

En ce qui concerne la tomodensitométrie cérébrale, les images radiologiques ne sont pas spécifiques. L'existence sur le scanner cérébral d'une atrophie cortico-sous-corticale bilatérale, marquée par un élargissement des sillons corticaux et une dilatation des ventricules, est fréquemment observée chez les sidéens avec atteinte du système nerveux central [9].
L'imagerie par résonance magnétique (IRM) est plus sensible que le scanner et représente un élément important du diagnostic. Les images évocatrices sont inconstantes, non spécifiques et peuvent être diversement associées [9]. Trois anomalies ont été souvent observées en séquence de pondération T2 :
– lésions floconneuses (atteintes focales aux contours flous) ;
– lésions punctiformes (petits foyers de 1 centimètre de diamètre) ;
– lésions diffuses (atteintes portant sur une large zone), le plus souvent.
La tomographie par émission de positons (PET scan) peut montrer, en utilisant différents traceurs, une diminution de la perfusion cérébrale globale ou prédominante dans les régions frontales chez les sujets séropositifs asymptomatiques et présentant des résultats normaux à l'IRM ou au scanner [9].
Quant à la spectroscopie de résonance magnétique (SRM), des travaux récents ont montré que le syndrome cognitif et moteur associé au VIH-1 s'accompagne d'une perturbation du métabolisme cérébral de la choline, détectée de manière non invasive par spectroscopie et localisée par résonance magnétique. Cet examen s'avérerait dès lors un outil de choix dans le diagnostic précoce de l'affection [27].

Examen neuropsychologique

L'examen neuropsychologique des patients atteints de syndrome cognitif et

moteur associé au VIH-1 peut être utile pour faire le diagnostic clinique, quantifier la gravité de l'atteinte, suivre l'évolution du syndrome et apprécier sa réponse au traitement. Il n'existe pas de tests neuropsychologiques spécifiques de cette affection. La sélection de batteries courtes mais sensibles est recommandée chez ces sujets fatigables, souvent soumis à de multiples investigations [22].

Les tests neuropsychologiques utilisés explorent assez largement les fonctions cognitives : l'attention, la mémoire, la concentration, la coordination visuo-spatiale et motrice [24]. Les tests doivent être adaptés au contexte socioculturel, à l'âge et au niveau d'instruction des sujets testés. Une baisse significative des performances à plusieurs de ces tests est en faveur d'une atteinte organique cérébrale. Il faut vérifier au préalable l'absence de troubles psychiatriques pouvant retentir sur les performances des patients : évaluation clinique de la dépression et de l'anxiété et recherche de facteurs exogènes comme l'alcoolisme ou la prise de drogues [24].

Plusieurs tests neuropsychologiques sont utilisés, pour évaluer les différentes fonctions cognitives, dans ces démences de type sous-cortical :

– le test de la fluence verbale de Benton, le Trail Making test A et B, le Stroop test et le code de Wechsler Adult Intelligent Scale (WAIS) évaluent la rapidité psychomotrice et l'attention ;

– l'empan chiffré du WAIS-R (révisé), les épreuves de rétention mnésique immédiate verbale et visuelle de Wechsler, les épreuves de rétention différée verbale et visuelle ainsi que l'épreuve de reconnaissance visuelle de la batterie 144 de Signoret apprécient la mémoire ;

– les similitudes du WAIS-R et les matrices progressives de Raven (PM42) estiment le raisonnement ;

– l'épreuve des cubes de Kohs évalue les capacités visuo-constructives [24].

Électrophysiologie

L'intérêt de l'électroencéphalogramme réside dans la précocité de la détection des anomalies diffuses lors des atteintes primaires de l'encéphale par le VIH-1 et dans la valeur des complexes lents biphasiques focaux ou disséminés comme marqueurs de l'atteinte de la substance blanche dans le syndrome cognitif et moteur associé au VIH-1 [28].

Les potentiels évoqués visuels, auditifs précoces et somesthésiques présentent des anomalies à tous les stades de l'infection au VIH-1. Les potentiels évoqués cognitifs, en particulier l'onde P300, sont généralement altérés au cours du syndrome cognitif et moteur associé au VIH-1, comme dans de nombreux autres processus démentiels sous-corticaux. Les anomalies peuvent même exister chez les patients aux stades précoces de l'infection. Des études longitudinales pourront confirmer si ces potentiels représentent un marqueur précoce de l'affection, donc un moyen de diagnostic et de surveillance des sujets atteints [29].

Traitement

Il n'existe pas de traitement spécifique du syndrome cognitif et moteur associé au VIH-1. Toutefois, les études réalisées ont montré une amélioration des fonctions cognitives aux tests neuropsychologiques, une réduction sensible de l'antigène P24 dans le liquide céphalo-rachidien des patients et une réduction significative de l'incidence de l'affection chez les patients traités précocement par la zidovudine (AZT). L'AZT est un analogue nucléosidique inhibiteur de la transcriptase inverse et traverse facilement la barrière hémato-encéphalique. L'association de l'AZT aux autres analogues nucléosidiques, comme la didanosine (DDI), semble donner de meilleurs résultats [30]. Mais avec l'apparition de phénomènes d'intolérance et de résistance à l'AZT, d'autres schémas thérapeutiques ont été envisagés en tenant compte des mécanismes physiopathologiques de l'affection, à savoir : le blocage de l'affection virale, le contrôle de la libération des substances toxiques produites par la microglie et les macrophages activés, la protection contre l'excitotoxicité, la lutte contre le stress oxydatif et le blocage des programmes cellulaires conduisant à la mort apoptotique des neurones [22]. L'apport des antiprotéases pourrait être primordial dans ce contexte. Les symptômes d'allure générale dont se plaignent les patients méritent un traitement symptomatique approprié. L'utilisation des médicaments psychotropes doit se faire avec parcimonie pour ces malades souvent très sensibles aux sédatifs, antidépresseurs et psychostimulants. La moitié de la dose usuelle est souvent prescrite. Dans certains cas, une psychothérapie d'accompagnement de type « conseil » est recommandée [22].

Conclusion

L'existence d'un syndrome cognitif et moteur associé au VIH-1 est un fait reconnu et le rôle du virus dans la survenue de cette affection est démontré [3]. Plusieurs facteurs font que les données épidémiologiques ne permettent pas de chiffrer avec précision l'incidence et la prévalence de l'affection. Le diagnostic, qui est un diagnostic d'exclusion, fait surtout appel à la clinique, à l'imagerie cérébrale et à l'examen du liquide céphalo-rachidien.

Cliniquement, l'affection, qui se présente sous la forme d'une démence sous-corticale, est déterminée par la triade troubles cognitifs, troubles moteurs et troubles du comportement qui s'aggravent avec l'évolution de la maladie [24].

Plusieurs hypothèses physiopathologiques ont été avancées. Le rôle des macrophages et des cellules microgliales infectés par le virus a été mis en évidence [9, 21]. Des mécanismes indirects ont été également évoqués, en particulier le stress excitotoxique dû à la production de cytokines pro-inflammatoires et la neurotoxicité de la gp 120. L'existence de la pâleur myélinique de la substance blanche ainsi que la présence de cellules géantes multinucléées et de cellules microgliales ont des valeurs diagnostiques plus ou moins spécifiques à l'examen neuropathologique.

Une prise en charge globale et précoce des patients symptomatiques infectés par le VIH-1 et une utilisation judicieuse et adaptée des molécules antivirales, associées éventuellement à d'autres molécules (des anticalciques par exemple), ouvrent des perspectives nouvelles dans le traitement du syndrome cognitif et moteur associé au VIH-1 [22] ■

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Références

1. Ho DD, Rota TR, Schooley RT, Allan JD, Groopman JE. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *N Engl J Med* 1985 ; 313 : 1493-7.

2. Grant I, Hampton JA, Hesselmink JR, Kennedy CJ, Richman DD, Spector SA. Evidence for early central nervous system involvement in the acquired immunodeficiency syndrome (AIDS) and other human immunodeficiency virus (HIV) infection. *Ann Intern Med* 1987 ; 107 : 828-36.

3. Belec L, Trotot P, Lescs MC, Gray F. Les lésions précoces du système nerveux central au cours de l'infection par le virus de l'immunodéficience humaine. *Médecine Science* 1992 ; 8 : 1057-64.

4. Snider WD, Simpson DM, Nielsen S, Gold JWM, Metroka CE, Posner JB. Neurological complications of acquired immune deficiency syndrome : analysis of 50 patients. *Ann Neurol* 1983 ; 14 : 403-8.

5. Navia BA, Jordan BD, Price RW. The AIDS dementia complex. I. Clinical features. *Ann Neurol* 1986 ; 16 : 517-23.

6. Navia BA, Cho ES, Petito CK, Price RW. The AIDS dementia complex. II. Neuropathology. *Ann Neurol* 1986 ; 16 : 525-36.

7. OMS. Syndrome d'immunodéficience acquise (sida) : échelle provisoire OMS proposée pour la determination des stades de l'infection et de la maladie à VIH. *Rel Epidemiol Hebd* 1990 ; 65 : 221-8.

8. Working group of the American Academy of Neurology AIDS Task Force. Nomenclature and research case definition for neurological manifestation of human immunodeficiency virus type I (HIV-I) infection. *Neurology* 1991 ; 41 : 778-85.

9. Gray F, Belec L, Geny C, Schouman Claeys E. Diagnostic des encéphalopathies diffuses de l'adulte au cours de l'infection à VIH (1re partie). *Presse Med* 1993 ; 22 : 1226-31.

10. Hénin D, Duyckaerts C, Chaunu MP, *et al.* Etude neuropathologique de 31 cas de syndrome d'immunodépression acquise. *Rev Neurol* 1987 ; 143 : 631-42.

11. Howlett WP, Nkya WM, Nmuni KA, Missalek WR. Neurological disorders in AIDS and HIV disease in the northern of Tanzania. *AIDS* 1989 ; 3 : 289-96.

12. Belec L, Testa J, Vohito MP, *et al.* Manifestations neurologiques et psychiatriques du sida en République centrafricaine. *Bull Soc Path Ex* 1989 ; 82 : 297-307.

13. Nzisabira L, Dumas M, Vaillat JM. Manifestations neurologiques associées au VIH-1 au Burundi. In : Dumas M, Giordano C, Gentilini M, Chieze F, éds. *Neurologie tropicale*. Montrouge : John Libbey Eurotext, 1993 : 133-6.

14. Catalan J, Meadows J, Singh A. Prevalence of HIV associated dementia in a central London health district in 1991. *Neurology* 1993 ; 43 (suppl.) : 27.

15. Perriens J, Mussa M, Luabeya MK, *et al.* Neurological complications of HIV 1 seropositive internal medicine in patients in Kinshasa-Zaïre. *J Acquir Immune Defic Synd* 1992 ; 5 : 333-40.

16. Janssen RS, Nwanyanwu OC, Selik R, Stehn Green JK. Epidemiology of human immunodeficiency virus encephalopathy in the United States. *Neurology* 1992 ; 42 : 1472-6.

Résumé

L'atteinte du système nerveux central est fréquente au cours de l'infection par le virus de l'immunodéficience humaine de type 1 (VIH-1). Plus de 40 % des cas de sida présentent une symptomatologie neurologique patente et les études neuroanatomiques ont révélé l'existence de lésions du système nerveux central dans 80 à 90 % des cas. Au cours de l'infection par le VIH-1, le système nerveux central peut être le siège d'une atteinte directe par le virus ou par une pathologie infectieuse et/ou tumorale consécutive à l'immunodéficience induite (infections opportunistes). L'encéphalopathie au VIH-1, dénommée actuellement « syndrome cognitif et moteur associé au VIH-1 », intéresse les patients de tous âges et est beaucoup plus fréquente au stade de l'immunodépression. Sa fréquence varie entre 4 et 65 % de la population infectée par le VIH-1.

Plusieurs hypothèses physiopathologiques ont été avancées. Le rôle des macrophages et des cellules microgliales infectés par le virus a été mis en évidence. Des mécanismes indirects ont également été évoqués, en particulier la libération de lymphokines (TNF, interleukines) et la neurotoxicité d'une glycoprotéine de l'enveloppe du VIH-1 (la glycoprotéine 120). Cliniquement, l'affection se présente sous la forme d'une démence sous-corticale déterminée par la triade troubles cognitifs, troubles moteurs et troubles du comportement. Le diagnostic clinique est un diagnostic d'exclusion. Les infections opportunistes et les tumeurs du système nerveux central doivent être éliminées par des examens d'imagerie cérébrale (tomodensitométrie, imagerie par résonance magnétique) ou par l'examen du liquide céphalo-rachidien. L'examen anatomopathologique révèle l'existence d'une pâleur myélinique de la substance blanche, la présence de cellules géantes multinucléées et de cellules microgliales.

L'examen neuropsychologique est utile pour confirmer le diagnostic, quantifier la réponse au traitement antirétroviral et évaluer l'importance des troubles cognitifs. Il n'existe pas de traitement spécifique de l'affection. L'utilisation des analogues nucléosidiques associés aux autres familles thérapeutiques existantes ouvre des perspectives nouvelles dans la prise en charge de ces patients.

17. Portegies P, Enting RH, de Gans J, *et al.* Presentation and course of AIDS dementia complex : 10 years of follow up in Amsterdam, the Netherlands. *AIDS* 1993 ; 7 : 669-75.

18. Laly J, Mabel L. Province de Kéraia. Profil clinique et sérologique des patients atteints du sida. *Sidalerte* 1995 ; 46 : 25.

19. McArthur JC, Hoover DR, Bacellar H, Miller EN, Cohen BA, Becker JT. Dementia in AIDS patients : incidence and risk factors. *Neurology* 1993 ; 43 : 2245-52.

20. Kouassi B, Giordano C, Boa Y, *et al.* Manifestations neurologiques associées à l'infection à VIH à Abidjan. In : Dumas M, Giordano C, Gentilini M, Chieze F, éds. *Neurologie tropicale*. Montrouge : John Libbey Eurotext, 1993 : 97-107.

21. Lipton SA, Gendelman HE. Dementia associated with the acquired immunodeficiency syndrome. *N Engl J Med* 1995 ; 332 : 934-40.

22. Harrisson MJG, McArthur JC. HIV-associated dementia complex. In : *AIDS and neurology*. Churchill Livingstone, 1995 : 31-64.

23. Wiley CA. Quantitative neuropathologic assessment of HIV-1 encephalitis. In : Oldstone MBA, Vitkovic L, eds. *HIV and dementia*. Berlin : Springer-Verlag, 1995 : 55-61.

24. Mayer E, Sevino O. Complexe cognitivo-moteur associé au VIH. Aspects neuropsychologiques. In : Eustache F, Agniel A, éds. *Neuropsychologie clinique des démences : évaluations et prises en charge*. Marseille : Solal, 1995 : 71-87.

25. Beyne P, Lisovoski F, Got P. La β_2-microglobuline dans le liquide céphalo-rachidien en neurologie. *Presse Med* 1995 ; 24 : 1071-4.

26. Griffin DE, McArthur JC, Cornabath DR. Neopterin and interferon gamma in serum and cerebrospinal fluid of patients with HIV associated neurologic disease. *Neurology* 1991 ; 41 : 69-74.

27. Tracey I, Carr CA, Guimaraes AR, Worth JL, Navia BA, Gonzalez RG. Brain choline-containing compounds are elevated in HIV-positive patients before the onset of AIDS dementia complex : a proton magnetic resonance spectroscopic study. *Neurology* 1996 ; 46 : 783-8.

28. Beaumanoir A, Burkland P, Gauthier G, Le Floch-Rohr J, Ochsner F, Waldvogel F. EEG dans 19 cas de sida avec atteinte de l'encéphale. *Neurophysiol Clin* 1988 ; 18 : 313-22.

29. Somma-Mauvais H, Regeis H, Gastaut JL, Gastaut JA, Farnarier G. Potentiels évoqués multimodaux dans l'infection par le virus de l'immunodéficience humaine. *Rev Neurol* 1990 ; 146 : 196-204.

30. Chretien F, Gray F, Bélec L, Levy Y. Protection of human immunodeficiency virus encephalitis by a switch from zidovudine to didanosine. *Antimicrob Agents Chemother* 1996 ; 40 : 278.

PREVENTION OF JARISCH–HERXHEIMER REACTIONS BY TREATMENT WITH ANTIBODIES AGAINST TUMOR NECROSIS FACTOR α

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ABSTRACT

Background In patients with louse-borne relapsing fever (*Borrelia recurrentis* infection), antimicrobial treatment is often followed by sudden fever, rigors, and persistent hypotension (Jarisch–Herxheimer reactions) that are associated with increases in plasma concentrations of tumor necrosis factor α (TNF-α), interleukin-6, and interleukin-8. We attempted to determine whether sheep polyclonal Fab antibody fragments against TNF-α (anti–TNF-α Fab) could suppress the Jarisch–Herxheimer reaction.

Methods We conducted a randomized, double-blind, placebo-controlled trial in 49 patients with proven louse-borne relapsing fever. Immediately before the intramuscular injection of penicillin, the patients received an intravenous infusion of either anti–TNF-α Fab or a control solution.

Results Ten of the 20 patients given anti–TNF-α Fab had Jarisch–Herxheimer reactions with rigors, as compared with 26 of the 29 control patients ($P=0.006$). The controls had significantly greater mean maximal increases in temperature (1.5 vs. 0.8°C, $P<0.001$), pulse rate (31 vs. 13 per minute, $P<0.001$), and systolic blood pressure (25 vs. 15 mm Hg, $P<0.003$), as well as higher mean peak plasma concentrations of interleukin-6 (50 vs. 17 μg per liter) and interleukin-8 (2000 vs. 205 ng per liter) ($P<0.001$ for both comparisons). Levels of TNF-α were undetectable after treatment with anti–TNF-α Fab.

Conclusions Pretreatment with sheep anti–TNF-α Fab suppresses Jarisch–Herxheimer reactions that occur after penicillin treatment for louse-borne relapsing fever, reduces the associated increases in plasma concentrations of interleukin-6 and interleukin-8, and may be useful in other forms of sepsis. (N Engl J Med 1996;335:311-5.)

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LOUSE-BORNE relapsing fever, caused by *Borrelia recurrentis* spirochetes, has been responsible for massive epidemics in North Africa, the Middle East, and Europe during this century. The epidemic at the end of World War II involved some 10 million people.[1] There is a potential for future epidemics wherever war and the movement of refugees or immigrants threaten a breakdown in public health. Since the mortality rate for untreated louse-borne relapsing fever has reached 70 percent in some epidemics,[1] antimicrobial treatment is essential. Although effective in eliminating spirochetes, this treatment precipitates a potentially life-threatening febrile inflammatory reaction in a majority of patients.[1,2] Fever plus other characteristic symptoms after chemotherapy was first described in secondary syphilis and has become known as the Jarisch–Herxheimer reaction.[3,4] This reaction has been described in a variety of bacterial infections, including brucellosis, leptospirosis, Lyme disease, and relapsing fevers. The clinical and pathophysiologic features of the reaction closely resemble those of a classic endotoxin reaction,[2] but unlike other types of acute sepsis syndrome, the Jarisch–Herxheimer reaction is predictable and less variable in its intensity. One to two hours after treatment with penicillin or tetracycline, patients with louse-borne relapsing fever become restless and apprehensive and intense rigors suddenly develop that last 10 to 30 minutes. The temperature, respiratory and pulse rates, and blood pressure all rise sharply. During the next few hours there is profuse sweating, a fall in blood pressure, and a slow decline in temperature.[1,2] The reaction is distressing to the patient and has a case fatality rate of approximately 5 percent.[1] Just before symptoms of the Jarisch–Herxheimer reaction develop there is a substantial increase in circulating levels of tumor necrosis factor α (TNF-α), interleukin-6, and interleukin-8.[5]

We have been impressed by the similarities in clinical features and pathophysiologic and cytokine disturbances between the Jarisch–Herxheimer reaction seen in louse-borne relapsing fever and severe sepsis from other causes. The sepsis syndrome, or "systemic inflammatory response syndrome,"[6] has a case fatality rate of up to 60 percent despite treatment with antimicrobial agents and supportive measures. Progression from sepsis to life-threatening shock is initiated by endotoxin released from the cell walls of gram-negative bacteria and by other viral, bacterial, protozoal, and fungal pyrogens or toxins. Studies in animals,[7-9] healthy humans,[10] and patients[11] suggest that TNF-α is the most important mediator in sepsis

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but that other cytokines, including interleukin-1β, interleukin-6, and interleukin-8, are also released. Since a variety of microbial components can stimulate macrophages to release cytokines, the systemic inflammatory response syndrome might best be treated by neutralizing the effects of the mediating cytokines with specific antibodies, soluble receptors, or natural receptor antagonists.[12] Animal models may not accurately represent disease processes in humans.[13] Clinical trials in patients with septic shock are difficult to conduct because of the heterogeneity of the patients' ages, infectious agents, and underlying disease and uncertainties about the site of infection. This diversity leads to multiple overlapping subgroups of patients unsuitable for statistical comparison.[14] Since the predictable pathophysiologic and cytokine responses of the Jarisch–Herxheimer reaction resemble models used for the systemic inflammatory response syndrome, we considered that therapeutic interventions similar to those used for the syndrome might be beneficial.

In the present study we investigated the effect of sheep Fab antibody fragments against TNF-α (anti–TNF-α Fab) on the incidence and severity of the Jarisch–Herxheimer reactions resulting from antibiotic treatment of louse-borne relapsing fever. The results may have relevance for the management of both this infection and other types of systemic inflammatory response syndrome.

METHODS

Patients

Patients with *B. recurrentis* spirochetes in their peripheral-blood film, who had been transferred to the Black Lion Hospital, Addis Ababa, Ethiopia, from other hospitals and health clinics in the city, were recruited for the study if they were between 12 and 60 years old, had no clinical evidence of other diseases, and gave informed consent for hospital admission, treatment, and investigation. The nature of the study was explained to them in their own language (usually Amharic). Those unwilling to join the study were offered the standard treatment (intramuscular penicillin) used throughout Ethiopia. Each patient's clinical history and the results of a physical examination were recorded on standard forms. Patients lay supine in bed throughout the study with intravenous cannulas in both arms.

The sample size of 20 patients given nonspecific sheep Fab antibodies and 20 given anti–TNF-α Fab was based on the results of a previous study in which 82 percent of patients had typical Jarisch–Herxheimer reactions after treatment with penicillin.[5] On the basis of the assumption that specific antibody was 50 percent effective in preventing the Jarisch–Herxheimer reaction, a sample of 40 patients would be sufficient to achieve a power of 80 percent with a type I error of 0.05 in a placebo-controlled trial.

Antibody

Lyophilized sheep polyclonal anti–TNF-α Fab and nonspecific control Fab were supplied by Therapeutic Antibodies, London. Antibodies raised in sheep by immunization with recombinant human TNF-α in Freund's adjuvant were precipitated with sodium sulfate and cleaved with papain to yield Fab and Fc. The total Fab fraction was isolated and lyophilized. Nonspecific antibodies from an unimmunized donor flock were processed similarly and used as control Fab. For each dose, the contents of four 1.5-g vials of lyophilized anti–TNF-α Fab, each containing approximately 0.25 g of specific anti–TNF-α Fab, were dissolved in 100 ml of 0.9 percent saline. In our patients, this corresponded to a dose of approximately 120 mg of total Fab per kilogram of body weight or 20 mg of specific anti–TNF-α Fab per kilogram. Four vials of control Fab were prepared by the same method, producing solutions indistinguishable in appearance from 0.9 percent saline.

Treatment

We randomly assigned patients to treatment with anti–TNF-α Fab, control Fab, or 0.9 percent saline alone in a 2:2:1 ratio, strictly in order of their admission to the hospital, using a table of random numbers without a block design. Each patient was assigned a study number referring to a numbered treatment pack that was prepared for administration by a senior laboratory technologist who played no part in the subsequent care or assessment of the patients. The saline group was added to allow assessment of the possible adverse effects of sheep Fab. Once base-line temperature had stabilized (fluctuated by less than 0.5°C within a 30-minute period), the test infusion was given over a period of 30 minutes. On completion of the infusion, 600,000 U of penicillin G procaine was administered by deep intramuscular injection into the anterior thigh and an infusion of 0.9 percent saline was continued at a rate of 1 liter every 8 hours for the next 24 hours.

Assessment

The Jarisch–Herxheimer reaction was graded as absent (no shivering), mild (shivering), moderate (intermittent rigors), or severe (severe, sustained rigors) by a clinician who was unaware of the patient's treatment. Base-line measurements of rectal temperature, blood pressure, and respiratory and pulse rates were recorded every 15 minutes for 5 hours beginning 1 hour before penicillin treatment; the measurements were then continued at 30-minute intervals for a further 4 hours. Core temperature was monitored with a rectal probe, and blood pressure was measured with an automatic sphygmomanometer.

Blood was withdrawn for spirochete and white-cell counts and cytokine assays 30 minutes before penicillin treatment and 1, 1.5, 2, 4, 8, and 24 hours afterward. White cells were counted in a chamber, and spirochete density was calculated by counting the number of spirochetes per 100 white cells and multiplying the value by the white-cell count. For the cytokine assays 10 ml of blood was collected into endotoxin-free tubes, immediately centrifuged at 100×*g* for five minutes, frozen at −20°C, and transported in dry ice to Saint Bartholomew's Hospital, London, for assays of TNF-α and interleukin-1β, interleukin-6, and interleukin-8 with commercial kits (Medgenix Diagnostics).

Statistical Analysis

The Mann–Whitney test was used to compare the maximal changes in temperature, systolic blood pressure, and respiratory and pulse rates between groups. The chi-square test with Yates' correction was used to compare the incidence of reactions and the proportion of patients whose blood films were clear of spirochetes at four and eight hours. The two-tailed t-test was used to analyze the differences in peak cytokine concentrations and white-cell counts. The severity of the reactions was compared with use of the Mann–Whitney test corrected for ties (equal values). The control group given saline and the control group given nonspecific Fab were combined for analysis because there was no significant difference between the groups in either physiologic variables or cytokine concentrations.

The study was approved by the research ethics committee of the Faculty of Medicine, Addis Ababa University.

RESULTS

During the five-week period of the study, August 5 to September 9, 1993, 51 patients were enrolled.

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Two were subsequently excluded from the analysis. One had a "spontaneous crisis" while under observation before penicillin treatment. He became feverish, and there was spontaneous clearance of spirochetes from the peripheral blood. The second patient was excluded because no spirochetes could be found in the blood smear taken immediately before penicillin treatment.

The base-line characteristics of the 20 patients treated with specific anti–TNF-α Fab and the 29 controls are shown in Table 1. There were no significant differences between groups.

TABLE 1. CHARACTERISTICS OF THE GROUPS BEFORE TREATMENT.*

CHARACTERISTIC	CONTROL GROUP (N=29)	ANTI–TNF-α FAB GROUP (N=20)
Female sex (no.)	2	1
Age (yr)	24±6	21±6
Weight (kg)	51±6	52±6
Duration of illness (days)	5±4	5±2
Temperature (°C)	39.2±1.0	39.6±0.8
Systolic blood pressure (mm Hg)	103±15	101±13
Respiratory rate (per min)	32±8	38±13
Pulse rate (per min)	105±18	113±16
White-cell count (per mm³)	3100±2700	1900±2300
Spirochete count (per mm³)	3300±3100	3500±2200

*Plus–minus values are means ±SD. There were no significant differences between the two groups at the 5 percent level by Student's t-test.

Jarisch–Herxheimer Reactions

The incidence and severity of Jarisch–Herxheimer reactions were lower in the group given anti–TNF-α Fab than in the controls. Thus, 90 percent of those receiving control infusions (26 of 29) had clinically evident reactions, as compared with 50 percent of those receiving anti–TNF-α Fab (10 of 20) ($P=0.006$ by the chi-square test). In the control group 38 percent had moderate reactions and 10 percent had severe reactions, whereas in the anti–TNF-α Fab group, only mild reactions were observed ($P=0.004$ by the Mann–Whitney test). The control patients had significantly greater mean maximal increases above base-line values in temperature (1.5 vs. 0.8°C, $P<0.001$), pulse rate (31 vs. 13 per minute, $P<0.001$), and systolic blood pressure (25 vs. 15 mm Hg, $P=0.003$) than the patients treated with anti–TNF-α Fab (Fig. 1). The temperature and pulse rate also fell faster toward normal values in patients given anti–TNF-α Fab (Fig. 1). Maximal changes in respiratory rates were not significantly different between the two groups (mean ±SD, 16±13 breaths per minute in the control group and 9±14 breaths per minute in the anti–TNF-α Fab group; $P=0.11$).

Cytokine Concentrations

In the control group, plasma TNF-α, interleukin-6, and interleukin-8 concentrations increased markedly after treatment with penicillin (Table 2). Levels of TNF-α could not be measured reliably in the patients who received anti–TNF-α Fab; however, these patients had much greater reductions in the peak concentrations of interleukin-6 and interleukin-8. There was no significant difference in the interleukin-1β response between the two groups.

Spirochete and White-Cell Counts

The length of time to the disappearance of spirochetes and the extent of the fall in the white-cell count typical of the Jarisch–Herxheimer reaction[1,2] were similar in the two groups. In the control group, the spirochetes were cleared within four hours in 14 percent of patients and within eight hours in 48 percent, as compared with 10 percent and 50 percent, respectively, in the group given anti–TNF-α Fab. The white-cell count declined by 3100±2700 and 1900±2300 cells per cubic millimeter, respectively, in the control and anti–TNF-α Fab groups.

Adverse Effects of Fab

One patient who received saline alone died with signs of widespread bronchopneumonia 19 hours after penicillin treatment. Adverse effects occurred in 7 of the 19 patients who received control Fab and in 4 of 20 given anti–TNF-α Fab. There were eight episodes of urticaria, two of dry cough, and one of bronchospasm in the group given control Fab, as compared with three episodes of urticaria and one of vomiting in the group given anti–TNF-α Fab. All these symptoms occurred while the Fab was being infused, and all responded promptly to treatment with either epinephrine (0.5 mg intramuscularly) or chlorpheniramine maleate (10 mg intravenously), or both.

DISCUSSION

Murine monoclonal antibodies directed against TNF-α have been reported to increase the mean arterial pressure in terminal septic shock[15] and to produce a dose-related attenuation of fever in cerebral malaria.[16] Recently, human–murine chimeric monoclonal antibodies directed against TNF-α have been shown to cause remissions in patients with other inflammatory conditions, such as Crohn's disease[17] and rheumatoid arthritis.[18] We used sheep polyclonal Fab antibody fragments against TNF-α.

The Jarisch–Herxheimer reaction of louse-borne relapsing fever is an interesting and predictable model of the acute inflammatory response associated with the release of large amounts of TNF-α, interleukin-6, interleukin-8, and other cytokines.[2,5] Ben-

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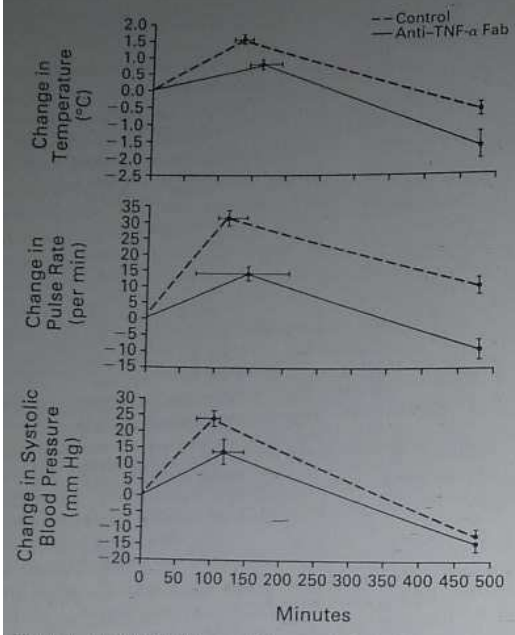


Figure 1. Mean (±SE) Maximal Changes in Body Temperature, Pulse Rate, and Systolic Blood Pressure during and after the Jarisch–Herxheimer Reaction in Control Patients and Those Treated with Anti–TNF-α Fab.

Time 0 indicates the time before penicillin was given. For the peak values, the medians (±1 quartile) are also given.

TABLE 2. PEAK PLASMA CONCENTRATIONS OF CYTOKINES DURING THE FIRST EIGHT HOURS AFTER PENICILLIN TREATMENT IN PATIENTS WITH LOUSE-BORNE RELAPSING FEVER.*

CYTOKINE	CONTROL GROUP (N=29)	ANTI–TNF-α FAB GROUP (N=20)	P VALUE
	ng/liter		
TNF-α	1764±1237	10±6†	<0.001
Interleukin-8	2000±1925	205±410	<0.001
Interleukin-6	50±44	17±17	<0.001
Interleukin-1β	15±15	9±11	Not significant

*Plus–minus values are means ±SD.

†The low or undetectable levels of TNF-α in this group were probably explained in part by the masking of epitopes by the therapeutic polyclonal anti–TNF-α Fab.

zylpenicillin attaches to penicillin-binding protein-1 in borrelia spirochetes, producing large surface blebs. The damaged spirochetes are rapidly phagocytosed by circulating polymorphonuclear leukocytes and extravascularly in the spleen.[19] The pyrogen released by borreliae causing relapsing fever has not been identified, but it does not appear to be endotoxin.[19] However, lipoproteins from two other spirochetes, *B. burgdorferi* and *Treponema pallidum*, induce the biosynthesis of tumor necrosis factor in murine macrophages.[20] Corticosteroids and antipyretic agents such as acetaminophen have little or no effect on the life-threatening cardiorespiratory complications of the Jarisch–Herxheimer reaction.[21] Meptazinol, an opioid agonist–antagonist, ameliorated some features of the reaction but did not prevent it.[22]

Treatment with anti–TNF-α Fab significantly reduced the incidence and severity of the Jarisch–Herxheimer reaction. The maximal increases in core temperature, pulse rate, and systolic blood pressure were significantly smaller in patients treated with anti–TNF-α Fab than in patients treated with saline or nonspecific Fab. Treatment with anti–TNF-α Fab profoundly reduced the peak plasma concentrations of interleukin-6 and interleukin-8, probably by blocking the biologic effects of TNF-α. Circulating levels of TNF-α were undetectable in patients who received anti–TNF-α Fab, probably because of the masking of epitopes by the therapeutic polyclonal antibody. It seemed possible that the increases in TNF-α levels during the Jarisch–Herxheimer reaction might have some protective biologic role and that the use of neutralizing antibodies might prevent the successful treatment of louse-borne relapsing fever. However, spirochetes were cleared from the circulation just as quickly in patients treated with anti–TNF-α Fab as in those given nonspecific Fab or saline.

Eleven of the 39 patients given 6 g of control Fab or anti–TNF-α Fab intravenously had mild anaphylactoid reactions. These may have been due to minimal contamination with Fc fragments or chymopapain, which are known to cause reactions.[23] In the future, chymopapain and its related enzyme will be coupled to a solid-phase support so that they can be extracted, and residual Fc fragments will be eliminated on an ion-exchange column or by prolonged digestion with papain. Another sheep polyclonal antibody (Digibind, Wellcome) has proved to be safe during many years of clinical use for digitalis-induced toxicity.

Further studies have been planned in which Fab purified by affinity chromatography will be used in an attempt to eliminate side effects and to allow measurement of the dose response. There is some evidence from studies in animals that mixtures of antibodies neutralizing various mediators involved in the systemic inflammatory response syndrome may be more beneficial than the use of antibodies against

a single initiator or mediator.[24] Because of their synergistic effects, a combination of antibodies directed against TNF-α and interleukin-1β or other cytokines may be optimal in the prevention of the Jarisch–Herxheimer reaction and perhaps in other systemic inflammatory response syndromes.[25,26]

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REFERENCES

1. Bryceson ADM, Parry EHO, Perine PL, Warrell DA, Vukotich D, Leithead CS. Louse-borne relapsing fever: a clinical and laboratory study of 62 cases in Ethiopia and a reconsideration of the literature. Q J Med 1970; 39:129-70.
2. Warrell DA, Pope HM, Parry EHO, Perine PL, Bryceson AD. Cardiorespiratory disturbances associated with infective fever in man: studies of Ethiopian louse-borne relapsing fever. Clin Sci 1970;39:123-45.
3. Jarisch A. Therapeutische Versuche bei Syphilis. Wien Med Wochenschr 1895;45:721-4.
4. Herxheimer K, Krause. Ueber eine bei Syphilitischen vorkommende Quecksilberreaktion. Dtsch Med Wochenschr 1902;28:895-7.
5. Negussie Y, Remick DG, DeForge LE, Kunkel SL, Eynon A, Gritfin GE. Detection of plasma tumor necrosis factor, interleukins 6, and 8 during the Jarisch-Herxheimer reaction of relapsing fever. J Exp Med 1992;175:1207-12.
6. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 1992; 101:1644-55.
7. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985;229:869-71.
8. Tracey KJ, Beutler B, Lowry SF, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986;234:470-4.
9. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 1987; 330:662-4.
10. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988; 318:1481-6.
11. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1987;1:355-7.
12. Christman JW. Potential treatment of sepsis syndrome with cytokine-specific agents. Chest 1992;102:613-7.
13. Abraham E, Raffin TA. Sepsis therapy trials: continued disappointment or reason for hope? JAMA 1994;271:1876-8.
14. Parrillo JE, Parker MM, Natanson C, et al. Septic shock in humans: advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Intern Med 1990;113:227-42.
15. Exley AR, Cohen J, Buurman WA, et al. Monoclonal antibody to TNF in severe septic shock. Lancet 1990;335:1275-7.
16. Kwiatkowski D, Molyneux ME, Stephens S, et al. Anti-TNF therapy inhibits fever in cerebral malaria. Q J Med 1993;86:91-8.
17. Derkx B, Taminiau J, Radema S, et al. Tumour-necrosis-factor antibody treatment in Crohn's disease. Lancet 1993;342:173-4.
18. Elliott MJ, Maini RN, Feldmann M, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. Lancet 1994;344:1105- 10.
19. Butler TC. Relapsing fever: new lessons about antibiotic action. Ann Intern Med 1985;102:397-9.
20. Radolf JD, Norgard MV, Brandt ME, Isaacs RD, Thompson PA, Beutler B. Lipoproteins of *Borrelia burgdorferi* and *Treponema pallidum* activate cachectin/tumor necrosis factor synthesis: analysis using a CAT reporter construct. J Immunol 1991;147:1968-74.
21. Butler T, Jones PK, Wallace CK. *Borrelia recurrentis* infection: single-dose antibiotic regimens and management of the Jarisch-Herxheimer reaction. J Infect Dis 1978;137:573-7.
22. Teklu B, Habte-Michael A, Warrell DA, White NJ, Wright DJ. Meptazinol diminishes the Jarisch-Herxheimer reaction of relapsing fever. Lancet 1983;1:835-9.
23. Smith MB, Hofmann VC. Anaphylactoid reaction to chymopapain. Anaesthesia 1989;44:767-9.
24. Cross AS, Opal SM, Palardy JE, Bodmer MW, Sadoff JC. The efficacy of combination immunotherapy in experimental *Pseudomonas* sepsis. J Infect Dis 1993;167:112-8.
25. Dinarello CA, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. JAMA 1993; 269:1829-35.
26. Spooner CE, Markowitz NP, Saravolatz LD. The role of tumor necrosis factor in sepsis. Clin Immunol Immunopathol 1992;62:S11-S17.

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Amir sultan (MD), 2014

3. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. N Engl J Med 1990;323:1438–44.
4. Karlsson M, Hovind-Hougen K, Svenungsson B, Stiernstedt G. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. J Clin Microbiol 1990;28:473–9.
5. Krüger WH, Pulz M. Detection of *Borrelia burgdorferi* in cerebrospinal fluid by the polymerase chain reaction. J Med Microbiol 1991;35:98–102.
6. Lebech A, Hansen K. Detection of *Borrelia burgdorferi* DNA in urine samples and cerebrospinal fluid samples from patients with early and late Lyme neuroborreliosis by polymerase chain reaction. J Clin Microbiol 1992;30:1646–53.
7. Keller TL, Halperin JJ, Whitman M. PCR detection of *Borrelia burgdorferi* DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. Neurology 1992;42:32–42.
8. Luft BJ, Steinman CR, Neimark HC, et al. Invasion of the central nervous system by *Borrelia burgdorferi* in acute disseminated infection. JAMA 1992;267:1364–7 (Erratum, JAMA 1992;268:872).
9. Amouriaux P, Asseou M, Margarita D, Baranton G, Saint Girons I. Polymerase chain reaction with the 30-kb circular plasmid of *Borrelia burgdorferi* B31 as a target for detection of the Lyme borreliosis agents in cerebrospinal fluid. Res Microbiol 1993;144:211–9.
10. Liebling MR, Nebbia MJ, Rodriguez A, Sigal LH, Jin T, Louie JS. The polymerase chain reaction for the detection of *Borrelia burgdorferi* in human body fluids. Arthritis Rheum 1993;36:665–75.
11. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid in Lyme arthritis. N Engl J Med 1994;330:229–34.
12. Centers for Disease Control. Clinical criteria for Lyme disease. MMWR 1990;39:19–21.
13. Persing DH, Rutledge BJ, Rys PN, et al. Target imbalance: disparity of *Borrelia burgdorferi* genetic material in synovial fluid from Lyme arthritis patients. J Infect Dis 1994;169:668–72.
14. Bradley JF, Johnson RC, Goodman JL. The persistence of spirochetal nucleic acids in active Lyme arthritis. Ann Intern Med 1994;120:487–9.
15. Malawista SE, Barthold SW, Persing DH. Fate of *Borrelia burgdorferi* DNA in tissues of infected mice after antibiotic treatment. J Infect Dis 1994;170:1312–6.

Pentoxifylline Fails to Prevent the Jarisch-Herxheimer Reaction or Associated Cytokine Release

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The Jarisch-Herxheimer reaction (JHR) observed after antibiotic treatment of relapsing fever caused by *Borrelia recurrentis* is associated with the systemic appearance of cytokines. The decrease of cytokine production and block of JHR was attempted by administering pentoxifylline prior to antibiotic treatment. Fifteen patients with confirmed relapsing fever were infused intravenously with pentoxifylline 90 min before intramuscular injection of penicillin; 4 patients were not treated with pentoxifylline. All patients developed JHR to varying degrees. Treatment with pentoxifylline failed to prevent fever, increase in pulse, respiration, or blood pressure, or decrease in white blood cell count. No reduction of circulating levels of tumor necrosis factor, interleukin 6, or interleukin-8 was observed with pentoxifylline treatment. Pentoxifylline did not prevent clearance of the *B. recurrentis* spirochetes. Thus, pentoxifylline treatment of patients with relapsing fever fails to prevent or diminish JHR or the associated cytokine release observed after appropriate antibiotic treatment.

The Jarisch-Herxheimer reaction (JHR) is a severe systemic reaction observed after treatment of spirochete infections, although it can be observed after treatment of other bacterial infections. The reaction consists of a rise in temperature, a decrease in white blood cell count, and a rise in pulse and respiration rates. While it occurs after treatment of many spirochete infections, the most severe reaction occurs after treatment of relapsing fever [1].

Relapsing fever is due to the bloodborne spirochete, *Borrelia recurrentis*. The spirochete is spread from host to host by ticks or lice and is endemic in eastern Africa. The disease results in recurring fevers with relapses every 3–5 days. Relapsing fever carries a high mortality rate if left untreated; however, standard antibiotic therapy induces JHR in up to 40% of treated patients [2].

We have previously reported that JHR is associated with the systemic release of cytokines [3]. Many reports have implicated the acute production of cytokines as the causative factors for the altered pathophysiology observed in several diseases. In this study, we sought to reduce the production of cytokines by pretreating patients with pentoxifylline, which has been re-

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ported to decrease cytokine production [4]. The treatment protocol tested whether pentoxifylline could reduce the production of cytokines in the face of JHR and thereby lessen its clinical symptoms.

Materials and Methods

Selection of patients and treatment protocol. Patients were identified at the Tikur Anbessa and Black Lion Hospitals, Addis Ababa University, by a history of fever, headache, and chills. A definitive diagnosis of relapsing fever was made by identification of the spirochetes in a Giemsa-stained peripheral blood smear. The duration of the symptoms, age, sex, and clinical parameters were determined by the clinical history of the patient. Of 19 patients identified, 15 were treated with pentoxifylline immediately prior to antibiotic treatment and 4 were treated with antibiotics alone. One of the pentoxifylline-treated patients was excluded from subsequent analysis because spontaneous lysis of the spirochetes occurred prior to antibiotic treatment. After intravenous pentoxifylline was given in a loading dose of 300 mg over 90 min, the relapsing fever was treated with 600,000 U of penicillin intramuscularly. This intravenous dose of pentoxifylline has been shown to decrease tumor necrosis factor (TNF) production in response to endotoxin [4]. Pentoxifylline treatment was continued by intravenous infusion for 16 h at a dose of 40 mg/h. Each patient subsequently received oral tetracycline (250 mg four times a day) for 3 days to ensure treatment success.

Data collection. Blood samples were collected at the following times: −1.5 h (prior to the start of pentoxifylline infusion), 0 h (before initiation of antibiotic treatment), and 1, 2, 3, 5, 8, and 24 h. Clinical data were collected at the same time points for blood pressure, pulse, respiration rate, and temperature. Blood was processed for white blood cell count, and a blood smear was examined to document the elimination of the spirochetes, defined as the absence of spirochetes in 50 microscopic fields. Plasma was obtained immediately and frozen for later cytokine analysis.

Cytokine analysis. Cytokines were measured as in our previous study [3]. Briefly, TNF was determined by the WEHI 164 subclone 13 biologic assay, interleukin (IL)-6 was measured by the B9 biologic assay, and IL-8 was measured with a sensitive and specific ELISA, which measures only IL-8 and not other chemokines, such as Groα, RANTES, or MIP-1.

Statistical analyses. Results between the groups were first compared by analysis of variance. Differences between the individual groups were then compared by Student's Newman-Keuls test. These analyses were done only with the pentoxifylline-treated patients, since there were too few non–pentoxifylline-treated patients for appropriate analysis.

Results

Presenting clinical signs and symptoms. The clinical data on patients presenting with relapsing fever are shown in table 1. All of the patients presented with headache and fever, and the majority also had chills. For all patients, a definitive diagnosis of relapsing fever was made by direct visualization of spirochetes in the blood smear. At the time of admission into the study, all patients were tachypnic (32 ± 2 breaths/min) and tachycardic (119 ± 4 beats/min) and had an elevated temperature (39.1 ± 0.2°C). For this analysis, both the pentoxifylline-treated and -untreated patient data were combined because the groups were similar until the infusion of pentoxifylline. Symptoms were present for 2–12 days prior to treatment.

Table 1. Clinical data for patients presenting with relapsing fe... who were subsequently treated or not treated with pentoxifylline pr... to administration of antibiotic for the infection.

	Pentoxifylline pretreatment	
	Yes	No
Male:female	13:1	4:0
Age (years)	24 (13–45)	21 (14–28)
% with symptoms		
Headache	100	100
Fever	100	100
Chills	71	100
Duration (days) of symptoms	4.4 (3–8)	7 (2–12)
No. (%) with blood smear positive for spirochetes	14 (100)	4 (100)
No. (%) with JHR	14 (100)	4 (100)
Mild	4 (29)	1 (25)
Moderate to severe	10 (71)	3 (75)
Time (h) to onset of JHR	1.6 (1.33–2)	1.5 (1.17–2)
Peak temperature (°C)	40.7 ± 0.2	40.8 ± 0.4
Peak pulse (beats/min)	137 ± 6	152 ± 4
Peak respiratory rate	44 ± 3	44 ± 3
Time (h) to clearance of spirochetes after antibiotics	4.6 (3–8)	6.0 (5–8)

NOTE. JHR, Jarisch-Herxheimer reaction. Data are mean ± SE or mean (range) unless indicated as %. There was no difference between pentoxifylline and no pentoxifylline groups for any parameter.

Development of JHR. Fourteen of the study patients were given intravenous pentoxifylline prior to treatment with intramuscular penicillin; an additional 4 patients were treated with penicillin only. All of the patients in this study developed JHR, and the prior administration of pentoxifylline did not decrease the incidence or severity of the reaction. The assessment of severity of the reaction was based on the clinical observations of vomiting, loss of continence, and development of chills and rigor. Severity was classified as moderate to severe in 71% of the patients treated with pentoxifylline and 75% of patients not given pentoxifylline. JHR was defined quantitatively (table 1). A classic feature of JHR is pyrexia, which develops even in the face of elevated temperature due to the underlying disease; the data in table 1 show that all of the patients had a rapid increase in temperature that was not inhibited by pentoxifylline.

After antibiotic treatment of relapsing fever, temperatures returned to normal within 24 h in all patients (36.7 ± 0.1°C). Both pulse and respiration rates increased significantly after antibiotic treatment and were unaffected by prior infusion of pentoxifylline (table 1). All of these clinical parameters during the acute phase of the disease (−1.5 to 8 h) were significantly

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elevated above those observed after successful treatment of the relapsing fever (24 h). The mean arterial blood pressure also increases during JHR as the white blood cell count falls. We observed both of these changes in our patients within 1–2 h after antibiotic treatment; again, pentoxifylline failed to prevent these parameters from changing (data not shown). While pentoxifylline treatment did not prevent JHR, it also did not interfere with clearance of the spirochetes, since the blood smears showed clearance of spirochetes within 8 h of antibiotic treatment (table 1).

Alterations in cytokine levels. We first reported during the 1989 season of relapsing fever, in the same clinical setting as the current study, a dramatic increase in the appearance of cytokine levels as JHR evolves [3]. The appearance of TNF, IL-6, and IL-8 over time was measured in the previous study. In our present study, all of the patients developed JHR and all had an increase in circulating cytokine levels. To directly compare the appearance of the cytokines in patients pretreated with pentoxifylline with those not pretreated, plasma cytokine profiles from the 1989 relapsing fever season are also presented [3]. Data for each patient are expressed as a percent of that patient's maximal response in order to normalize the data. Figure 1 shows that there was no significant inhibition of any of these cytokine levels by pentoxifylline. In fact, the curves are nearly superimposable with regard to the kinetics and magnitude of the cytokine response. As in many other studies, TNF is the first cytokine to appear, with IL-6 and IL-8 lagging slightly behind. TNF was rapidly cleared from the blood and by 5 h had nearly returned to baseline levels. It should be noted that the TNF in these patients was measured with a bioassay; therefore, the TNF in the bloodstream represents biologically active TNF and not inactive TNF complexed with soluble receptors. By 24 h, TNF, IL-6, and IL-8 were detectable in only 3 of the 18 patients; for all three cytokines, the levels were significantly less than peak levels.

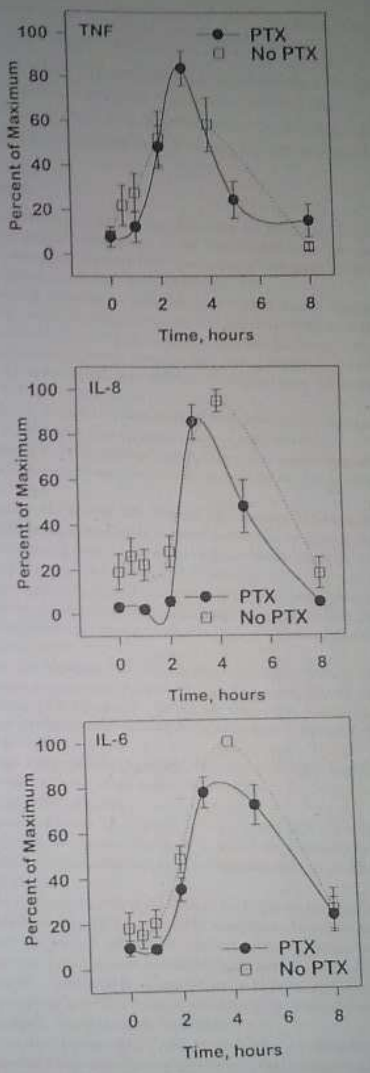


Figure 1. Increases in circulating cytokine levels during Jarisch-Herxheimer reaction. Circulating cytokine levels (interleukin [IL]-6 and -8 and tumor necrosis factor [TNF]) were measured and expressed as % of each patient's maximum value. Pentoxifylline (PTX) levels are from this study, while no PTX levels were determined during previous year. All 3 cytokines showed dramatic increase that was not blunted by pentoxifylline. Each value is mean ± SE for 13 to 14 patients in pentoxifylline group and 15 in no pentoxifylline group.

Discussion

Cytokines have been implicated in the pathogenesis of a wide spectrum of diseases, including sepsis, autoimmune diseases, and transplant rejection. It has been suggested that inhibition of the production of cytokines may prove a useful therapeutic modality for the treatment of these diseases. Many experimental approaches have been tried to inhibit either the production or action of cytokines and thereby improve outcome. These treatment options may work very well during in vitro testing conditions or in experimental animal models of disease but then fail to show efficacy in clinical trials. There remains a critical need to better understand the basic mediators of disease and to investigate the efficacy of drugs proposed as new therapeutic agents.

Pentoxifylline has been used extensively in animal studies, in which contradictory results are often observed. Using the lipopolysaccharide (LPS) model of sepsis, pentoxifylline has

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been reported to decrease TNF production and improve survival [5]; however, in another study, TNF production was reduced but there was no improvement in survival [6]. Conflicting results have also been reported in pig models of peritonitis: In one study, pentoxifylline was reported to have beneficial effects [7]; another study reported limited beneficial effects and specifically documented that pentoxifylline did not reduce TNF levels [8]. Pentoxifylline failed to prevent microvascular injury in a rat model of peritonitis [9].

Despite these negative or conflicting results in experimental animals, pentoxifylline has been proposed as a treatment for several diseases because it has been effective in reducing TNF production in LPS-stimulated cells while increasing IL-6 levels [10]. This differential inhibition of cytokine production is also observed when pentoxifylline is ingested orally and the isolated mononuclear cells are stimulated with LPS [11]. Inhibition of TNF was noted when malaria antigens were used to stimulate human or murine cells [12].

It has been reported that there was no reduction in the side effects of OKT3 therapy when patients were given pentoxifylline [13]. In another study using endotoxin-infused healthy volunteers as a model for sepsis, pentoxifylline reduced the appearance of TNF in the serum but did not reduce IL-6 or cortisol levels [4]. Additionally, none of the clinical responses to endotoxin were blocked. Another study using oral pentoxifylline prior to infusion of endotoxin failed to show any reduction in fever or plasma cytokine levels [14]. Our results are very similar to these studies [4, 13, 14] in several respects.

First, pentoxifylline did not prevent any of the clinical manifestations of JHR from appearing. Second, treatment of the underlying disease (transplant rejection or relapsing fever) was not affected by pentoxifylline. Third, we did not observe any reduction in IL-6 levels with pentoxifylline treatment. However, we also did not detect any reduction in TNF production after treatment of relapsing fever, while TNF was reduced in some of the previous studies [4]. The stimulus for the cytokine release in JHR is not known, but it is clearly related to rapid spirochete removal from blood, resulting in overwhelming stimulation of the reticuloendothelial system. It is possible that pentoxifylline will not block stimulation of cells other than monocytes [15] or that different stimuli are not affected by pentoxifylline.

These conflicting human and animal studies highlight the need for continued investigations. Future studies must determine if there is a reduction in the clinical parameters of the disease, if there are any alterations in the mediators felt to be responsible for the disease, and if treatment of the underlying disease has still been successful. Our data clearly indicate that pentoxifylline will not prevent acute physiologic changes associated with spontaneous release of cytokines. The use of JH of relapsing fever as a human model of cytokine release gi a highly reproducible model of terms of severity and kineti

References

1. Warrell DA, Perine PL, Krause DW, Bing DH, MacDougal SJ. Pathophysiology and immunology of the Jarisch-Herxheimer–like reaction louse-borne relapsing fever: comparison of tetracycline and slow-rele penicillin. J Infect Dis 1983; 147:898–909.
2. Zein ZA. Louse borne relapsing fever (LBRF): mortality and frequen of Jarisch-Herxheimer reaction. J R Soc Health 1987; 107:146–7.
3. Negussie Y, Remick DG, DeForge LE, Kunkel SL, Eynon A, Griffin G Detection of plasma tumor necrosis factor, interleukins 6 and 8 durin the Jarisch-Herxheimer reaction of relapsing fever. J Exp Med 199 175:1207–12.
4. Zabel P, Wolter DT, Schonharting MM, Schade UF. Oxpentifylline endotoxaemia [see comments]. Lancet 1989; 2:1474–7.
5. Schade UF. Pentoxifylline increases survival in murine endotoxin shoc and decreases formation of tumor necrosis factor. Circ Shock 1990; 31 171–81.
6. Lechner AJ, Roubens LR, Potthoff LH, Treadway TL, Matuschak GM Effects of pentoxifylline on tumor necrosis factor production and sur vival during lethal *E. coli* sepsis vs. disseminated candidiasis with fungal septic shock. Circ Shock 1993; 39:306–15.
7. Tighe D, Moss R, Hynd J, et al. Pretreatment with pentoxifylline improves the hemodynamic and histologic changes and decreases neutrophil adhe siveness in a pig fecal peritonitis model [see comments]. Crit Care Med 1990; 18:184–9.
8. Law WR, Nadkarni VM, Fletcher MA, et al. Pentoxifylline treatment of sepsis in conscious Yucatan minipigs. Circ Shock 1992; 37:291–300.
9. Flamm and FJ, Sibbald WJ, Girotti MJ, Martin CM. Pentoxifylline does not prevent microvascular injury in normotensive, septic rats. Crit Care Med 1995; 23:119–24.
10. Waage A, Sorensen M, Stordal B. Differential effect of oxpentifylline on tumour necrosis factor and interleukin-6 production [letter, comment]. Lancet 1990; 335:543.
11. Neuner P, Klosner G, Schauer E, et al. Pentoxifylline in vivo down-regulates the release of IL-1 beta, IL-6, IL-8 and tumour necrosis factor-alpha by human peripheral blood mononuclear cells. Immunology 1994; 83:262–7.
12. Prada J, Prager C, Neifer S, Bienzle U, Kremsner PG. Production of interleukin-6 by human and murine mononuclear leukocytes stimulated with Plasmodium antigens is enhanced by pentoxifylline, and tumor necrosis factor secretion is reduced. Infect Immun 1993; 61:2737–40.
13. Alegre ML, Gastaldello K, Abramowicz D, et al. Evidence that pentoxifylline reduces anti-CD3 monoclonal antibody–induced cytokine release syndrome. Transplantation 1991; 52:674–9.
14. Martich GD, Danner RL, Ceska M, Suffredini AF. Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. J Exp Med 1991; 173:1021–4.
15. Schandene L, Vandenbussche P, Crusiaux A, et al. Differential effects of pentoxifylline on the production of tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) by monocytes and T cells. Immunology 1992; 76:30–4.

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Q J Med 1997; 90:213–221

The effect of antibody against TNFα on cytokine response in Jarisch-Herxheimer reactions of louse-borne relapsing fever

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Summary

Severe Jarisch Herxheimer reaction (J-HR) precipitated by antibiotic treatment of louse-borne relapsing fever (LBRF) is associated with a transient, marked rise in circulating tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6) and interleukin 8 (IL-8). Ovine polyclonal anti-TNFα antibody fragments (Fab) were used in a randomized double blind placebo controlled trial in an attempt to prevent this reaction. Within 4 h after penicillin, in controls ($n=29$), a several-fold rise in cytokines occurred, concomitant with a fall in spirochaetes and maximal clinical manifestations of the J-HR. An intravenous infusion of anti-TNFα Fab, 30 min before penicillin in 20 patients reduced peak plasma levels of IL-6 and IL-8 (but not IL-1β) compared with controls ($p=0.01$ and <0.001, respectively) and the incidence of the J-HR, indicating some neutralization of TNFα. An apparent fall in TNFα reflected interference of anti-TNFα in the immunoassay.

Introduction

The systemic inflammatory response syndrome (SIRS)[1] can be initiated by a number of viral, bacterial, protozoal and fungal toxins, including lipopolysaccharides released from the cell walls of Gram-negative bacteria. Studies in animals,[2,3,4] human volunteers[5] and patients[6] suggest that TNFα is the most important mediator in this syndrome, but that other cytokines, including IL-1β, IL-6 and IL-8, are also involved. Despite treatment with antimicrobials and supportive measures, SIRS continues to have a mortality of up to 60%, and it has been suggested that it might best be treated by neutralizing one or more of the mediating cytokines with, for example, specific antibodies.[7] The effectiveness of this approach remains unproven, because animal models may be inappropriate,[8] while in clinical studies the great diversity of SIRS leads to multiple overlapping subgroups of patients unsuitable for statistical comparison.[9]

We believe that an ethically acceptable and predictable model of SIRS is required to enable the accurate assessment of the potential role of antibodies directed against a single or multiple[10] cytokines. The Jarisch-Herxheimer reaction (J-HR) which usually follows antibiotic treatment of louse-borne relapsing fever (LBRF) meets some of the requirements of such a model. Clinical manifestations of the reaction are predictable in their timing and nature and closely resemble those of a classical endotoxin reaction.[11] Furthermore, Negussie and his colleagues showed that the J-HR which followed penicillin therapy and the resultant clearance of

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spirochaetes from the circulation was associated with a several-fold rise in circulating levels of TNFα, IL-6 and IL-8.[12]

The present double-blind, placebo-controlled study was designed to determine whether ovine antibody fragments (Fab) directed against TNFα would suppress circulating cytokine levels and hence the J-HR—since this might be relevant to the treatment of SIRS and other clinical conditions in which high levels of circulating TNFα have been found. This paper relates primarily to plasma cytokine levels, and for reasons of brevity the clinical findings are reported in detail elsewhere.[13]

Methods

Patients

A total of 49 patients, (46 males and 3 females), were recruited from hospitals and health clinics in Addis Ababa. They were between 12 and 60 years old with no clinical evidence of other diseases. All gave informed consent. The diagnosis of LBRF was based on clinical suspicion confirmed by finding *Borrelia recurrentis* spirochaetes in their peripheral blood film stained with Wright's stain. Clinical history and examination were recorded on standard proformas, and patients lay supine in bed throughout the study with an intravenous cannula inserted into both arms for blood sampling and drug administration.

Antibody

Sheep were immunized with recombinant human TNFα in Freund's adjuvant and the antibodies were precipitated with sodium sulphate and cleaved with papain to give Fab and Fc. These were supplied by Therapeutic Antibodies Inc. as lyophilized ovine polyclonal anti-TNFα Fab. Non-specific antibodies from a donor flock were similarly processed and supplied as ovine control Fab. The infusions were prepared by someone not involved in the care or assessment of the patients, who dissolved the contents of 4 × 1.5 g vials of lyophilized anti-TNFα or control Fab in 100 ml of 0.9% saline.

Protocol and clinical measures

Patients were randomized for treatment with anti-TNFα Fab (20 patients), control Fab (19 patients) or 0.9% saline alone (10 patients). After stabilization of core temperature (i.e. $\pm 0.5\,^{\circ}C$ for 30 min), the test infusion was given over a 30-min period. A curative dose of procaine penicillin (600 000 units) was then administered by deep intramuscular injection into the anterior thigh, and an infusion of 0.9% saline was continued at a rate of 1 L per 8 h for 24 h. Blood samples (10 ml) were taken into sterile endotoxin-free lithium heparin tubes immediately before starting the first infusion (−0.5 h), at the time of the penicillin injection (0 h) and after a further 1, 1.5, 2, 4, 8 and 24 h. The samples were separated by centrifugation immediately and the plasma was kept at $-20\,^{\circ}C$ until assayed at St Bartholomew's Hospital for TNFα, IL-1β, IL-6 and IL-8. Commercial kits for IFNγ and the two soluble TNFα receptors, sTNF-R1 (p55) and sTNF-R2 (p75) were generously donated, which enabled samples from nine of the patients (six from the control and three from the treated groups) to be assayed. Thin blood smears were prepared at the same time intervals on glass microscope slides, stained using Wright's stain and examined by light microscopy by an independent observer for the presence and number of *B. recurrentis*. Baseline measurements of rectal temperature (using a rectal probe), of blood pressure (by an automatic sphygmomanometer), and of the respiratory and pulse rates were recorded at frequent intervals throughout. The clinical severity of the J-HR was graded as none (no shivering); mild (shivering); moderate (intermittent rigors) or severe (severe sustained rigors) by a clinician who, like the rest of the clinical team, was blinded to the patient's treatment.

Cytokine assays

These were all performed by one person (REC) using commercial immunoassay kits (Medgenix, Belgium) according to the manufacturer's instructions. Each kit is based on several monoclonal antibodies directed against different epitopes on the macromolecule to be measured, as a means of reducing hyperspecificity and incubation times and of increasing sensitivity.

Immunoradiometric Assay (IRMA) for TNFα

Standards or samples (200µl) were added to plastic tubes coated with several monoclonal antibodies directed against different epitopes on TNFα (the capture antibodies), followed immediately by monoclonal antibodies labelled with ^{125}I (the signal antibodies). Standards are provided in human serum with levels between 0–5000 ng/l, and one ng of the Medgenix standard is equivalent to 32 U of the International Reference Standard (NIBSC 87/650). After 16 to 24 h at room temperature, the contents of the tubes were aspirated (or decanted) to waste and the tubes were washed carefully and counted using a Hydrogramma 16 (Innotron). The assay measures total TNFα (i.e. free TNFα as well as that bound to natural soluble receptors).[14] The assay has a minimum detectable level (MDL) of 5 ng/l and serum or plasma TNFα levels in normal subjects

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range from less than 5 to about 20 ng/l with a mean of 6.3 ng/l.

IRMA for IL-1β

This assay is virtually identical to the TNFα assay. Standards range from 0 to 5000 ng/l and each ng of standard is equivalent to 200 U of an International Reference Preparation (NIBSC 86/552). The MDL is 5 ng/l and values are usually undetectable in plasma or serum from normal subjects but may, on occasion, be as high as 15 ng/l.

IRMA for IL-6

This assay is similar to the above except that it was done in two steps to help avoid a high-dose hook effect. Samples and standards (200 µl) were incubated in the antibody-coated tubes for 16–20 h and then aspirated (or decanted) to waste before addition of 200 µl ^{125}I-labelled monoclonal antibodies. Standards range between 0 and 5000 ng/l and 1 ng is equivalent to 3.5 U of a Reference Preparation (NIBSC 88/514). Levels of IL-6 in 157 normal plasma samples were below the levels of detection (6 ng/l) in 39 and ranged from 6 to 31 ng/l in the remainder.

IRMA for IFNγ

This assay is based on the same principle as the TNFα assay. Standards range from 0 to 90 IU/ml and one IU/ml of the Medgenix standard is stated by the manufacturer to be equivalent to 1 IU/ml NIH Gg 23-901-530 standard. The assay has an MDL of 0.2 IU/ml and a mean value of 0.55 ± 0.14 has been reported in 20 normal controls.

Immunoenzymometric Assay (IEMA) for IL-8

The general format differed in that the capture monoclonal antibodies were used to coat the walls of microtitre plates while the signal antibodies were labelled with horseradish peroxidase. The resultant assay was very sensitive, with a MDL of 0.7 ng/l, and used standards ranging between 0 and 750 ng/l, where 1 ng of standard is equivalent to 0.14 U of the International Reference Standard (NIBSC 89/520). Normal values range from <8 to 47 ng/l.

IEMA for sTNF-R1

This has the same format as the IL-8 assay. Standards were provided in bovine serum with concentrations between 0 and 77 µg/l. sTNF-R2 and TNFα, at concentrations of 1500 µg/l and 400 µg/l, respectively, did not cross-react with sTNF-R1 in the assay. The MDL was 50 ng/l, and levels in 129 normal sera ranged from 0.3 µg/l to 2.9 µg/l with a mean of 1.2 µg/l.

IEMA for sTNF-R2

This assay is virtually the same as that for sTNF-R1 with sTNF-R2 standards (in bovine serum) ranging between 0 and 142 µg/l. There was no interference in the assay due to sTNF-R1 and TNF, at 1500 µg/l and 400 µg/l, respectively. The MDL was 100 ng/l and serum levels in 117 normal subjects ranged from 1.91 to 8.51 µg/l with a mean of 4.17 µg/l.

Statistical analysis

The Mann–Whitney U test was used to analyse the differences in admission and peak cytokine concentrations. For comparison with the data obtained in the group receiving anti-TNFα Fab, the saline control and non-specific Fab groups were combined for analysis, as there was no significant differences between their clinical findings and cytokine levels.

Ethical approval

Ethical approval for the study was given by the Research Ethics Committee, Faculty of Medicine, Addis Ababa University.

Results

There were no significant differences in either the clinical or plasma cytokine findings for patients receiving saline or control Fab. The two have, therefore, been combined to provide what will be referred to as the control group (comprising 29 patients). The remaining 20 patients who received ovine anti-TNFα Fab are referred to as the test group.

Clinical findings

In this paper, for simplicity, the patients are only graded according to the severity of their J-HR as defined earlier. Of the 29 controls, three had no reaction, while 12, 11 and three experienced mild, moderate and severe J-HRs, respectively. In contrast, 10 of the test group had no reaction and the other 10 only mild J-HR. Both the incidence and severity of the J-HR in the two groups differed significantly ($p<0.01$ and <0.005, respectively) with the Fab-receiving patients experiencing less severe reactions. Systolic blood pressure, pulse rate and temperature increased less in the test group compared with the control group.[13]

Basal cytokine levels

All 49 patients had cytokine assays performed on at least one basal plasma sample collected before treatment began. There was no significant difference in these basal cytokine levels between the control

Table 1 Basal plasma cytokine levels in patients with LBRF

	TNFα (ng/l)	sTNF-R1 (μg/l)	sTNF-R2 (μg/l)	IL-1β (ng/l)	IL-6 (ng/l)	IL-8 (ng/l)	IFNγ (IU/ml)
Number of samples	76	18	16	98	98	98	18
Median	200	17.5	250	ND	2000	37	0.4
(10th–90th percentile)	(100–580)	(8–50)	(120–250)	(0–10)	(900–6400)	(9–350)	(0.2–0.8)
Quoted upper limit of kit normal range	20	2.9	8.5	15	31	47	0–8
Samples with median above upper limit (%)	100%	100%	100%	6%	100%	51%	0%

Basal levels of TNFα and IL-6 were markedly above the quoted upper limit of normal; there was no significant difference between basal cytokine levels in the control and test group; 50% of IL-8 levels were raised and IL-1β levels were mostly unaffected. Nine patients had IFNγ and soluble-receptor assays performed on two basal samples; median admission levels for s-TNF-R1 and sTNF-R2 were 6 and 29 times the upper limit of normal, respectively, and IFNγ was normal.

and test groups. The results are summarized in Table 1. Every sample tested had TNFα and IL-6 levels markedly above the upper limit of the normal range, with median values more than 10 and 60 times higher, respectively. IL-8 levels were elevated in about 50% of samples while baseline values of IL-1β appeared unaffected. In general, basal admission levels remained stable or fluctuated only slightly between −0.5 and 0 h.

Nine of the patients also had IFNγ and soluble-receptor assays performed on two basal samples. The median of admission levels for IFNγ was normal and those for sTNF-R1 and sTNF-R2 were markedly raised, with values 6 and more than 29 times the upper limit of normal, respectively (Table 1).

Cytokine profiles during the J-HR

Control patients

Figure 1 shows a typical response in a patient who received a saline infusion before penicillin and developed a severe J-HR. Almost immediately after penicillin treatment, the spirochaete count started to fall and by 8 h spirochaetes were undetectable in the peripheral blood film (in the other patients, spirochaete clearance was unaffected by the test Fab and was the same in both groups[13]). After a delay of about 1 h a rise in TNFα and IL-6 levels was detected, followed by IL-8 (at 1.5 h) and IL-1β between 2 and 3 h. The increase in plasma cytokine levels coincided with or preceded clinical signs of J-HR, but peaked after the rigors had subsided. In this patient, IL-1β and IL-8 levels had returned to baseline by 24 h, and TNFα and IL-6 were lower.

The median plasma levels and percentiles (10th–90th) for TNFα, IL-1β, IL-6 and IL-8 over time for the 29 control patients are listed in Table 2. IL-1β was undetected in 8/29 patients throughout the study period, and levels were much lower and restricted to a narrower range than the other cytokines. Data on the exact time that cytokine levels started to rise are not accurate, because insufficient blood samples were collected at the appropriate times. Plasma levels were raised significantly above baseline for TNFα ($p<0.0001$) and IL-6 ($p=0.0001$) by 1.5 h, for IL-8 by 2 h ($p=0.0003$) and for IL-1β at 4 h ($p=0.0002$). Median levels for all four cytokines continued to rise until a peak concentration was reached at 4 h, after which levels started to fall and were below admission levels at 24 h. TNFα, IL-6 and IL-8 were detected throughout the study period (−0.5 to 24 h) in every patient, including two who did not have clinical evidence of a J-HR. There was no obvious relationship (although the relative sample size was small) between plasma peak cytokine levels and the severity of the J-HR as shown for each patient in the control group (Figure 2).

There were additional assays in six controls. IFNγ plasma concentration levels remained normal throughout the study period. sTNF-R1 and sTNF-R2 levels were markedly elevated throughout, and no obvious rise nor fall in levels, was apparent.

Control group (n=29) vs. test group (n=20)

The typical rise to a peak and then fall of circulating cytokine levels seen in the control group (Figure 3a) was not detected in the test group (Figure 3b). Thus, 'measured' plasma levels of TNFα fell dramatically during the 30-min infusion of anti-TNFα from a median baseline level at −0.5 of 225 ng/l to 3 ng/l at 0 h and remained low throughout the study period. There was no significant change in the median circulating cytokine levels from baseline for IL-8 and IL-1β throughout the study period, but IL-6 levels rose from a median baseline of 2.8 μg/l to 6.5 μg/l ($p=0.001$) at 2 h. Cytokine levels for this group are listed in Table 2. Median peak levels occurred at the same time (4 h) as in the control group, but were significantly lower for TNFα, IL-6 and IL-8, and are

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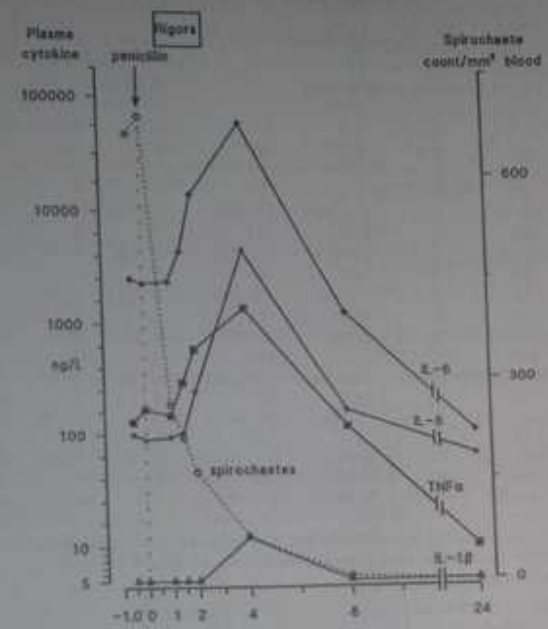


Figure 1. Typical response in a patient receiving a control saline infusion 30 min before penicillin. After penicillin, spirochaetes fell abruptly, and after about 1 h circulating levels of TNFα, IL-6, IL-8, and IL-1β started to rise, and peaked at 4 h. This patient experienced sustained rigors which occurred as cytokine levels were increasing and subsided before peak levels were achieved.

compared in Table 3. The J-HR occurred in 50% of the test group and was mild.

In additional assays, IFNγ and sTNF-R1 and sTNF-R2 levels in three patients receiving test Fab were similar to those in the six controls.

In vitro studies of the effect of anti-TNFα Fab on the measurement of TNFα

On endogenous TNFα

Duplicate aliquots of plasma were prepared from the series of samples collected from a control patient who had received only a saline infusion and was known to have had a marked rise in TNFα following his penicillin injection. One series of aliquots was preincubated with control Fab and the other with anti-TNFα Fab in an amount calculated to be similar to that encountered *in vivo* in test subjects. The samples were then assayed in the usual way. As expected, TNFα levels increased markedly from a mean basal level of 780 ng/l to a peak of nearly 5000 ng/l in the samples containing control Fab, but were dramatically lower with a mean basal level of 50 ng/l and a peak of 160 ng/l in samples containing anti-TNFα Fab. Clearly the latter interfered with the assay.

On TNFα standards

The IRMA for TNFα is based on the use of capture monoclonal antibodies adsorbed to the assay tubes, and signal antibodies labelled with ^{125}I which are added to the tubes immediately after the standards (or sample). The more TNFα present in a standard, the more will bind to the capture antibodies and provide a link so that the amount of radioactivity (cpm) in the tube after washing is directly related to the amount of TNFα in the sample. Each TNFα standard provided in the kit was preincubated with saline, Fab control or specific anti-TNFα Fab and then assayed in the usual way. The maximal amount of TNFα (i.e. with the highest cpm) was detected in the standards preincubated with saline or control Fab, while TNFα was almost undetectable in the standards containing anti-TNFα. Finally, a high TNFα standard was preincubated with doubling dilutions of control or anti-TNFα Fab prior to assay. As shown in Figure 4, there was a gradual increase in the amount of TNFα apparently being measured as the amount of anti-TNFα was reduced.

Table 2 Median plasma cytokine concentrations

Time (h)		Combined control group ($n=29$)				Test group ($n=20$)			
		TNFα (ng/l)	IL-1β (ng/l)	IL-6 (μg/l)	IL-8 (ng/l)	TNFα (ng/l)	IL-1β (ng/l)	IL-6 (μg/l)	IL-8 (ng/l)
−30	Median	170	0	1.7	54	225	0	2.8	29.5
	Percentile*	(70–580)	(0–11)	(0.6–6.8)	(7–420)	(120–600)	(0–9)	(1.3–6.8)	(12.5–405)
0	Median	180	0	2.1	42	3	0	2.5	33.5
	Percentile	(80–540)	(0–18)	(0.75–5)	(6–350)	(0.5–8.5)	(0–4)	(1.4–6.4)	(11–435)
1	Median	230	0	1.9	46	4	0	1.8	25
	Percentile	(120–520)	(0–13)	(1.2–6)	(10–380)	(1.5–20)	(0–9)	(1.1–5.7)	(8.5–483)
1.5	Median	520	0	4.8	61	4	0	3.9	19.5
	Percentile	(270–1400)	(0–15)	(2–16)	(6–390)	(0.5–10.5)	(0–8)	(1.3–15)	(5–483)
2	Median	800	0	13	240	4	0	6.5	22.5
	Percentile	(430–2200)	(0–16)	(4.4–36)	(25–2700)	(0–11.5)	(0–10)	(2.8–24.5)	(5.5–538)
4	Median	1350	8	30	925	4	0	12	33
	Percentile	(520–3500)	(0–40)	(5–135)	(90–4600)	(2–11)	(0–28)	(2–36)	(8–715)
8	Median	518	2	3	160	6	0	3.6	18
	Percentile	(140–2200)	(0–27)	(0.1–13)	(24–2100)	(0–11.5)	(0–7)	(0.06–10.4)	(5.5–433)
24	Median	50	0	3	25	5	0	0.11	23.5
	Percentile	(16–190)	(0–3)	(0.04–0.6)	(8–100)	(1–11)	(0–5)	(0.04–0.06)	(6–325)

In the combined control group ($n=29$), median TNFα and IL-6 levels were raised significantly above baseline by 1.5 h, for IL-8 by 2 h and IL-1β by 4 h. Median peak levels occurred at 4 h for all of these cytokines.
* Range of 10th–90th percentiles.

Discussion

Negussie and his colleagues were the first to report that severe J-HRs, which occurred in 14 of their 17 patients (82%) with LBRF treated with penicillin, are associated with, and probably caused by, a transient, marked rise in plasma levels of TNFα followed by IL-6 and IL-8. The plasma levels of these cytokines increased between four- and ten-fold compared to admission, as determined by bioassay (for TNFα and IL-6) or an immunoassay (for IL-8). The three patients who did not develop a typical J-HR showed no cytokine response. We have, in general, confirmed these findings in a larger control group. Although it is not possible to compare actual cytokine levels directly, because of differences in the assays and standards used, the profile of the cytokine release in the two studies was remarkably similar. Median values for TNFα, IL-6 and IL-8 at each time-point for both studies had increased significantly above baseline by 1.5 h for TNFα and IL-6, and by 2 h, IL-8 showed a four-fold increase. Each cytokine had peaked at 4 h, when TNFα, IL-6 and IL-8 had increased 8-, 18- and 17-fold over baseline, respectively. However, the three control patients in our study who did not develop symptoms of a J-HR showed a typical cytokine response, which is in agreement with Cuevas and colleagues.[15] IFNγ levels were normal throughout the study and there was no relationship to the J-HR.

IL-1β assays were also performed in the present study, but admission plasma levels were normal, and only 12 of the 29 control subjects (41%) showed a rise following penicillin injection and then only of a minor degree. These findings accord with those of Cannon and his colleagues[16] who used a radioimmunoassay to determine plasma levels of IL-1β in patients with septic shock and in normal subjects infused with endotoxin.

Great care must be taken in interpreting assay results, since various factors can influence different types of assay to different degrees. For example, the two soluble receptors to TNFα found in the circulation (sTNF-R1 and sTNF-R2) are known to be raised markedly in septic shock, and were present in extremely high concentrations in the nine patients whose samples were assayed in this study. Engelberts[14] and his colleagues have shown that such levels had profound and differing effects on a WEHI 164 bioassay, an enzymoimmunoassay and an IRMA for TNFα. However, the TNFα IRMA used in the present study was chosen because the results obtained are not influenced by the presence of soluble receptors.

One reason for undertaking the present study is that the J-HR that complicates antibiotic treatment of LBRF resembles other forms of SIRS in its clinical features and cytokine changes. Also, the J-HR has the advantage of predictability, which contrasts with previous clinical trials in SIRS, where it is unlikely that patients would be diagnosed and available for

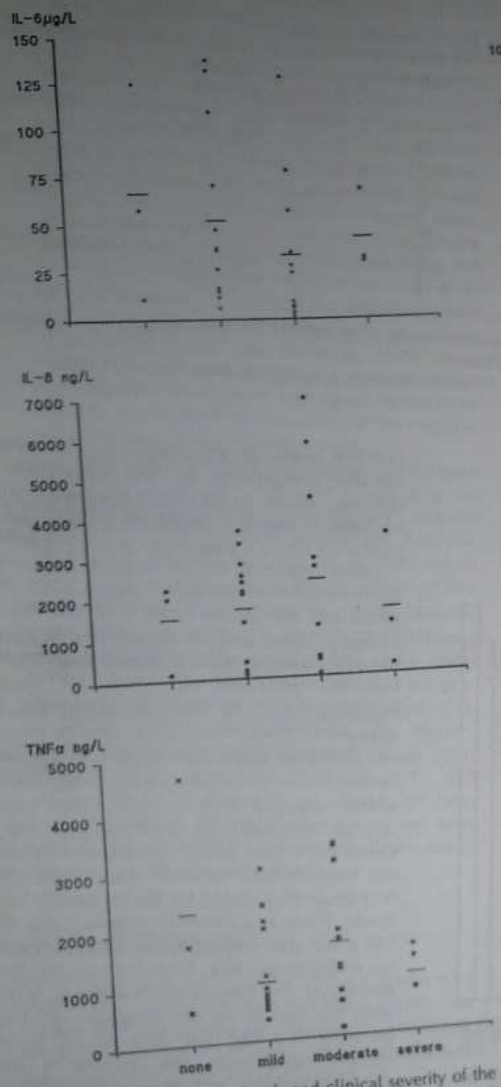


Figure 2. Cytokine peak levels and clinical severity of the J-HR in the control group. Clinical severity of the J-HR was graded as none (no shivering), mild (shivering), moderate (intermittent rigors) and severe (sustained rigors). There was no obvious relationship between peak plasma cytokine levels and the severity of the reaction, but the relative sample size was small.

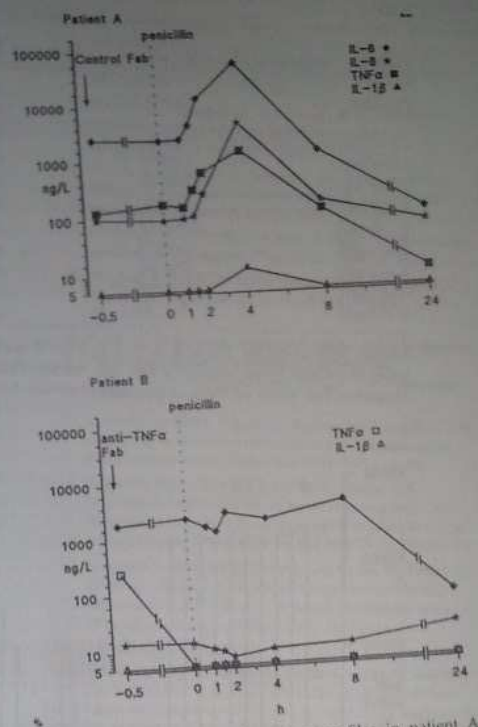


Figure 3. Comparison of cytokine profiles in patient A receiving control Fab and on patient B, receiving anti-TNFα Fab. In Patient A cytokine levels rose to a peak and then fell back to below admission levels. During the 30 min infusion of anti-TNFα (patient B), TNFα levels fell suddenly and remained low throughout the study period, peak IL-6 and IL-8 levels were suppressed.

treatment before the onset of their inflammatory response. Recent experience with the use of specific monoclonal antibodies for the treatment of septic shock has been disappointing, especially when such antibodies have been directed against lipopolysaccharides (LPS).[17] This lack of success may reflect the heterogeneous nature of septic shock. Alternatively, as we have suggested,[18] greater care may need to be taken with the selection of monoclonal antibodies suitable for use in SIRS, and this would be facilitated greatly by the availability of a suitable model.

We have used Fab prepared from polyclonal antibodies obtained from sheep immunized with rhTNFα for use in the present double-blind placebo-controlled trial. Nature invariably mounts a polyclonal response to antigens and, in our experience, such antibodies are usually more effective clinically and require less rigorous selection than their monoclonal counterparts.[18] It was hoped that Fab, with their much more rapid rate and larger volume of

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Table 3 Comparison of median plasma cytokine peak levels in control and test

Cytokine	Group (*n*)	Median	10th–90th percentile	*p*
TNFα (ng/l)	Control 29	1350	520–3500	
TNFα (ng/l)	Test 20	4*	2–11	
IL-1β (ng/l)	Control 29	8	0–40	<0.0001
IL-1β (ng/l)	Test 20	0	0–28	
IL-6 (μg/l)	Control 29	30	5–135	NS
IL-6 (μg/l)	Test 20	12	2–36	
IL-8 (ng/l)	Control 29	925	90–4600	0.01
IL-8 (ng/l)	Test 20	33	8–715	<0.001

Median peak levels for TNFα, IL-6, IL-8 and IL-1β occurred at the same time (4 h) in both groups but were significantly lower in patients given anti-TNFα Fab. NS, not significant.
*Apparent low levels of TNFα in the test group may be due to interference by the anti-TNFα Fab in the assay.

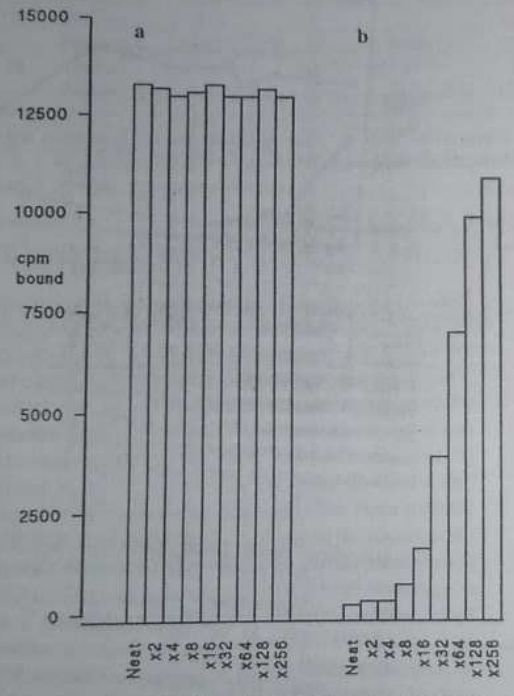


Figure 4. Effect of different concentrations of **a** control Fab and **b** anti-TNFα Fab on measured TNFα. A high TNFα standard was preincubated with doubling dilutions of **a** and **b** and then assayed in the usual way. Maximal TNFα (highest cpm) was detected in the standard preincubated with **a** and was detected in increasing amounts as the amount of anti-TNFα preincubated with the standard was reduced.

distribution than intact immunoglobulins, might offer advantages when used in an attempt to block the biological effects of TNFα. This cytokine probably operates largely in the interstitial rather than the vascular compartment of the extracellular fluid volume. This is, as far as we are aware, the first occasion in which a polyclonal product has been used clinically in a large group of patients with SIRS.

Ovine anti-TNFα Fab produced a rapid and profound reduction in 'measured' plasma levels of TNFα which had approached baseline by the end of the 30-min infusion, and remained low throughout the rest of the study. A series of *in vitro* studies indicated that the polyclonal Fab had, as would be expected, bound to most, or all, of the surface epitopes in TNFα. As a result, neither the capture nor the signal antibodies provided in the immunoradiometric kit could bind to the endogenous TNFα whose disappearance from the circulation was, therefore, probably apparent rather than real. The rate of clearance of circulating Fab-TNFα complexes has not been studied; however, their rapid clearance may be another reason for the apparently low TNFα levels. There is substantial evidence that the anti-TNFα Fab also suppressed the biological activity of the endogenous TNFα. Thus, both the incidence and severity of the clinical manifestations of the I-HR were less than in the control group, and circulating levels of IL-6 and IL-8 (but not of IL-1β) were reduced significantly. The changes in interleukin levels are probably real, because anti-TNFα Fab does not interfere with their assay. In addition, rats receiving an intraperitoneal dose of monoclonal antibody directed against TNFα prior to receiving a bolus dose of endotoxin had markedly altered plasma cytokine profiles resembling those demonstrated in the I-HR patients receiving Fab.[19] In these animal experiments, antibody against TNFα abolished plasma TNFα bioactivity and considerably reduced the IL-6 detected in plasma. Macromolecules such as TNFα are thought to exert their biological effects by means of an active site(s) which binds to, and activates, specific cell-wall receptors. The most likely mecha-

ism by which anti-TNFα Fab impairs these effects is to bind to an epitope(s) in close proximity to the active site, and thereby hinder its binding to cell receptors—the steric hindrance mechanism. Fisher and his colleagues[20] have infused a monoclonal antibody directed against TNFα into patients with sepsis, and shown that plasma TNFα levels could no longer be detected by means of an L929 cell-line bioassay, but could be detected by an IRMA. These apparently divergent findings are easy to explain by means of the steric hindrance mechanism. Thus, the monoclonal antibody had been chosen with great care to ensure that it impaired the biological effect of TNFα (i.e. it bound to an epitope in the immediate vicinity of the active site). However, TNFα has a multiplicity of different epitopes, so that it is not surprising that the capture and signal monoclonal antibody used in the IRMA could bind to the cytokine and, therefore, enable its measurement.

A further study suggests that the steric hindrance mechanism is not the only one which may be involved. Kwiatkowski and his colleagues[21] recently infused a carefully selected monoclonal antibody into patients who had high circulating TNFα values due to cerebral malaria. Again the endogenous TNFα could no longer be assayed using a WEHI bioassay (as expected according to the steric hindrance theory) but, in contrast to the Fisher study, TNFα levels increased when measured by an IRMA. This suggests that the monoclonal antibody was also binding to (and thereby delaying the movement of) the cytokine, out from the vascular compartment where most TNFα is usually formed. Even if the TNFα had retained all its bioactivity, it still might not have produced its usual biological effects if it had been restrained or sequestered within the circulation rather than reaching the interstitial fluid.

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References

1. Bone RC, Balk RA, Cerra FB, *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992; **101**:1644–55.
2. Beutler B, Milsark IW, Cerami A. Passive immunisation against cachectin/tumour necrosis factor protects mice from lethal effects of endotoxin. *Science* 1985; **229**:869–71.
3. Tracey KJ, Beutler B, Lowry SF, *et al.* Shock and tissue injury induced by recombinant human cachectin. *Science* 1986; **234**:470–4.
4. Tracey KJ, Fong Y, Hesse DG, *et al.* Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987; **330**:662–4.
5. Michie HR, Manogue KR, Spriggs DR, *et al.* Detection of circulating tumour necrosis factor after endotoxin administration. *N Engl J Med* 1988; **318**:1481–6.
6. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome with meningococcal disease. *Lancet* 1987; **1**:355–7.
7. Christman JW. Potential treatment of sepsis syndrome with cytokine-specific agents. *Chest* 1992; **102**:613–17.
8. Abraham E, Raffin TA. Sepsis therapy trials. Continued disappointment or reason for hope? *JAMA* 1994; **271**:1876–8.
9. Parillo JE, Parker MM, Natanson C. Septic shock in humans: Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann Intern Med* 1990; **113**:227–42.
10. Cross AS, Opal SM, Palardy JE, Bodmer MW, Sadoff JC. The efficacy of combination immunotherapy in experimental Pseudomonas sepsis. *J Infect Dis* 1993; **167**:112–18.
11. Bryceson ADM, Parry EHO, Perine PL, Warrell DA, Vukotich D, Leithead S. Louse-borne relapsing fever. A clinical and laboratory study of 62 cases in Ethiopia and a reconsideration of the literature. *Q J Med* 1970; **39**:129–70.
12. Negussie Y, Remick DG, DeForge LE, Kunkel SL, Eynon A, Griffin GE. Detection of plasma tumour necrosis factor, interleukins 6 and 8 during the Jarisch-Herxheimer reaction of louse-borne relapsing fever. *J Exp Med* 1993; **175**:1207–12.
13. Fekade D, Knox K, Hussein K, Melka A, Lalloo D, Coxon RE, Warrell DA. Sheep Fab antibodies to tumour necrosis factor and the prevention of Jarisch-Herxheimer reactions. *N Engl J Med*; 1996; **335**:311–15.
14. Engelberts I, Stephens S, Francot GJM, van der Linden CJ, Buurman W. Evidence for different effects of soluble TNF-receptors on various TNF measurements in human biological fluids. *Lancet* 1991; **338**:515–16.
15. Cuevas LE, Borgnolo G, Hailu B, Smith G, Almaviva M, Hart CA. Tumour necrosis factor, interleukin-6 and C-reactive protein in patients with louse-borne relapsing fever in Ethiopia. *Ann Trop Med Parasitol* 1995; **89**:49–54.
16. Cannon JG, Tompkins RG, Gelfand JA, *et al.* Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990; **161**:79–84.
17. Greenman RL, Schein RMH, Martin MA. A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of Gram-negative sepsis. *JAMA* 1991; **266**:1097–102.
18. Landon J, Chard T, Coxon RE. *An introduction to the clinical applications of antibodies. Therapeutic Antibodies.* London, Springer-Verlag, 1995:1–32.
19. Sharma RJ, Macallan DC, Sedgwick P, Remick DG, Griffin GE. Kinetics of endotoxin induced acute phase response in the rat: Gene regulation and its modulation by monoclonal antibody to TNFα. *Am J Physiol* 1992; **262**:R786–93.
20. Fisher CJ, Opal SM, Dhainaut J-F, *et al.* Influence of an anti-tumour necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. *Crit Care Med* 1993; **21**:318–27.
21. Kwiatkowski D, Molyneux ME, Stephens S, *et al.* Anti-TNF therapy inhibits fever in cerebral malaria. *Q J Med* 1993; **86**:91–8.

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ATRIAL FIBRILLATION AND EMBOLIC COMPLICATIONS
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ATRIAL FIBRILLATION AND EMBOLIC COMPLICATIONS

M. MARU

SUMMARY

Clinical records of 136 cardiac patients with atrial fibrillation (AF) followed in the cardiac clinic of the Black Lion Hospital were analysed. The mean age of the patients was 41 ± 13 years, with a male to female ratio of 1.0 : 1.6. Rheumatic heart disease (RHD) was found in 66.3%, hypertension in 10.3%, cardiomyopathy in 8.8% and ischaemic heart disease in 6.6%. Embolic episodes occurred in 26 (19.1%) cases with five deaths. Twenty-one patients had cerebrovascular accident while five had femoral artery occlusion. The major cause of embolisation was rheumatic valvular heart diseases (65.5%), especially mitral stenosis. In order to reduce the high risk of systemic emboli, it is recommended that patients with AF and associated recent onset of congestive heart failure, previous history of thromboembolism and hypertension should be anticoagulated if there are no contraindications.

INTRODUCTION

Atrial fibrillation (AF) is a common arrhythmia with a prevalence of approximately two per cent in general population(1). It is more common in the elderly, affecting about five per cent of persons over 60 years of age(2). The overall prevalence of AF in the Framingham cohort study was 21.5/1000 men and 17.1/1000 women. The prevalence rises with age from 2.0/1000 for the 25 - 34 age group to 30.0/1000 for the 55 - 60 age group(3).

Systemic embolisation arising from the heart frequently has disastrous consequences, particularly when it involves the cerebral circulation(4, 5), and therapy is of no consequence once the insult has occurred. Therefore, the recognition of the commonest aetiologic causes and predisposing factors of cardiac embolisation would help in instituting preventive therapy.

The purpose of this study was to review some of the clinical features of AF, with particular attention to the aetiologic background or associated cardiac disorders and its embolic complication.

MATERIALS AND METHODS

The study was based on review of the clinical records of patients with AF being followed in the cardiac clinic of Black Lion Hospital from September 1993 to August 1994. All cases were examined and followed by the author. All had complete blood count (CBC), chest x-ray and echocardiography. T_3, T_4 and TSH were done when indicated. The age, sex, clinical features and ECG findings were recorded on a preset protocol.

The diagnosis of AF was based on the absence of visible P-waves on the electrocardiogram, which were replaced by irregular "f" waves (or no signs of atrial activity) accompanied by a grossly irregular R-R interval with normal QRS complexes (6).

RESULTS

A total of 900 cardiac patients were being followed in the cardiac clinic. Out of these, rheumatic valvular heart disease was found in 47%, hypertension and hypertensive heart disease in 11.3%, cardiomyopathy in 8.8%, ischaemic heart disease in 7.7% and congenital heart disease in 5.8%.

Atrial fibrillation was found in 136 (15.1%) of the 900 patients. The age and sex distribution of the 136 patients is shown in Table 1. The male to female ratio was 1.0 :1.6. The age range was from 15 - 80 years with a mean age of 41 ± 13 years. Forty six percent were less than 35 years of age. The cardiac diseases associated with AF are shown in Table 2. Rheumatic heart disease (RHD) was found in 90 (66.3%) and hypertension in 14 (10.3%) of the patients.

Table 1

Age and sex distribution of 136 patients with atrial fibrillation.

Age(yrs)	15-24	25-34	35-44	45-54	55-64	65≥	
Sex M	14	11	13	5	3	6	52
F	21	17	9	10	17	10	84
Total	35	28	22	15	20	16	136
%	25.7	20.6	16.2	11.0	14.7	11.8	100

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Table 2

Diseases associated with atrial fibrillation in 136 patients

Types of disease	No	%
Rheumatic heart disease	90	66.3
Hypertension	14	10.3
Cardiomyopathy	12	8.8
Ischaemic heart disease	9	6.6
Ischaemic heart disease with hypertension	4	2.9
Hyperthyroidism	5	3.7
Cor pulmonale	1	0.7
Syphilitic aortitis	1	0.7
Total	136	100

The duration of illness ranged from less than six months to over 10 years. In 50% of the cases the duration was less than four years. The duration of the AF could not be ascertained because AF was already present when the patients were seen the first time. Of the different rheumatic valvular lesions, the most frequently found were mitral stenosis in forty one, combined mitral regurgitation and mitral stenosis in twenty seven and mitral regurgitation alone in thirteen cases (Table 3).

Table 3

The different rheumatic lesions in patients with atrial fibrillation

Type of valve lesion	No	%
Mitral stenosis	41	45.6
Mitral regurgitation and mitral stenosis	27	30.0
Mitral regurgitation	13	14.5
Aortic regurgitation, aortic stenosis and mitral stenosis	4	4.4
Aortic and mitral regurgitation	3	3.3
Stenosis and regurgitation	2	2.2
Total	90	100.0

Twenty six (19.1%) patients with AF had systemic embolisation. Out of these, rheumatic mitral valve disease was found in seventeen cases, cardiomyopathy in three, hypertension in three, ischaemic heart disease in two and hypertension and ischaemic heart disease in one. Twenty one patients had stroke of sudden onset, while five had femoral artery occlusion with development of gangrene of the toes in three cases. Seven patients died during the study period. Four died suddenly and three died with severe heart failure.

DISCUSSION

The incidence of AF in different cardiac disorders ranges from 10 -40%(1,7).In this study AF was found in 15.1% which is less than that reported from the Sudan(8) and Nigeria(9, 10). The distribution and type of heart disease was similar to that reported from sub-Saharan Africa(8,9,10) with the exception that endomyocardial fibrosis was completely absent in our cases. In the developed countries, coronary heart disease and hypertension are the commonest causes of AF(3). The higher incidence of RHD (66.3%) and the lower incidence of hypertension (10.3%) compared to previous Ethiopian studies(11,12) may be explained by the fact that hypertensive patients in our hospital are followed in the cardiac as well as nephrology clinics.

As clearly demonstrated by previous clinical and necropsy studies, AF associated with valvular and non-valvular heart disease is a recognised risk factor for the development of systemic embolisation. The incidence of emboli varies from 9 - 27% in clinical reports and increases to 41% in necropsy studies(3,13,14). In this series an incidence of 19.1% is similar to the above findings.

The risk of embolisation in rheumatic valvular heart disease with AF is much higher than that with sinus rhythm and other categories of heart diseases with AF. The risk of emboli also becomes greater with older patients(15,16) and with lower cardiac indices but appears to correlate poorly with left atrial size or clinical classification(16,17).

However, recent studies have identified three clinical(18) and two echocardiographic(19) predictors. The three clinical predictors of subsequent thromboembolism in patients with AF are: hypertension, recent onset of congestive heart failure and previous history of thromboembolism. The two echocardiographic predictors are left ventricular dysfunction and left atrial dilatation.

All our patients were seen after they developed the embolic episode and therefore there was no possibility to assess their cardiac status. However, 50% of the patients were in NYHA classes III and IV.

Several controlled clinical studies have demonstrated that valvular and non-valvular heart diseases with AF have a 4-7 fold risk of systemic embolisation (20, 21) when compared to comparable groups of patients without AF, an indication that AF as such is the most important factor in the occurrence of embolism. Fleming and Baily(17) in a level IV study found 25% incidence of emboli among 500 patients with AF, while in 217 patients with AF treated with warfarin, only five embolic episodes occurred over nine and half year period, yielding an incidence rate of 0.8% patient-year. Other placebo controlled randomised clinical studies(22) have also clearly shown that the high risk of systemic embolisation can be significantly reduced by chronic anticoagulation with warfarin in patients without any contraindications.

Therefore, it is recommended that patients with AF associated with hypertension, history of previous thromboembolism, recent onset of congestive heart failure and atrial dilatation by echocardiography should be put on prophylactic anticoagulation as long as there are no contraindications. It is also recommended that appropriate

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public health measures be taken by ministries of health in the developing countries so as to reduce the incidence of rheumatic fever and to identify cases of hypertension early and to treat them in order to reduce their disastrous consequences.

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REFERENCES

1. Dunn, M., Alexander, J., Silva, R. and Hildner, F. Antithrombolic therapy in atrial fibrillation. *Chest.* 1989; **95**:1185 - 275
2. Kitchens, G.M. and Flegel, K.M. Atrial fibrillation, stroke and anticoagulation. What is to be done? *J. gen. intern. Med.* 1986; **1**:126-9.
3. Kannel, W.B., Abott, R.D., Savage, D.D. and McNamara, R.M. Epidemiological features of chronic atrial fibrillation. The Framingham Study. *N. Engl. J. Med.* 1982; 306:1018-22.
4. Cerebral Embolism Task Force. cardiogenic brain embolism. The second report of the cerebral task force. *Arch. Neurol.* 1989; 46:727-43.
5. Cerebral Embolism Task force. Cardiogenic brain embolism. *Arch. Neurol.* 1986; **43**:77-84.
6. Marriott, H.J.L. Practical electrocardiography - ed. The Williams Wilkins Co. Baltimore. 1988; 147-199.
7. Gregory, W.B., Atwood, G.E., Hirsch, G., Sherman, D.G., Hughes, R.A. and Connally, S.G. Stroke prevention in non-valvular atrial fibrillation. *Ann. intern. Med.* 1991; **115**: 727-736.
8. Halim, A.M. and Jacques, J.E. Rheumatic heart disease in the Sudan. *Brit. Heart J.* 1961; **23**:383-386.
9. Cole, T.O. and Feyestan, C.O. Atrial fibrillation. *East Afr. Med. J.* 1974; **51**:715-719.
10. Beet, E.A. Rheumatic heart disease in Northern Nigeria. *Trans. roy. Soc. trop. Med. Hyg.* 1964; **50**:587-591
11. Abraham, G. Patterns of cardiovascular diseases among adult hospitalised Ethiopians. *Ethiop. Med. J.* 1982; **20**: 63-67.
12. Maru, M. The changing pattern of cardiovascular diseases in Ethiopia. *East Afr. Med. J.* 1993; **70**:772-776.
13. Hinton, R.C., Kistler, G.I., Fallow, G.G., Fielich, A.L. and Fisher, C. M. Influence of aetiology of atrial fibrillation on incidence of systemic embolization. *Amer. J. Cardiol.* 1977; **40**:509-573.
14. Aberg, H. Atrial fibrillation. *Acta. Med. Scand.* 1989; **185**:373-379.
15. Dewar, H.A. and Weightman, D. Study of mitral valve disease and atrial fibrillation. *Brit. Heart. J.* 1983; **49**:133-140.
16. Coulshed, N.,Epstein, E.J., McKendricks, C.S., Galloway, R.W. and Walker, E. Systemic embolization in mitral valve disease. *Brit. Heart J.* 1970; **32**:26-34.
17. Flemming, H.A. and Baily,S.M. Mitral valve disease, systemic embolization and anticoagulants. *Postgrad. Med. J.* 1975; **47**:599-604.
18. The Stroke prevention in Atrial Fibrillation Investigators. Predictors of thromboembolism in atrial fibrillation:1. Clinical features of patients at risk. *Ann. intern.Med.* 1992; **116**:1-5.
19. The stroke prevention in Atrial Fibrillation Investigators. Predictors of thromboembolism in atrial fibrillation. Echocardigraphic features of patients at risk. *Ann. intern. Med.* 1992; **116**:6-12.
20. Szekey,P. Systemic embolic and anticoagulant prophylaxis in rheumatic heart disease. *Brit. Heart J.* 1964; **1**:1209-12.
21. Wolf, P.A., Dawber, T.R., Thomas, H.E. Jr. and Kannel, W.B. Epidemiological assessment of chronic atrial fibrillation and risk of stroke. The Framingham study. *Neurology (Minneap.).* 1978; **28**:973-977.
22. Petersen, P., Boysen, G., Godtfredsen, J., Andersen, E.D. and Andersen, B. Placebo controlled randomised trial of Warfarin and aspirin for the prevention of the thromboembolic complications in chronic atrial Fibrillation. The Copenhagen AFASAK Study. *Lancet* 1989; **1**:175-9.

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Borrelia recurrentis Characterization and Comparison with Relapsing-Fever, Lyme-Associated, and Other *Borrelia* spp.

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***Borrelia recurrentis*, the cause of louse-borne relapsing fever, has until recently been considered noncultivable, which has prevented characterization of this spirochete. We successfully cultivated 18 strains from patients with louse-borne relapsing fever and present the initial characterization of these isolates. Electron microscopy revealed spirochetal cells with pointed ends, an average wavelength of 1.8 μm, an amplitude of 0.8 μm, and 8 to 10 periplasmic flagella. The G+C ratio was 28.4 mol%. Whole DNA-DNA hybridizations showed similarity between the isolates of *B. recurrentis* but not with *Borrelia hermsii*, *Borrelia parkeri*, *Borrelia turicatae*, or the Lyme-associated borreliae. Sequencing studies of both the flagellin and 16S RNA genes revealed that the greatest similarity was between *B. recurrentis* and *Borrelia duttonii*. Analysis of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of strains revealed four groups based on the position of a major protein band (one of the groups showed some heterogeneity and was subdivided into four subgroups). Pulsed-field gel electrophoresis revealed five distinct patterns.**

In the last century, successive epidemics of louse-borne relapsing fever spread from Asiatic Russia into Europe, causing death in from 2 to 40% of the cases. Now, this disease has all but vanished in all areas except the Andean foothills, the Ethiopian highlands, and nearby Rwanda. Even in the turmoil in the former Yugoslavia, the appearance of louse-borne typhus was not accompanied by relapsing fever. Why the disease has persisted in only a few isolated foci and has become invisible elsewhere remains an enigma.

The vector of this disease is the infected human body louse (*Pediculus humanus humanus*). Lice become infected by feeding on a spirochetemic patient. The ingested spirochetes enter the gut and cross the epithelium into the hemolymph. Transmission to humans occurs by contamination of abraded skin with hemolymph from a crushed infected louse. Clinically, louse-borne relapsing fever is characterized a 5- to 7-day incubation period, one to five relapses of fever, and spirochetemia (notably fewer spirochetemias than in tick-borne relapsing fever). Febrile episodes typically last 2 to 9 days, with an afebrile interval of about 9 days. The initial fever is usually the longest and most severe and is followed by milder relapses. Very high spirochete counts are usually associated with a poorer prognosis. Mortality is higher in louse-borne relapsing fever (up to 40% in untreated cases and 10% in treated cases) than in the tick-borne variety; this may be related to higher counts of circulating spirochetes. It is possible that as seen in outbreaks, there is a spectrum of severity.

We were able to culture *Borrelia recurrentis* for the first time since it was described in 1867 by Obermeier (12). Preliminary characterization of *B. recurrentis* and a comparison of this organism with relapsing-fever, Lyme-associated, and other borrelial species are described in this paper.

TABLE 1. Strains used in this study

Organism	Strain(s)	Location
B. recurrentis	A1 to A18[a]	Addis Ababa, Ethiopia
B. burgdorferi sensu stricto	B31	United States
B. garinii	20047	France
B. afzelii	VS461	France
B. japonica	HO14	Japan
Group VS116	UK	United Kingdom
Group PotiB2	PotiB2	Portugal
B. miyamotoi	HT31	Japan
B. hermsii	HS1	United States
B. parkeri		United States
B. turicatae		United States

[a] Strain A1 has been deposited in the American Type Culture Collection as strain ATCC 700241.

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TABLE 2. *B. recurrentis* strains grouped according to plasmid patterns and protein profiles

Plasmid group	Protein profile(s)						
	Protein group 1	Protein group 2	Protein group 3	Protein subgroup 4a	Protein subgroup 4b	Protein subgroup 4c	Protein subgroup 4d
Type 1	A1, A2, A3	A5					
Type 2	A4		A6, A7, A8, A9	A10			
Type 3					A11, A12		
Type 4						A13, A14, A15, A16	
Type 5							A17, A18

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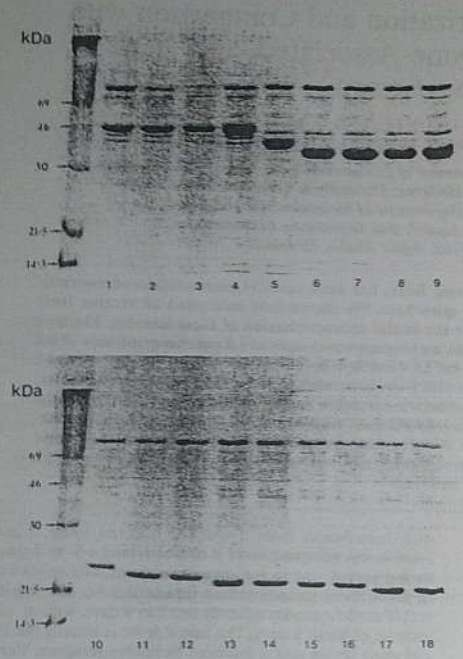


FIG. 1. Coomassie blue-stained SDS-PAGE protein profiles of *B. recurrentis* A1 to A18. Lanes 1 through 4, protein group 1 strains; lane 5, protein group 2 strain; lanes 6 through 9, protein group 3 strains; lane 10, protein subgroup 4a strain; lanes 11 and 12, protein subgroup 4b strains; lanes 13 through 16, protein subgroup 4c strains; lanes 17 and 18, protein subgroup 4d strains.

MATERIALS AND METHODS

Louse-borne relapsing-fever patients. All patients from which cultures were obtained were demonstrated to be spirochetemic by blood film microscopy by using Wright's stain. One patient was female, and the remaining 17 patients were male. The ages of the patients ranged from <12 to 36 years. Most were laborers or jobless. Body lice were found on the clothing in all cases. The duration of clinical symptoms varied between 3 and 7 days.

Bacterial strains and culture conditions. The strains used in this study are listed in Table 1. All isolates were cultured in BSK II medium (3). Relapsing-fever strains were maintained by thrice weekly subculturing, and the remaining strains were subcultured on a weekly basis. The strains of Lyme-associated and tick-borne relapsing-fever borreliae were of unknown passage number. The strains of *B. recurrentis* had passage numbers between 8 and 15; this was the minimum number of passages necessary to obtain sufficient material to undertake the study.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Borrelial cells were harvested from BSK II medium by centrifugation at 3,500 × *g*, washed twice, and suspended in phosphate-buffered saline (Oxoid) to give a protein concentration of approximately 1.8 mg/ml (as determined with a Bio-Rad Bradford protein assay kit). Cells were diluted in electrophoresis sample buffer containing (final concentrations) 75 mM Tris-HCl, 100 mM dithiothreitol, 2% (vol/vol) SDS, and 10% (vol/vol) glycerol. This mixture was boiled for 5 min, divided into aliquots, and frozen at −20°C until it was required.

Samples were boiled again for 5 min immediately before 5-μl volumes were loaded into wells on minigels (type SE250 Mighty Small system; LKB, Ltd.). The optimal sample concentration for gel loading was determined by dilution. Borrelial proteins were stacked by using a 4% stacking gel and then were separated by discontinuous SDS-PAGE by using 12.5% gels run at 150 V (gels were prepared from a 30% [wt/vol] acrylamide–0.8% [wt/vol] bisacrylamide stock solution [Protogel; Flowgen Instruments, Ltd.]).

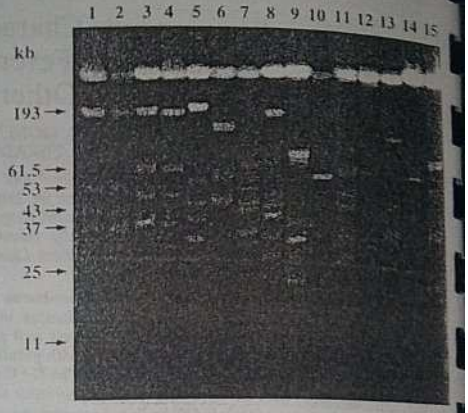


FIG. 2. PFGE patterns comparing each plasmid type of *B. recurrentis* w... tick-borne and Lyme-associated borreliae. Lane 1, *B. recurrentis* type 1; lane 2, ... *recurrentis* type 2; lane 3, *B. recurrentis* type 3; lane 4, *B. recurrentis* type 4; lan... 5, *B. recurrentis* type 5; lane 6, *B. hermsii*; lane 7, *B. parkeri*; lane 8, *B. turicata*; lane 9, *B. miyamotoi*; lane 10, *B. burgdorferi* sensu stricto; lane 11, *B. garinii*; lan... 12, *B. afzelii*; lane 13, *B. japonica*; lane 14, *B. burgdorferi* UK (group VS116); lan... 15, *B. burgdorferi* PotiB2.

Immunoblotting. SDS-PAGE gels as described above were blotted onto ... trocellulose membranes as described elsewhere (11). After these membranes h... been blocked overnight in 5% dried milk (Marvel) in Tris-buffered saline (... mM Tris, 150 mM sodium chloride; pH 7.5), they were incubated at ro... temperature overnight with 1:1,000 dilutions of monoclonal antibody H9724 (... After washing, each membrane was incubated with a 1:3,000 dilution of g... anti-mouse alkaline phosphatase conjugate (Bio-Rad), washed, and develope... by using a solution containing *p*-nitroblue tetrazolium chloride (0.3 mg/ml) ... 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (0.15 mg/ml), and the ... action was stopped by washing in distilled water (11). The molecular weight ... the flagellin band could then be determined by comparison to molecular weig... standards (Rainbow markers; Amersham International).

Coomassie blue staining. Minigels were stained by using a Coomassie bl... stain (2.5 g of Coomassie blue R250 in 1 liter of 95% [vol/vol] ethanol, filter... before use)–10% (vol/vol) glacial acetic acid solution (1:1) overnight. The g... were destained twice for 1.5 h with 100 ml of 95% (vol/vol) ethanol and 150 ... of 5% (vol/vol) glacial acetic acid, then with 33 ml of 95% (vol/vol) ethanol ... 133 ml of 5% (vol/vol) glacial acetic acid for 2 h, and finally with 117 ml of ... (vol/vol) glacial acetic acid and 50 ml of distilled water overnight.

Pulsed-field gel electrophoresis (PFGE). Cells were harvested from BSK ... medium by centrifugation at 3,500 × *g* for 20 min, washed twice with a w... solution (75 mM NaCl, 25 mM EDTA; pH 7.5), and resuspended in 1 ml of ... wash solution. The optical density at 600 nm was adjusted to 2. Blocks w... prepared by using a 1:1 dilution of cells and 1% (vol/vol) low-melting-p... agarose (Promega) poured into 100-μl molds. DNA was extracted by incubat... at 50°C overnight with 1 ml of 0.5 M EDTA (pH 8.5)–1% (wt/vol) sarcos... proteinase K (10 μg/ml) per block. Following extraction, the blocks were wash... four times with TE (10 mM Tris-Cl [pH 7.6], 1 mM EDTA) and stored at 4°C in ...

Two-hundred-milliliter 1.2% agarose gels were loaded with blocks, sealed w... agarose, and run in 0.5× TBE (0.045 M Tris-borate, 0.001 M EDTA) by u... a Pharmacia Gene Navigator. Lambda ladder, delta 39 (Promega), 8- to 48-

TABLE 3. Molecular sizes of *B. recurrentis* plasmids

Plasmid group	Plasmid sizes (kb)
Type 1	193, 53, 43, 37, 25, 1...
Type 2	192, 53, 43, 37, 11
Type 3	185, 61.5, 46, 37, 11
Type 4	183, 61.5, 46, 37, ... 30
Type 5	194, 53, 43, 37, 31, ...

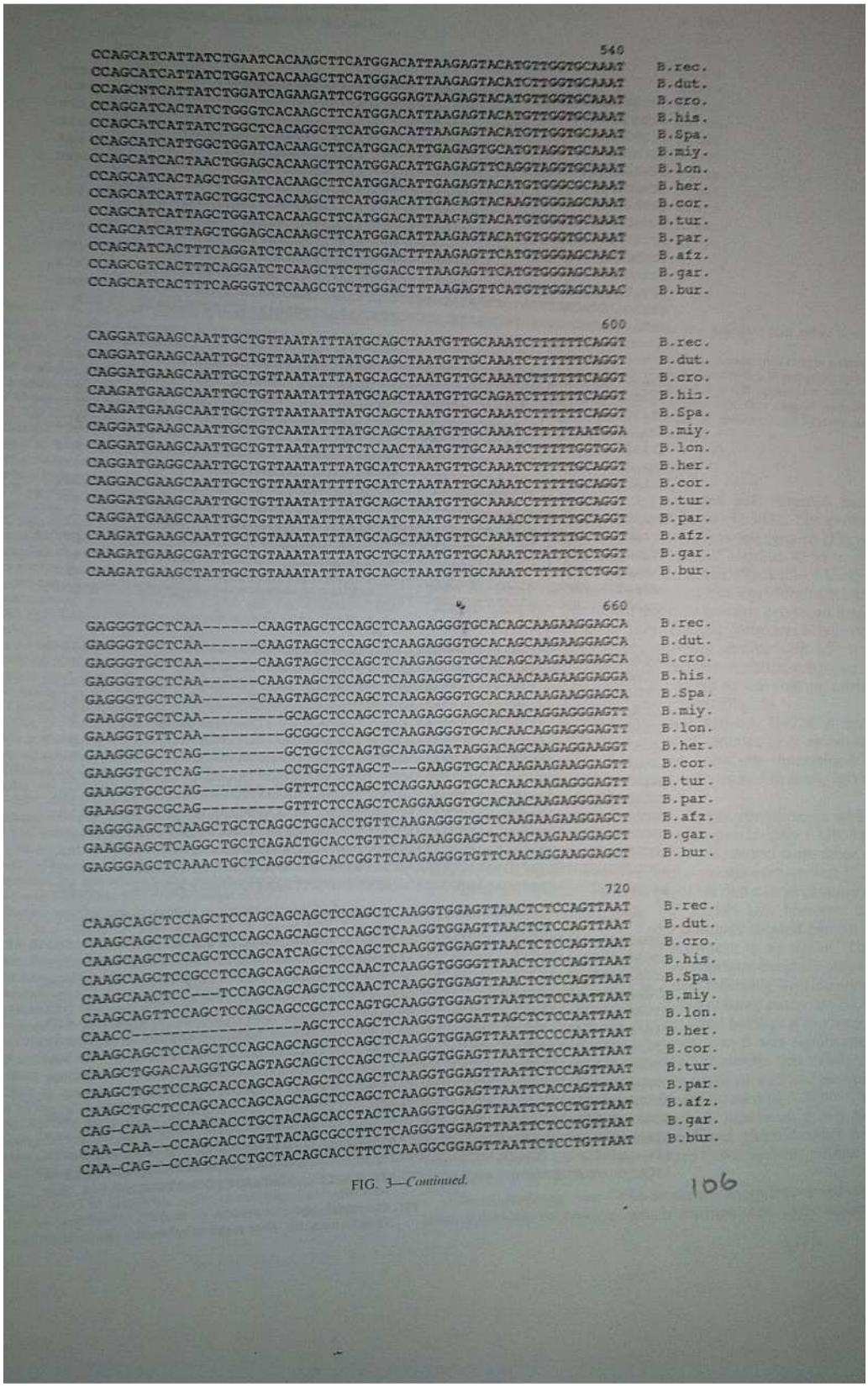
```
                                                                  300
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GATGAAGTAGAGAAAGTGTTGGTGAGAATGAAAGAGCTTGCTGTTCAATCTGGTAATGGT   B.dut.
GATGAAGTAGAGAAAGTGTTGGTGAGAATGAAAGAGCTTGCTGTTCAATCTGGTAATGGT   B.cro.
GATGAAGTAGAAAAAGTGTCGGTGAGAATGAAGGAGCTCGCGGTTCAATCTGGTGGGGGT   B.his.
GCTGAAGTAGAGANAGTGTTGGTGAGAATGAAAGAGGTTGCTGCTCAATCTGGTAATGGT   B.Spa.
AACGAGGTAGAGAAAGTATTAGTAAGAATGAAAGAACTTGCTGTTCAGTCTGGTAATGGT   B.miy.
AATGAAGTAGAGAGAGTATTAGTAAGAATGAAAGAACTTGCTGTTCAATCTGGTAATGGT   B.her.
GATGAAGTAGAGAAAGTATTAGTAAGAATGAAAGAACTTGCTGTTCAGTCTGGTAATGGT   B.cor.
AATGAAGTAGAGAAAGTATTAGTAAGAATGAAAGAACTTGCTGTGCAGTCTGGTAATGGT   B.tur.
AATGAAGTAGAGAAAGTATTAGTAAGAATGAAAGAACTTGCTGTTCAGTCTGGTAATGGT   B.par.
AATGAAGTAGAAAAAGTTTTAGTAAGAATGAAAGAATTAGCAGTTCAATCAGGTAACGGA   B.afz.
AATGAAGTAGAAAAAGTTTTAGTAAGAATGAAAGAATTAGCAGTTCAATCAGGTAACGGT   B.gar.
AATGAAGTAGAAAAAGTCTTAGTAAGAATGAAGGAATTGGCAGTTCAATCAGGTAACGGC   B.bur.

                                                                  360
ACATATTCAGATGCAGACAGAGGTTCTATTCAGATTGAAGTTGAACAACTTACAGATGAG   B.rec.
ACATATTCAGATGCAGACAGAGGTTCTATTCAGATTGAAGTTGAACAACTTACAGATGAG   B.dut.
ACATATTCAGATGCAGTCAGAGGTTCTATTCAGATTGAAGTTGAACAACTTACAGATGAG   B.cro.
ACATATTCAGATGCAGACAGGGGTTCTACTCAGAGTGAAGATGAACAACTCACAGATGAG   B.his.
ACATATTCAGATGCAGACAGAGGTTCTATTCAGATTGAAATTGAGCAACTTACAGATGAG   B.Spa.
ACATACTCAGATTCAGATAGAGGGTCTATTCAGATTCAAATTGAACAACTTACAGATGAA   B.miy.
ACATATTCAGATGCAGACAGAGGTTCTATTCAAATTGAAATTGAACAACTTACAGATGAA   B.lon.
ACATATTCAGATGCAGACAGAGGTTCTATTCAAATTGAAATTGAGCAACTTACAGATGAA   B.her.
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ACGTATTCAGACTCAGACAGAGGTTCTATACGAATTGAAATAGAGCAACTTACAGACGAA   B.afz.
ACATATTCAGACGCAGACAGAGGTTCTATACAAATTGAAATAGAGCAACTTACAGACGAA   B.gar.
ACATATTCAGATGCAGACAGAGGTTCTATACAAATTGAAATAGAGCAACTTACAGACGAA   B.bur.

                                                                  420
ATCAATAGAATTGCTGATCAAGCACAATATAACCATATGCATATGTTATCTAATAGATCA   B.rec.
ATCAATAGAATTGCTGATCAAGCACAATATAACCATATGCATATGTTATCTAATAGATCA   B.dut.
ATCAATAGAATTGCTGATCAAGCACAATATAACCATATGCATATGTTATCTAATAGATCA   B.cro.
CTCAATAGAATTGCTGATCAAGCACNATATAACCATATCCATNTGTTATCAAATAGATCA   B.his.
ATCAATAGAATTGCTGATCAGGCACAATATAACCATATGCATATGTTATCTAATAGATCA   B.Spa.
ATAAACAGAATTGCTGATCAGGCTCAATACAACCAAATGCATATGTTATCTAATAAGTCA   B.miy.
ATTAACAGAGTTGCTGATCAGGCTCAATACAACCAGATGCATATGTTATCTAACAAATCA   B.lon.
ATCAACAGAATTGCTGATCAGGCTCAATACAACCAAATGCATATGTTGTCCAACAAGTCA   B.her.
ATTAACAGAATTTCTGATCAAGCTCAATACAATCAAATGCATATGTTGTCTAATAAATCA   B.cor.
ATCAACAGAATTGCTGATCAAGCTCAATACAACCAAATGCATATGTTGTCCAACAAGTCA   B.tur.
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ATTAATAGAATTGCTGATCAGGCTCAATATAACCAAATGCACATGTTGTCAAACAAATCT   B.afz.
ATTAATAGAATTGCTGATCAAGCTCAATATAACCAAATGCACATGTTGTCAAACAAATCT   B.gar.
ATTAATAGAATTGCTGATCAAGCTCAATATAACCAAATGCACATGTTATCAAACAAATCT   B.bur.

                                                                  480
TCTGCTGAGCATGTAAGAACAGCTGAAGAGCTTGGAATGCAACCTGTAAAGATTAATACA   B.rec.
TCTGCTGAGCATGTAAGAACAGCTGAAGAGCTTGGAATGCAACCTGTAAAGATTAATACA   B.dut.
TCTGCTGAGCATGTAAGAACAGCTGAAGAGCGTGGAATGCAACCTGTAAAGATTAATACT   B.cro.
CCTGCTGAGAATGTAAGAACAGCTGGAGAGCTTGGAATGCAACCTGGGAAGATTAATACA   B.his.
GCTGCTGAGAATGTAAGAACAGCTGAAGAGCTTGGAATGCAACCTGTAAAGATTAATACA   B.Spa.
GCTGCTCAAAATGTAAAAACTGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAACACA   B.miy.
TCTGCTCAAAATGTAAAAACTGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAATACA   B.lon.
GCTGCTCAAAATGTAAAAACAGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAACACA   B.her.
GCTGCTGAGAATGTAAAAACAGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAACACA   B.cor.
GCTGCTCAAAATATAAAAACAGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAACACA   B.tur.
GCTGCTCAGAATATAAAAACAGCTGAAGAGCTTGGAATGCAACCTGCAAAAATTAACACA   B.par.
GCTTCCCAAAATGTAAAAACAGCTGAAGAGCTTGGAATGCAGCCTGCAAAAATTAACACA   B.afz.
GCTTCCCAAAATGTAAGAACAGCTGAAGAACTTGGAATGCAACCTGCAAAAATCAACACA   B.gar.
GCTTCTCAAAATGTAAGAACAGCTGAAGAGCTTGGAATGCAGCCTGCAAAAATTAACACA   B.bur.
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FIG. 3—*Continued.*



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                                                                    540
CCAGCATCATTATCTGAATCACAAGCTTCATGGACATTAAGAGTACATGTTGGTGCAAAT    B.rec.
CCAGCATCATTATCTGGATCACAAGCTTCATGGACATTAAGAGTACATCTTGGTGCAAAT    B.dut.
CCAGCNTCATTATCTGGATCAGAAGATTCGTGGGGAGTAAGAGTACATGTTGGTGCAAAT    B.cro.
CCAGGATCACTATCTGGGTCACAAGCTTCATGGACATTAAGAGTACATGTTGGTGCAAAT    B.his.
CCAGCATCATTATCTGGCTCACAGGCTTCATGGACATTAAGAGTACATGTTGGTGCAAAT    B.Spa.
CCAGCATCATTGGCTGGATCACAAGCTTCATGGACATTGAGAGTGCATGTAGGTGCAAAT    B.miy.
CCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGTGCAAAT    B.lon.
CCAGCATCACTAGCTGGATCACAAGCTTCATGGACATTGAGAGTACATGTGGGCGCAAAT    B.her.
CCAGCATCATTAGCTGGCTCACAAGCTTCATGGACATTGAGAGTACAAGTGGGAGCAAAT    B.cor.
CCAGCATCATTAGCTGGATCACAAGCTTCATGGACATTAACAGTACATGTGGGTGCAAAT    B.tur.
CCAGCATCATTAGCTGGAGCACAAGCTTCATGGACATTAAGAGTACATGTGGGTGCAAAT    B.par.
CCAGCATCACTTTCAGGATCTCAAGCTTCTTGGACTTTAAGAGTTCATGTGGGAGCAACT    B.afz.
CCAGCGTCACTTTCAGGATCTCAAGCTTCTTGGACCTTAAGAGTTCATGTGGGAGCAAAT    B.gar.
CCAGCATCACTTTCAGGGTCTCAAGCGTCTTGGACTTTAAGAGTTCATGTTGGAGCAAAC    B.bur.

                                                                    600
CAGGATGAAGCAATTGCTGTTAATATTTATGCAGCTAATGTTGCAAATCTTTTTTCAGGT    B.rec.
CAGGATGAAGCAATTGCTGTTAATATTTATGCAGCTAATGTTGCAAATCTTTTTTCAGGT    B.dut.
CAGGATGAAGCAATTGCTGTTAATATTTATGCAGCTAATGTTGCAAATCTTTTTTCAGGT    B.cro.
CAAGATGAAGCAATTGCTGTTAATATTTATGCAGCTAATGTTGCAGATCTTTTTTCAGGT    B.his.
CAAGATGAAGCAATTGCTGTTAATAATTATGCAGCTAATGTTGCAAATCTTTTTTCAGGT    B.Spa.
CAGGATGAAGCAATTGCTGTCAATATTTATGCAGCTAATGTTGCAAATCTTTTTAATGGA    B.miy.
CAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTTGGTGGA    B.lon.
CAGGATGAGGCAATTGCTGTTAATATTTATGCATCTAATGTTGCAAATCTTTTTGCAGGT    B.her.
CAGGACGAAGCAATTGCTGTTAATATTTTTGCATCTAATATTGCAAATCTTTTTGCAGGT    B.cor.
CAGGATGAAGCAATTGCTGTTAATATTTATGCAGCTAATGTTGCAAACCTTTTTGCAGGT    B.tur.
CAGGATGAAGCAATTGCTGTTAATATTTATGCATCTAATGTTGCAAACCTTTTTGCAGGT    B.par.
CAAGATGAAGCAATTGCTGTAAATATTTATGCAGCTAATGTTGCAAATCTTTTTGCTGGT    B.afz.
CAAGATGAAGCGATTGCTGTAAATATTTATGCTGCTAATGTTGCAAATCTATTCTCTGGT    B.gar.
CAAGATGAAGCTATTGCTGTAAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCTGGT    B.bur.

                                                                    660
GAGGGTGCTCAA------CAAGTAGCTCCAGCTCAAGAGGGTGCACAGCAAGAAGGAGCA    B.rec.
GAGGGTGCTCAA------CAAGTAGCTCCAGCTCAAGAGGGTGCACAGCAAGAAGGAGCA    B.dut.
GAGGGTGCTCAA------CAAGTAGCTCCAGCTCAAGAGGGTGCACAGCAAGAAGGAGCA    B.cro.
GAGGGTGCTCAA------CAAGTAGCTCCAGCTCAAGAGGGTGCACAACAAGAAGGAGGA    B.his.
GAGGGTGCTCAA------CAAGTAGCTCCAGCTCAAGAGGGTGCACAACAAGAAGGAGCA    B.Spa.
GAAGGTGCTCAA----------GCAGCTCCAGCTCAAGAGGGAGCACAACAGGAGGGAGTT    B.miy.
GAAGGTGTTCAA----------GCGGCTCCAGCTCAAGAGGGTGCACAACAGGAGGGAGTT    B.lon.
GAAGGCGCTCAG----------GCTGCTCCAGTGCAAGAGATAGGACAGCAAGAGGAAGGT    B.her.
GAAGGTGCTCAG----------CCTGCTGTAGCT---GAAGGTGCACAAGAAGAAGGAGTT    B.cor.
GAAGGTGCGCAG----------GTTTCTCCAGCTCAGGAAGGTGCACAACAAGAGGGAGTT    B.tur.
GAAGGTGCGCAG----------GTTTCTCCAGCTCAGGAAGGTGCACAACAAGAGGGAGTT    B.par.
GAGGGAGCTCAAGCTGCTCAGGCTGCACCTGTTCAAGAGGGTGCTCAAGAAGAAGGAGCT    B.afz.
GAAGGAGCTCAGGCTGCTCAGACTGCACCTGTTCAAGAAGGAGCTCAACAAGAAGGAGCT    B.gar.
GAGGGAGCTCAAACTGCTCAGGCTGCACCGGTTCAAGAGGGTGTTCAACAGGAAGGAGCT    B.bur.

                                                                    720
CAAGCAGCTCCAGCTCCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAACTCTCCAGTTAAT    B.rec.
CAAGCAGCTCCAGCTCCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAACTCTCCAGTTAAT    B.dut.
CAAGCAGCTCCAGCTCCAGCATCAGCTCCAGCTCAAGGTGGAGTTAACTCTCCAGTTAAT    B.cro.
CAAGCAGCTCCGCCTCCAGCAGCAGCTCCAACTCAAGGTGGGGTTAACTCTCCAGTTAAT    B.his.
CAAGCAGCTCCGCCTCCAGCAGCAGCTCCAACTCAAGGTGGAGTTAACTCTCCAGTTAAT    B.Spa.
CAAGCAACTCC---TCCAGCAGCAGCTCCAACTCAAGGTGGAGTTAATTCTCCAATTAAT    B.miy.
CAAGCAGTTCCAGCTCCAGCAGCCGCTCCAGTGCAAGGTGGAGTTAATTCTCCAATTAAT    B.lon.
CAACC-------------------AGCTCCAGCTCAAGGTGGATTAGCTCTCCAATTAAT    B.her.
CAAGCAGCTCCAGCTCCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAATTCCCCAATTAAT    B.cor.
CAAGCAGCTCCAGCTCCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAATTCTCCAATTAAT    B.tur.
CAAGCTGGACAAGGTGCAGTAGCAGCTCCAGCTCAAGGTGGAGTTAATTCTCCAGTTAAT    B.par.
CAAGCTGCTCCAGCACCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAATTCACCAGTTAAT    B.afz.
CAAGCTGCTCCAGCACCAGCAGCAGCTCCAGCTCAAGGTGGAGTTAATTCTCCTGTTAAT    B.gar.
CAG-CAA--CCAACACCTGCTACAGCACCTACTCAAGGTGGAGTTAATTCTCCTGTTAAT    B.bur.
CAA-CAA--CCAGCACCTGTTACAGCGCCTTCTCAGGGTGGAGTTAATTCTCCTGTTAAT    B.bur.
CAA-CAG--CCAGCACCTGCTACAGCACCTTCTCAAGGCGGAGTTAATTCTCCTGTTAAT    B.bur.
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FIG. 3—*Continued.*

106

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                                                    1014
ATGATTGCACAAGCAAATCAAGTACCTCAA                                  B.rec.
ATGATTGCACAAGCAAATCAAGTACCTCAA                                  B.dut.
ATGATT                                                          B.cro.
ATGATT                                                          B.his.
ATGATT                                                          B.Spa.
ATGATTGCACAAGCAAATCAAGTGCCTCAATATGTATTGTCATTGCTTACATAG          B.miy.
ATGATT                                                          B.lon.
ATGATTGCACAAGCAAATCAAGTACCTCAA                                  B.her.
ATGATTGCACAAGCAAATCAAGTACCTCAA                                  B.cor.
ATGATTGCACAAGCAAATCAAGTACCTCAA                                  B.tur.
ATGATT                                                          B.par.
ATGATT                                                          B.afz.
ATGATT                                                          B.gar.
ATGATTGCGCAGGCTAATCAAGTTCCCCAATATGTTTTGTCATTGCTTACATAA          B.bur.
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FIG. 3—Continued.

G+C content of DNA. The G+C content of *B. recurrentis* was determined by high-performance liquid chromatography. DNA dissolved in water was denatured by boiling for 5 min and chilled on ice, and 10 μl (3 to 5 μg) of DNA was digested with P1 nuclease (DNA-GC kit; Seikagaku Kogyo, Tokyo, Japan) according to the manufacturer's instructions; 10-μl volumes of hydrolysate and a standard were analyzed by high-performance liquid chromatography (Shimadzu model LC-10AS instrument fitted with a type AQ-312 column; YMC Co., Ltd., Tokyo, Japan). The mobile phase was 10 mM phosphate buffer (pH 3.5) at a flow rate of 1.5 ml/min.

Comparison of *B. recurrentis* DNA with DNAs from other borreliae. DNA was extracted from cells harvested from BSK II medium by incubating the cells for 3 h at 56°C in lysis buffer containing 10 mM Tris (pH 8), 60 mM EDTA (pH 8), 5% SDS, and 50 μg of proteinase K per ml. The DNA was extracted with phenol, precipitated with ethanol, and resuspended in water. Following 30 min of incubation at 37°C with RNase A (2 μg/ml), the DNA was again precipitated with ethanol, resuspended in water, and stored at −20°C until it was used.

DNA was quantified and diluted to produce dilution curves ranging from 200 ng to 50 pg for each of the louse-borne relapsing-fever strains and other species of tick-borne and Lyme-associated borreliae. A nylon membrane (Hybond-N; Amersham International) was divided into a grid, and 1-μl volumes of each dilution of DNA were dotted onto the membrane, allowed to dry, and fixed with a UV cross-linker.

B. recurrentis A1 was prepared as a probe by using random prime labelling with fluorescein-11-dUTP (Gene Images kit; Amersham International) according to the manufacturer's instructions. Fluorescein-labelled probe was not stored before use. Biotin-labelled probe from the same strain was also prepared (Photoprobe; Vector Laboratories). Briefly, equal volumes of a solution containing 1 μg of denatured DNA per ml and Photoprobe were mixed, and the mixture was irradiated with a sunlamp for 15 min. Unreacted probe was deproteinated by adding 1 volume of 0.1 M Tris-HCl (pH 9.5). The mixture was extracted with butanol twice (discarding the upper organic phase) and precipitated with ethanol, and the pellet was dissolved in sterile distilled water. Biotin-labelled probe was stored at −20°C until it was used.

Nylon membranes were prehybridized for 30 min to 1 h at 60°C by using 0.125 to 0.3 ml of 5× SSC (diluted from a 20× SSC stock solution [1× SSC is 0.015 M sodium citrate plus 0.15 M sodium chloride])–0.1% (vol/vol) SDS–5% (wt/vol) dextran sulfate–1/20 dilution of liquid block (supplied with the Gene Images kit) per cm^2. Denatured probe was added to the prehybridization buffer (10 ng/ml), and hybridization was allowed to proceed overnight at 60°C.

Spent hybridization solution was discarded and replaced with preheated 1× SSC–0.1% SDS (2 to 5 ml/cm^2 of membrane) for 15 min at 60°C. This was replaced with 0.5× SSC–0.1% (vol/vol) SDS, and the preparation was incubated as described above. Bound probe was detected by using a Gene Images CDP-star detection module (Amersham International) for fluorescein-labelled probe according to the manufacturer's instructions. The preparations were exposed to hyperfilm MP (Amersham International) for 30 min before being developed. Membranes hybridized with photobiotinylated probe were first blocked for 2 h at 37°C with 10% (wt/vol) dried milk (Marvel) in TBS (0.1 M Tris [pH 7.5], 0.15 M sodium chloride) and then were detected by using Vectastain (Vector Laboratories), washed three times in TBS (10 min each time), and exposed to *p*-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (50 and 15 mg/ml, respectively, in 100 ml of Tris-magnesium chloride buffer [0.1 M Tris, 0.5 mM magnesium chloride; pH 9.5]). The reaction was stopped by immersing the membrane in distilled water.

Electron microscopy. *B. recurrentis* A1 was grown in BSK II medium, harvested by centrifugation at 3,500 × *g* for 20 min, and washed once in phosphate-buffered saline (3,500 × *g*, 20 min), and the pellet was resuspended and fixed in 3% (vol/vol) glutaraldehyde in 0.1 M cacodylate buffer, recentrifuged, and incubated at 4°C for 1 h. Cells were postfixed in 2% (wt/vol) osmium tetroxide, dehydrated in ascending grades of ethanol, and embedded in Spurr resin. Ultrathin sections were stained with aqueous uranyl acetate and Reynold's lead citrate prior to examination with an electron microscope.

To prepare for negative staining, the pellet was resuspended (1:9) in either 2% (vol/vol) aqueous phosphotungstic acid (neutralized with 1 M potassium hydroxide) supplemented with 0.01% (vol/vol) glycerol or 1% ammonium molybdate neutralized with concentrated ammonium hydroxide. One drop of suspension was allowed to air dry at room temperature on carbon-coated Formvar films supported on copper grids prior to examination.

RESULTS

SDS-PAGE. Coomassie blue-stained gels revealed much heterogeneity in the area at and below 45 kDa. The protein patterns divided *B. recurrentis* strains into four groups (Fig. 1). One group (four strains) had a major band at 45 kDa; the second group consisted of a single strain with a major band at 40 kDa; the third group (four strains) had a predominant band at 35.5 kDa; and the remaining nine isolates produced major bands between 21.5 and 24 kDa. The last group was subdivided into four subgroups to accommodate strains with identical molecular weights. Subgroup 4a had one member, subgroup 4b was comprised of two strains, subgroup 4c had four members, and subgroup 4d contained two strains (Fig. 1).

Immunoblotting with monoclonal antibody H9724. A band located at 41 kDa was detected in all isolates of *B. recurrentis* (data not shown). The size of the band was determined by comparison to molecular weight standards and to strain B31 of *Borrelia burgdorferi* sensu stricto.

PFGE. All of the strains had a linear chromosome which migrated in the gel and had a size of approximately 1 Mb. The patterns observed for *B. recurrentis* were distinct from those of tick-borne relapsing-fever and Lyme-associated borreliae (Fig. 2). All of the isolates of *B. recurrentis* carried a large plasmid of approximately 192 kb (range, 183 to 194 kb); however, the patterns observed for 25- to 62-kb plasmids showed more heterogeneity. An 11-kb plasmid was present in all strains. This band was not always detectable on ethidium bromide-stained gels; however, it could be visualized following hybridization. All of the strains produced one of five plasmid patterns (Table 2). The presence of a major protein band belonging to group 4 (24 kDa or less) and plasmid types 3 to 5 was significantly different from the results obtained for strains that exhibited higher molecular weights for major proteins and had plasmid types 1 or 2 (chi-square test with Yates correction, 0.0009). The sizes of the plasmids found in each *B. recurrentis* group are given in Table 3. The chromosomal and plasmid bands between 25 and 194 kb resolved as sharp bands and thus probably represent linear DNA (10, 13).

DNA sequencing. The DNA sequence analysis revealed distinct signature nucleotides which distinguished Lyme-associ-

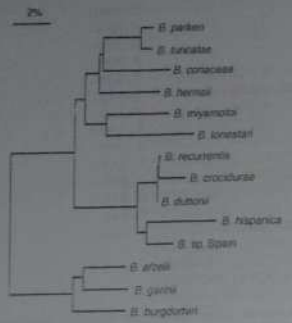


FIG. 4. Phylogenetic tree for the genus *Borrelia* constructed by using the neighbor-joining method and flagellin sequences.

ated borreliae from relapsing-fever borreliae; for example, there were signature nucleotides at positions 130, 193, 195, 196, 287, and 288. The relapsing-fever strains from Africa could be distinguished from those found in America by base changes at positions 65, 70, 190, and 200. *B. recurrentis* could be differentiated from *Borrelia duttonii* and indeed all of the other spirochetal strains analyzed by differences at positions 242, 525, 959, and 1481. Interestingly, all of these differences were substitutions of thymidine for cytosine. These data are based on those of Marti Ras et al. (26). Details of the complete 16S rRNA gene sequences for relapsing-fever and Lyme-associated borreliae have been reported elsewhere (26), and the sequence of *B. recurrentis* A1 has been deposited in the GenBank database under accession no. U42300.

A 984-base portion of the flagellin gene was sequenced, and the results were compared with *B. duttonii* 406K data. Only one base substitution was found, at position 497; adenosine was present in *B. recurrentis*, while *B. duttonii* contained a guanidine. The flagellin nucleotide sequence data have been deposited in the DDBJ, EMBL, and GenBank databases under accession no. D86618. A multiple alignment of these data with data for relapsing-fever, Lyme-associated, and other borreliae is shown in Fig. 3. A phylogenetic tree was constructed by using the neighbor-joining method, which revealed a cluster comprised of *B. recurrentis*, *B. duttonii*, and *Borrelia crocidurae* (Fig. 4). This cluster was more distantly related to *Borrelia hispanica* and the recently described Spanish relapsing-fever species (1). However, the American relapsing-fever strains clustered together and with *Borrelia miyamotoi* and *Borrelia lonestari*. The Lyme-associated species formed a third distinct cluster (Fig. 4).

G+C content of *B. recurrentis* DNA. The G+C content of strain A1 was 28.4 mol%. This is within the range of 27 to 32 mol% reported for members of the genus *Borrelia* (14).

Comparison of *B. recurrentis* DNA with DNAs from other borreliae. Both probe labelling and detection methods gave similar results for whole DNA-DNA hybridizations. *B. recurrentis* failed to react with the tick-borne relapsing-fever strains of *Borrelia hermsii*, *Borrelia parkeri*, and *Borrelia turicatae*. None of the Lyme-associated borreliae (*B. burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia japonica*, group VS116) hybridized with *B. recurrentis*, even when 200 ng of target DNA was used. All strains of *B. recurrentis* hybridized with the probe, detecting levels of 1 to 10 ng of DNA.

Electron microscopy. Electron microscopy revealed characteristic borrelial cells (Fig. 5a). The cells had tapering ends (Fig. 5b) and an average wavelength of 1.8 μm with an amplitude of 0.8 μm. The length was variable, but was approximately 12 to 22 μm. The cells had 8 to 10 periplasmic flagella and diameters of 0.27 to 0.34 μm (Fig. 5c through e). Flagellar counts of transverse sections revealed 10 flagella (Fig. 5c), while flagellar counts of the ends of cells revealed 8 flagella; however, insertion points could not be distinguished (Fig. 5d). Negatively stained preparations of the central portion of spirochetal cells revealed 16 flagella, and presumably the flagella were released at the point of crossover.

DISCUSSION

In general, there was similarity between the previously described morphology (20) and the electron microscopic results of this study. The cells had tapering ends (Fig. 5b) typical of those described for the genus *Borrelia*; however, the ends were slightly less pointed than those described for *B. burgdorferi* (21, 22). The lengths of the cells and the diameters were consistent with earlier findings (20). The wavelength of strain A1 was identical to the wavelength reported earlier (20); however, the amplitude was 0.8 μm, in contrast to the 0.3-μm amplitude previously reported (20). Another major difference was the number of flagella. Strain A1 of *B. recurrentis* had between 8 and 10 periplasmic flagella. The previously studied strains were reported to possess 15 to 20 and 25 to 30 unsheathed flagella (20, 22). The strains described in previous studies were multiply passaged and mouse adapted, and the original country of origin was not stated. Higher numbers of flagella (15 to 26 flagella) have been observed in a newly described tick-borne relapsing-fever *Borrelia* species isolated from Spain (1). Strain differences may account for the difference in numbers of flagella.

It is likely that infestation by a multiplicity of lice may be a more efficient vector for relapsing fever than a single tick bite. Also, once borreliae have been introduced into a human host, there is no need for flagellum-associated attachment determinants. Hence, a possible explanation for the lower number of flagella in *B. recurrentis* than in the tick-borne strains resides in the presumption that the louse is a more efficient disease vector than the tick. Interestingly, flagellum-less mutants of *B. burgdorferi* were as capable as an isogenic flagellated strain in inducing protective antibodies in mice (29).

The dissimilarity between *B. recurrentis* and American tick-borne relapsing-fever strains, Lyme-associated strains, and other borreliae was highlighted by the whole DNA-DNA hybridization studies. These studies demonstrated a high degree of similarity among the 18 culturable strains of *B. recurrentis*.

Comparison of the DNA sequence of *B. recurrentis* with the sequence of a strain of an African tick-borne relapsing-fever borrelia (*B. duttonii*) revealed that these organisms are closely related, but differ in 4 bp in the 16S rRNA gene (26) and in 1 bp in the flagellin gene. The differences in the 16S RNA gene and the flagellin gene sequences of *B. recurrentis* and *B. duttonii* suggest that there is selective pressure to maintain the flagellin gene without mutation. Phylogenetic trees based on either the 16S RNA gene sequence or the flagellin gene sequence gave similar cluster patterns, confirming the relationship of *B. recurrentis*, *B. duttonii*, and *B. crocidurae*, which are more distantly related to relapsing-fever strains of Spanish origin. American relapsing-fever strains fell into a separate cluster together with *B. miyamotoi* and *B. lonestari*, while the Lyme disease-associated borreliae clustered separately. Whether the similarity between *B. recurrentis* and *B. duttonii* reflects

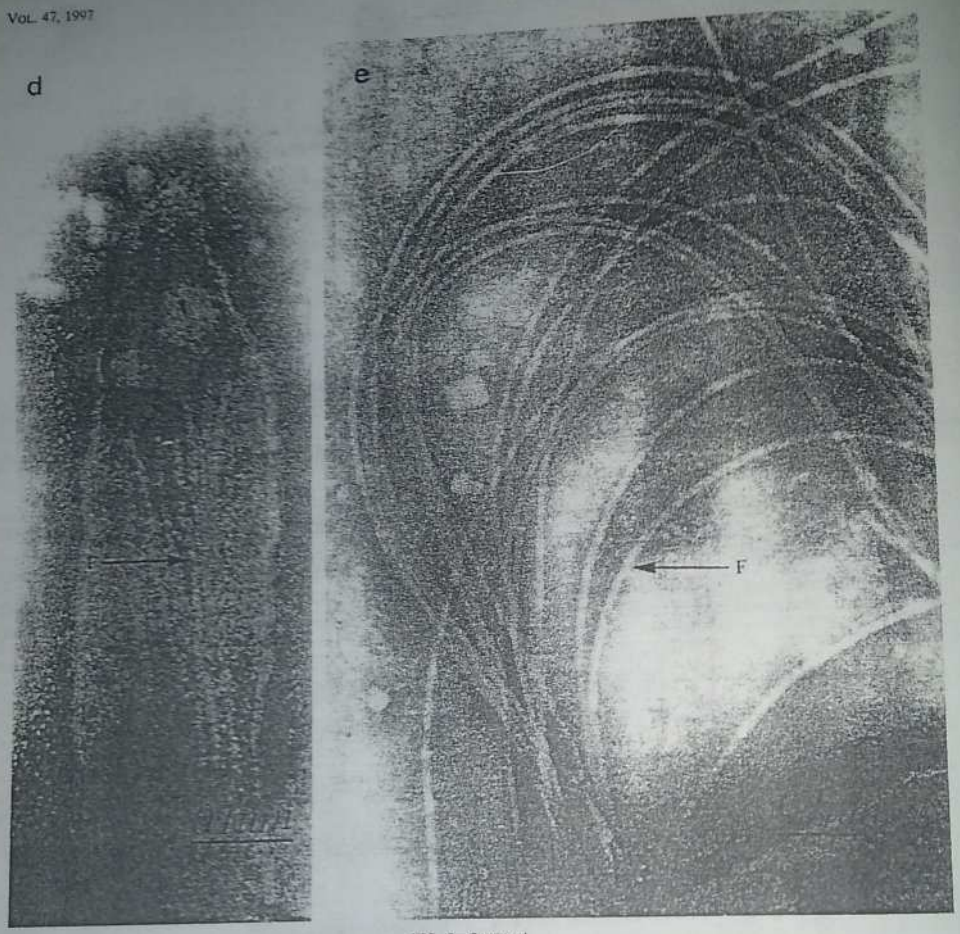


FIG. 5—*Continued.*

borrelial strains (6, 7, 13, 23). High-molecular-weight minichromosomes that are 180 and 170 kb long have been described previously in *B. hermsii* and *B. turicatae*, respectively (13), and were also demonstrated in *B. recurrentis* in our analysis. However, the high-molecular-weight minichromosomes in *B. recurrentis* were slightly larger (183 to 194 bp).

Both linear plasmids and linear chromosomes have been detected in relapsing-fever borreliae. Replicons with similar structures are more frequently associated with eukaryotic cells than with prokaryotes. Molecular characterization of these structures may provide clues to the evolutionary relationship between prokaryotes and eukaryotes and may also provide evidence for genetic exchange between these kingdoms (19). Workers have noted similarity between the AT-rich sequences at the ends of the specific linear plasmids of *B. burgdorferi* and tick-borne relapsing-fever strains and iridovirus (8, 18), suggesting that the linear replicons may have a common origin and may have been subject to transkingdom exchange. This is more likely as the tick is a common vector of both African swine fever (iridovirus) and relapsing fever. Since *B. recurrentis* is louse borne, the possible presence of these common sequences would have taxonomic and evolutionary implications, particularly in illuminating the relationship to tick-borne disease.

The serospecificity of borreliae in each clinical relapse is determined by the outer membrane proteins. The genetic mechanism of this antigenic variation has been studied in previously isolated borreliae other than *B. recurrentis* (5). It has been suggested that there is a minichromosome shuttle mechanism similar to that found with sleeping sickness trypanosomes. In *B. hermsii* the variable membrane lipoprotein (VMP)

is the responsible antigen (28). Studies of *B. hermsii* have suggested three possible mechanisms of VMP variation: (i) a complete silent *vmp* gene may replace a complete expressed *vmp* by intermolecular recombination; (ii) a silent complete VMP gene located on another plasmid may partially replace expressed *vmp* (the donor VMP sequence may be an incomplete pseudogene); or (iii) the donor gene may be on the same replicon as the active *vmp*, and the serotype switch may be achieved by intramolecular recombination in the expressed plasmid (28).

Areas of homology exist at the telomeres of the different *vmp* genes of *B. hermsii* and, indeed, have been shown to have homology with the telomeres of other relapsing-fever species, including *B. turicatae* and *B. crocidurae* (5a). Similarity has also been demonstrated between these telomeric sequences and those of other borreliae, including the OspC gene of *B. burgdorferi* sensu lato (24, 25) and a VMP-like gene in *B. myamotoi* (17). Future use of primers for conserved telomeric sequences to amplify and sequence the VMP genes of our cultivable strains would allow us to compare these organisms with other relapsing-fever borreliae. Similarly, probes would allow us to locate the genes responsible for the antigenic variants of *B. recurrentis*. It would then be possible to characterize different antigenic variants and identify whether the differences in observed plasmid profiles seen in this study are associated with major genetic reorganizations. This has not been the case with *B. hermsii*. Both protein profiles and plasmid patterns of *B. recurrentis* allowed isolates to be grouped. The association seen in Table 2 suggests that these two events may be related. It is plausible that as strains undergo antigenic variation, the major protein responsible for serotype specificity is altered, and this event may be accompanied by a concomitant genetic reorganization, resulting in a changed plasmid profile. Up to five relapses have been reported with louse-borne relapsing fever (9); whether this correlates with the five plasmid profiles seen in Fig. 2 remains speculative.

Further study of *B. recurrentis* present in clinical material and lice and a comparison of the results with results obtained with our cultivable strains would establish whether certain culture-adapted variants are selected by our in vitro methods (a culture-associated VMP of approximately 20 kDa has been described for *B. hermsii* [27]) and would also establish the range of VMPs expressed in primary disease (relapse variants would require the use of animal models) and the range of VMPs expressed in the louse vector.

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REFERENCES

1. Anda, P., W. Sánchez-Yebra, M. del Mar Vitutia, E. P. Pastrana, I. Rodriguez, N. S. Miller, P. B. Backenson, and J. L. Benach. 1996. A new *Borrelia* species isolated from patients with relapsing fever in Spain. Lancet 348:162–165.
2. Baltazard, M., M. Bahamanyar, and C. Mofidi. 1947. Fièvres Récurrentes transmises a la fois par ornithodores et par poux. Ann. Inst. Pasteur 73:1066–1071.
3. Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. 57:521–525.
4. Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessier. 1986. A borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. 52:549–554.
5. Barbour, A. G. 1990. Antigenic variation of a relapsing fever *Borrelia* species. Annu. Rev. Microbiol. 44:155–171.
5a. Barbour, A. G. 1997. Personal communication.
6. Baril, C., C. Richaud, G. Baranton, and I. S. Saint-Girons. 1989. Linear chromosome of *Borrelia burgdorferi*. Res. Microbiol. 140:507–516.
7. Bergström, S., A. G. Barbour, C. F. Garon, P. Hindersson, I. Saint-Girons, and T. G. Schwan. 1991. Genetics of *Borrelia burgdorferi*. Scand. J. Infect Dis. Suppl. 77:102–107.
8. Bergström, S., C. F. Garon, A. G. Barbour, and J. MacDougall. 1992. Extrachromosomal elements of spirochetes. Res. Microbiol. 143:623–628.
9. Calern, C. 1946. Relapsing fever on the Isthmus of Panama. Am. J. Trop Med. 26:761–769.
10. Casjens, S., M. Delange, H. L. Ley III, P. Rosa, and W. M. Huang. 1995. Linear chromosomes of Lyme disease agent spirochetes: genetic diversity and conservation of gene order. J. Bacteriol. 177:2769–2780.
11. Cutler, S. J., D. J. M. Wright, and V. H. Luckhurst. 1993. Simplified method for the interpretation of immunoblots for Lyme borreliosis. FEMS Immunol. Med. Microbiol. 6:281–286.
12. Cutler, S. J., D. Fekade, K. Hussein, K. A. Knox, A. Melka, K. Cann, A. R. Emilianus, D. A. Warrell, and D. J. M. Wright. 1994. Successful in-vitro cultivation of *Borrelia recurrentis*. Lancet 343:242. (Letter.)
13. Ferdows, M. S., P. Serwer, G. A. Griess, S. J. Norris, and A. G. Barbour. 1996. Conversion of a linear to a circular plasmid in the relapsing fever agent *Borrelia hermsii*. J. Bacteriol. 178:793–800.
14. Fukunaga, M., Y. Takahashi, Y. Tsuruta, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao. 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int. J. Syst. Bacteriol. 45:804–810.
15. Fukunaga, M., and Y. Koreki. 1996. A phylogenetic analysis of *Borrelia burgdorferi* sensu lato isolates associated with Lyme disease in Japan by flagellin gene sequence determination. Int. J. Syst. Bacteriol. 46:416–421.
16. Fukunaga, M., K. Okada, M. Nakao, T. Konishi, and Y. Sato. 1996. Phylogeny of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. Int. J. Syst. Bacteriol. 46: 898–905.
17. Hamase, A., Y. Takahashi, K. Nohgi, and M. Fukunaga. 1996. Homolog of variable major protein genes between *Borrelia hermsii* and *Borrelia miyamotoi*. FEMS Microbiol. Lett. 140:131–137.
18. Hinnebusch, J., and A. G. Barbour. 1991. Linear plasmids of *Borrelia burgdorferi* have a telomeric structure and sequence similar to those of a eukaryotic virus. J. Bacteriol. 173:7233–7239.
19. Hinnebusch, J., and K. Tilly. 1993. Linear plasmids and chromosomes in bacteria. Mol. Microbiol. 10:917–922.
20. Hovind Hougen, K. 1974. Electron microscopy of *Borrelia morisetti* and *Borrelia recurrentis*. Acta Pathol. Microbiol. Scand. Sect. B 82:799–809.
21. Hovind Hougen, K. 1984. Ultrastructure of spirochetes isolated from *Ixodes ricinus* and *Ixodes dammini*. Yale J. Biol. Med. 57:543–548.
22. Hulínská, D., J. Jirous, M. Valesová, and J. Herzogová. 1989. Ultrastructure of *Borrelia burgdorferi* in tissues of patients with Lyme disease. J. Basic Microbiol. 29:73–83.
23. Kitten, T., and A. G. Barbour. 1992. The relapsing fever agent *Borrelia hermsii* has multiple copies of its chromosome and linear plasmids. Genetics 132:311–324.
24. Marconi, R. T., D. S. Samuels, T. G. Schwan, and C. F. Garon. 1993. Identification of a protein in several *Borrelia* species which is related to OspC of the Lyme disease spirochetes. J. Clin. Microbiol. 31:2577–2583.
25. Margolis, N., D. Hogan, W. Cieplak, T. G. Schwan, and P. A. Rosa. 1994. Homology between *Borrelia burgdorferi* OspC and members of the family of *Borrelia hermsii* variable major proteins. Gene 143:105–110.
26. Marti Ras, N., B. Lascola, D. Postic, S. J. Cutler, F. Rodhain, G. Baranton, and D. Raoult. 1996. Phylogenesis of relapsing fever *Borrelia*. Int. J. Syst. Bacteriol. 46:859–865.
27. Meier, J. T., M. I. Simon, and A. G. Barbour. 1985. Antigenic variation is associated with DNA rearrangements in a relapsing fever borrelia. Cell 41:403–409.
28. Restrepo, B. I., C. J. Carter, and A. G. Barbour. 1994. Activation of a vmp pseudo gene in *Borrelia hermsii*: an alternative mechanism of antigenic variation during relapsing fever. Mol. Microbiol. 13:287–299.
29. Sadziene, A., P. A. Thompson, and A. G. Barbour. 1996. A flagella-less mutant of *Borrelia burgdorferi* as a live attenuated vaccine in the murine model of Lyme disease. J. Infect. Dis. 173:1184–1193.

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LETTER TO THE EDITORS-IN-CHIEF

ABSENCE OF THE FACTOR V LEIDEN MUTATION IN ETHIOPIANS

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Several reports have shown striking geographic differences in the distribution of the factor V Leiden allele where a mutation at nucleotide 1691 causes a substitution of Arg^{506} to Gln^{506} in the factor V protein (1-6). In a study by Rees et al. (2) the mutant allele was not detected in subjects from the African countries Kenya, Senegal, and Zambia, whereas the allele frequency varied from 0 to 7.0 % in the European countries studied.

The factor V Leiden mutation was analysed in 101 healthy, unrelated Ethiopian men and women living in Addis Ababa. Genomic DNA from whole blood was used to amplify part of the factor V gene by polymerase chain reaction as previously described (7-8). The G to A mutation at codon 506 was detected using the restriction enzyme *MnlI* followed by agarose gel electrophoresis and size determination of the reaction products.

None of the 202 alleles from the Ethiopian subjects carried the Leiden mutation. Our results support the previously reported heterogenous distribution of the factor V Leiden allele, and confirm that it is absent in an ethnic group of African origin.

REFERENCES

1. DAHLBÄCK, B. Resistance to activated protein C, the Arg^{506} to Gln mutation in the factor V gene, and venous thrombosis. Thromb Haemost *73*, 739-742, 1995.

Key words: activated protein C resistance, factor V, mutation, Ethiopian
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Amir sultan (MD), 2014

2. REES, D.C., COX, M., and CLEGG, J.B. World distribution of factor V Leiden. Lancet *346*, 1133-1134, 1995.
3. ARRUDA, V.R., ANNICHINO-BIZZACCHI, J.M., COSTA, F.F., and REITSMA, P.H. Factor V Leiden (FVQ506) is common in Brazilian population. Am J Hematol *39*, 242-243, 1995.
4. OZAWA, T., NIIYA, K., and SAKURAGAWA, N. Absence of factor V Leiden in the Japanese. Thromb Res *81*, 595-596, 1996.
5. FERRER-ANTUNES, C., PALMEIRO, A., GREEN, F., ROSA, H., FELICIANO, B., and SILVA, E. Polymorphisms of fibrinogen, factor VII and factor V genes comparison of allele frequencies in different ethnic groups. Thromb Haemost *73*, 1379, 1995.
6. TAKAMIYA, O., ISHIDA, F., KODAIRA, H., and KITANO, K. APC-resistance and MnlI genotype (Gln 506) of coagulation factor V are rare in Japanese population. Thromb Haemost *74*, 996, 1995.
7. BERTINI, R.M., KOELMAN, B.P.C., KOSTER, T., ROSENDAAL, F.R., DIRVEN, R.J., de RONDE, H., van der VELDEN, P.A., and REITSMA, P.H. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature *369*, 64-67, 1994.
8. KIRSCBAUM, N.E., and FOSTER, P.A. The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance. Thromb Haemost *74*, 874-878, 1995.

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SYMPTOMATIC TREATMENT OF NEUROLATHYRISM WITH TOLPERISONE HCL (MYDOCALM): A RANDOMIZED DOUBLE BLIND AND PLACEBO CONTROLLED DRUG TRIAL

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ABSTRACT: *The efficacy and safety of oral Tolperisone HCL was evaluated in double blind, placebo-controlled, randomized trial in 72 patients with neurolathyrism in stages I, II, and III of the disease at Kolla Duba Health Centre of Dembia District of North Gondar between January and April 1995. Taken orally daily for 12 weeks, tolperisone HCL (Mydocalm) in a dose of 150 milligrams (mgs) twice daily significantly improved subjective complaints such as muscle cramps, heaviness of the legs, startle attacks, flexor spasms and repeated falls. An overall subjective improvement was observed in 75% of the patients on tolperisone HCL and 39% of the placebo group (P=0.002). When objectively assessed spastic muscle tone in the abductors, stiffness of Achilles and spontaneous ankle clonus were significantly reduced in tolperisone HCL group (P values = 0.001, 0.04, and 0.0001, respectively). Walking ability and speed of walking was also significantly improved. The drug is most effective in relieving symptoms of stage I and stage II disease. Some adverse effects like muscle pain, generalized body weakness and dizziness were recorded in patients taking the drug but all were minor and self limited, none requiring discontinuation of treatment. It is concluded that tolperisone is a well tolerated and efficacious drug for symptomatic treatment of neurolathyrism.*

INTRODUCTION

Neurolathyrism is a neurotoxic disorder caused by prolonged and heavy consumption of seeds of the grass-pea *Lathyrus sativus* (1-7). The toxin responsible is the neuro-excitatory, non-protein amino acid named beta N-Oxalyl-alpha and beta Diamino Propionic acid (B-ODAP), synonym, Beta-N-Oxylylamino-L-Alanine Acid (BOAA) (2-4). It affects the central motor system (pyramidal cells and tracts) with the resulting irreversible, usually non progressive spastic paraparesis or rarely quadriparesis (4,5).

Lathyrism has been known since the time

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of Hippocrates, 460-377 BC, (6). Although this neurological disorder has occurred in the past in epidemics in North Africa, in the Middle East, Asia and Indian subcontinent, it is now endemic only in Ethiopia, India and Bangladesh (1,3,7,8).

The disease has been known for many years in Ethiopia where it has been given descriptive local names like "Sebre" "Ye guaya beshita" or "guaya" , all with the implications of "leg breaking" (paralytic) nature (9). The first scientific description of lathyrism in Ethiopia was done by Ferro-Luzzi in 1947 from Serae area of Eritrea where a locust-caused famine had occurred in 1946 (10). Subsequently Rizzotti in 1952 reported lathyrism in patients from Northern and Western Ethiopia (11).

The disease received renewed attention in 1976/77 because of a famine caused epidemics of lathyrism that occurred in Northern and Central Ethiopia especially in Dembia and Fogera subdistricts of Gondar (4,7,8,12).

A complete door-to-door survey that was carried out in 1985-88 in the same areas endemic for lathyrism revealed an estimated mean disease prevalence of 1-3% (12). Most patients developed the disease in the epidemics of 1976/77.

Sporadic cases also occur. The mean annual incidence rate of neurolathyrism in Dembia district, North Gondar was estimated to be 1.7/10,000 (12).

At present, there are thousands of patients with the disease, but without any medical intervention, as there is no established mode of therapy. Neurolathyrism mainly affects, young and productive farmers who need their physical fitness for survival, so it has a profound socio-economic implication (8).

Drug trials have shown, tolperisone HCL, (Mydocalm, Chemical name: 2,4 - dimethyl- 3 - piperidino propriophenone, Gedeon Richter, Budapest, Hungary), to be effective in such patients in Bangladesh alleviating the symptoms of lathyrism (13).

The present study therefore aimed at doing a similar therapeutic trial in Ethiopian patients by a randomized, double blind and placebo controlled trial, before it is recommended for clinical use.

PATIENTS AND METHODS

This double blind, placebo controlled randomized drug trial was carried out between January 1995 and April 1995 at Kolla-Duba health centre of Dembia district located 30 km away from Gondar town. The later is found 750 Km north-west of the capital, Addis Ababa. Kolla-Duba was chosen because of its proximity to Gondar town and the availability of many neurolathyrism patients in the surrounding villages.

The sample size was calculated based on the following assumptions: main outcome measures of reduction of muscle spasticity and improvement of muscle power, treatment effect of tolperisone HCL to be 50% and placebo effect of 15%. After Arcsine transformation of the expected success, 36 patients would be required in each group with power of 85% and confidence interval of 95% (14, EPI INFO 5 Software).

We diagnosed 300 patients who came from 34 farmer's associations and four rural towns of Dembia district of North Gondar who met the criteria for neurolathyrism. We have then stratified these patients into 4 grades depending on the degree of disability according to Acton (2), with some modification by Redda (8):

Stage 1: mild spastic gait with no use of stick; increased stiffness and exaggerated deep tendon reflexes (DTR) of the lower limbs; ankle clonus present, Babinski's sign

present.

Stage 2: spastic gait, with use of one stick, lower limbs exhibit increased stiffness; mild rigidity; exaggerated DTR; ankle clonus present, Babinski's sign present.

Stage 3: spastic gait, with use of two sticks; crossed adductor gait; lower limbs exhibit exaggerated DTR; ankle clonus present; Babinski's sign present.

Stage 4: crawling or bedridden state; loss of leg use, with contracture; arms strong; pyramidal signs present.

We randomly selected 30 patients each from stages I and II and 12 patients from stage III. Those with stage 4 disease were not included in the study for technical reasons. Patients from the three stages were randomly assigned to either tolperisone HCL (experimental) or vitamin B complex (placebo) treatment groups using random numbers generated by a calculator; each group consisted of 36 patients.

Selection and exclusion criteria: Patients were diagnosed to have neurolathyrism when found to have leg weakness and spasticity of subacute or insidious onset and a history of heavy grass pea consumption prior to and/or at the onset of the disease (8,12).

Exclusion criteria included sensory deficit in the extremities, marked asymmetric leg weakness, continuing bladder and bowel dysfunction and presence of peripheral gangrene in the legs (12). Patients with any known major organic illnesses like clinically overt liver cirrhosis and those who disagreed to participate in the study were also excluded.

The ethical approval was obtained from the ethical, research and publication committee of the Gondar College of Medical Sciences. We have also obtained written consent from all patients enrolled in the study.

A pilot study using the experimental drug, tolperisone HCL (Mydocalm) 150 mg twice daily with close monitoring was carried for 4 weeks on 10 patients to see the safety of the drug when used on an out patient basis, and the applicability of the parameters used to measure outcome.

The study was designed by a senior consultant Neurologist (second investigator) and carried out at the health centre by an Internist (first investigator). There were also two general practitioners for technical help. Periodic telephone consultations were obtained from the neurologist.

Patients in the active treatment group received tolperisone HCL (Mydocalm, Chemical name: 2,4 - dimethyl-3-piperidino propriophenone, Gideon Richter, Budapest, Hungary), 150 mg twice daily. Those in the placebo group received vitamin B complex administered one table twice daily, which is of the same size and colour to the active treatment, both dispensed in identical plastic bags of International Dispensary Association.

The treatment was given by two general practitioners while the evaluation was done by the second investigator every other week. Both the investigators as well as the study subjects were blinded with regard to the types of treatment given.

Assessment of the patients was done in two ways:

1. Using patients own subjective assessment based on the following parameters: muscle cramps , heaviness of the legs, startle attacks and flexor spasms, all graded as none, mild, moderate or severe; frequency of falls quantified as none, occasional, few and often; need for support graded as none, one cane or two canes; walking ability graded as no change, good improvement or very good improvement and patients overall subjective assessment graded

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2. REES, D.C., COX, M., and CLEGG, J.B. World distribution of factor V Leiden. Lancet *346*, 1133-1134, 1995.
3. ARRUDA, V.R., ANNICHINO-BIZZACCHI, J.M., COSTA, F.F., and REITSMA, P.H. Factor V Leiden (FVQ506) is common in Brazilian population. Am J Hematol *39*, 242-243, 1995.
4. OZAWA, T., NIIYA, K., and SAKURAGAWA, N. Absence of factor V Leiden in the Japanese. Thromb Res *81*, 595-596, 1996.
5. FERRER-ANTUNES, C., PALMEIRO, A., GREEN, F., ROSA, H., FELICIANO, B., and SILVA, E. Polymorphisms of fibrinogen, factor VII and factor V genes comparison of allele frequencies in different ethnic groups. Thromb Haemost *73*, 1379, 1995.
6. TAKAMIYA, O., ISHIDA, F., KODAIRA, H., and KITANO, K. APC-resistance and MnlI genotype (Gln 506) of coagulation factor V are rare in Japanese population. Thromb Haemost *74*, 996, 1995.
7. BERTINI, R.M., KOELMAN, B.P.C., KOSTER, T., ROSENDAAL, F.R., DIRVEN, R.J., de RONDE, H., van der VELDEN, P.A., and REITSMA, P.H. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature *369*, 64-67, 1994.
8. KIRSCBAUM, N.E., and FOSTER, P.A. The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance. Thromb Haemost *74*, 874-878, 1995.

There was a significant difference between the two treatment groups with regard to the need for support at the 12th week: no cane (grade I), 24 (67%) patients in the experimental and 15 (42%) patients in the placebo; one or two canes (grades II or III) 12 (33%) patients in the experimental and 21 (58%) patients in the placebo treatment groups, [(P = 0.03), RR (95% CI) = 0.57 (0.33,0.98)].

The likelihood of having improvement in walking ability was significantly higher in tolperisone HCL group, P = 0.001, RR (95% CI) = 0.38 (0.22, 0.68). Walking ability improved in the experimental treatment group as early as the 2nd week of treatment but more marked at the 12th week. No patient attained very good improvement in the placebo group.

The overall subjective assessment at 12th week revealed that 22 (61%) of placebo treated patients reported no change while 9 (25%) of tolperisone HCL treated patients reported so, P=0.002, RR (95% CI) = 0.40 (0.21, 0.74).

Twenty seven (75%) of patients in tolperisone HCL group were found to have subjective improvement at the end of the study while the placebo group showed 14 (38%) improvement. (P = 0.002). Most of the patients, 10 out of 14, who claimed to have improvement in the placebo group belonged to grade I.

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Table 1. The demographic and clinical characteristics of patients in the two groups, Dembia District, North Gondar, January - April 1995.

Variables	Tolperisone HCL	Vitamin B complex	P-value
Age (years)			
Median	27	25	
Range	12-65	10-63	
Male:Female	3.5:1.0	5.0:1.0	0.5
Sex			
Male	28 (78)	30 (83)	0.5
Female	8 (22)	6 (17)	
Year of attack			
1976/77	21 (58)	19 (53)	0.6
Thereafter	15 (42)	17 (47)	
Food shortage and ate a lot of "Guaya"			
Yes	36 (100)	36 (100)	1.0
No	-	-	
Percentage of "Guaya" consumed			
≤ 50	12 (33)	15 (42)	0.5
> 50	24 (67)	21 (58)	
Degree of disability			
Grade I	15 (42)	15 (42)	1.0
Grade II	15 (42)	15 (42)	1.0
Grade III	6 (17)	6 (17)	1.0

N.B. Numbers in parenthesis indicate percentages.

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Table 2. Initial subjective evaluation of the two treatment groups, Dembia District, North Gondar, January - April 1995.

Parameters	Tolperisone HCL No. (%)	Placebo No. (%)	P-value
Muscle Gramp			
None or mild	9 (25)	11 (30)	0.6
Moderate or severe	27 (75)	25 (70)	
Heavy legs			
None or mild	9 (25)	10 (28)	0.7
Moderate or severe	27 (75)	26 (72)	
Startle attacks			
None or mild	15 (42)	14 (39)	0.8
Moderate or severe	21 (58)	22 (61)	
Flexor spasm			
None or mild	8 (22)	11 (31)	0.4
Moderate or severe	28 (78)	25 (70)	
Repeated falls			
None or mild	16 (44)	14 (39)	0.6
Moderate or severe	20 (56)	22 (61)	
Need for support			
No cane	15 (42)	15 (42)	1.0
One or two canes	21 (58)	21 (58)	

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The proportion of patients with severe muscle cramp, severe heaviness of the legs, severe startle attacks, severe flexor spasm and frequent falls were lowered with treatment but the differences were not statistically significant, (P values = 0.2, 0.09, 0.08, 0.3 and 0.1 respectively).

Table 5 shows the results of physician's assessment at the 12th week. Objective assessment of power in the legs at the end of the treatment showed that 31 (86%) of patients in the tolperisone HCL group had either normal or moderately strong muscle power while 13 (36%) of patients in the placebo group had moderately strong muscle power, and the difference was significant, (P = 0.00001).

All three patients (one in the experimental and two in the placebo) who initially presented with very weak muscle power remained so after treatment. Twenty (56%) of patients in the tolperisone group attained a normal power while no patient in the placebo group has attained normal power, (P < 0.001).

Ankle clonus was absent at the 12th week in 22 (61%) of patients in tolperisone HCL treatment group compared with 6 (17%) in placebo, (P=0.0001). Those with frequent spontaneous clonus decreased from 8 (22%) to 4 (11%) in tolperisone HCL group and from 11 (31%) to 7 (19%) in the placebo group, but the difference was not significant, (p = 0.3).

Adductor muscle spasticity and Achilles tendon stiffness at the end of the trial was significantly reduced to none or milder degree in experimental treatment group. Those with 4+ adductor spasticity and Achilles stiffness didn't show significant difference between the two treatment groups, (P = 0.1).

Nine patients changed their grade from II to I in tolperisone HCL treatment group while no patient did so in placebo group at week twelve, (P = 0.03). Two patients from tolperisone HCL group were changed from grade III to grade II while no patient in placebo group did so at the end of treatment.

Both speed of walking and general walking ability were significantly improved with experimental treatment. The effect of treatment started at the 2nd week and progressively increased up to the 4th to 8th weeks. Thereafter there was no change in the response.

The side effects encountered are shown in Table 6. All the complaints subsided after the 4th week, none of them required discontinuation of treatment. One patient in the tolperisone HCL group required antiacid treatment for dyspepsia.

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Table 5. Grading of clinical response: final physician's assessment, North Gondar, January - April 1995.

Parameters	Tolp. HCL No. (%)	Placebo No. (%)	RR	(95% CI)	p value
Power in legs					
V. weak or week	5 (14)	23 (64)	0.22	(0.09,0.51)	0.00001
Mod. Strong or normal	31 (86)	13 (36)			
Ankle clonus					
None or occa.	27 (75)	15 (42)	0.43	(0.23,0.80)	0.004
few or frequent	9 (25)	21 (58)			
Adductor stiffness					
0 to 2+	26 (27)	11 (33)	0.40	(0.23,0.71)	0.004
3+ or 4+	10 (28)	25 (67)			
Achilles stiffness					
0 to 2+	25 (67)	11 (33)	0.44	(0.26,0.75)	0.001
3+ or 4+	11 (33)	25 (67)			
Disability					
Grade I	24 (67)	15 (42)	0.57	(0.33,0.98)	0.03
Grade II or III	12 (33)	21 (58)			
Speed of walking					
Very limited or limited	11 (33)	30 (83)	0.37	(0.22,0.61)	0.000007
Slightly limited or normal	25 (67)	6 (17)			
Walking ability					
No change	3 (8)	31 (86)	0.12	(0.04,0.35)	0.000001
Improvement or normal	33 (92)	5 (14)			

Tolp. = tolperisone, RR= relative risk, CI= confidence interval, V.= very, occa.= occasional

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Table 5. Side effects encountered (tolperisone group), North Gondar, January - April 1995.

Symptoms	Frequency No. (%)	Duration (Weeks)
Muscle pain	18 (50)	1-4
Gene. body weakness	7 (19)	1-4
Dizziness	4 (11)	2-3
Constipation	4 (11)	2-4
Headache	3 (8)	1-2
Sleep disturbances	3 (8)	1-3
Weakness in the legs	3 (8)	2 (3)-4 (6)
Others	3 (8)	1 (3)-2 (4)

Others = Gastro-Intestinal Upset, Urinary Incontinence and Urine Colour Change, One Each.
* = The side effects of the placebo were neglegible.

DISCUSSION

Lathyrism is practically an irreversible disease (7). It continues to claim more and more victims in direct proportion to increasing cultivation and consumption of grass pea (8). Lathyrus, though considered to be a poor person's food, is cultivated extensively in areas of Ethiopia subject to adverse agricultural conditions, particularity drought and flooding (4). It is also cultivated as profitable cash crop (12). Poorer farmers succumb to the disease with perma nent disability, without opportunities for social and physical rehabilitation (8).

There is no curative treatment for lathyrism (1,7,13). Surgical incision of tendo-Achilles and adductor muscles to release contracture is at its experimental stage (13), and not feasible for most patients.

Although provision of low BOAA varieties of lathyrus is a reasonable solution to this important public health problem (8), looking for safe and effective symptomatic treatment measures to improve quality of life of these crippled cases is of paramount importance.

Tolperisone HCL was found to be effective for symptomatic treatment of lathyrism in Bangladesh (13). This drug inhibits multi synaptic reflex activity, reduces strychnine induced hyper reflexia and the convulsive effects of electro-shock and has marked

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anti-nicotinic activity. tolperisone HCL also has an inhibitory effect on muscle contraction (17).

We have done placebo controlled, double blind, randomized drug trial, a similar trial to that of A Haque et al. in north west Bangladesh (13), to see the effect of tolperisone HCL in Ethiopian patients with similar results.

This study showed that tolperisone HCL significantly reduced muscle cramp and heaviness at the 12th week. In the first week, it seemed that muscle cramp has increased in the experimental group because half of the patients in this group complained of muscle pain, but the later was sharp and confined mainly to the back. Subsequently, after the 4th week, this has disappeared followed by improvement of the initial cramp and heaviness.

Those who had mild to moderate startle attacks, flexor spasm and repeated falls at the end of treatment were significantly higher in tolperisone HCL group. However those with severe symptoms had modest improvement which was not statistically significant.

Even though 9 patients dropped sticks after active treatment, all of them were from grade II. Only 2 patients from grade III changed to grade II, and none to grade I. Most patients tend to use canes at the end of treatment even though they could walk without it.

Few patients who presented with already very weak muscle power didn't improve with tolperisone HCL. The same was true for the patients who presented with frequent spontaneous ankle clonus, grade 4+ adductor and Achilles stiffness and very limited speed of walking, all features of grade III and grade IV disease. Based on the above findings, the drug is most beneficial in those patients with grade I and II disability. This is not discouraging because most of lathyrism patients (> 90%) belong to stages I and II (12).

The effect of the drug started as early as the 2nd week (first evaluation day), and thereafter the effect progressively increased up to the 4th to 8th week, after which time there was no marked change. Determination of optimum duration of treatment requires further follow up.

We have shown that the drug is safe with very minimal and self limited side effects. It can be used on ambulatory patients.

In conclusion, tolperisone HCL is effective drug for symptomatic treatment of neurolathyrism, similar to the finding in Bangladesh. It is more effective in the early stages of the disease.

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REFERENCES

1. Prasad LS, Sharan RK. Lathyrism. In: Vinken PJ, Bruyen GW, eds. *Handbook of clinical neurology: Intoxication of the nervous system*. Amsterdam, North Holland Publishing CO, 1979:505-14.
2. Acton HW. An investigation into the causation of lathyrism in man. *Ind Med Gaz* 1922;57:241-7.
3. Spencer PS, Schaumburg HH. Lathyrism: A neurotoxic disease. *Neuro-behavioral Toxicology and Teratology* 1983;5:625-9.
4. Redda Tekle-Haimanot, Yemane Kidane, Elizabeth Wuhib, et al. Patterns of *Lathyrus sativus* consumption and beta-N-oxalyL- alpha-beta- diaminopropionic acid (B-ODAP) content of food samples in lathyrism endemic regions of North West Ethiopia. *Nutritional Research* 1993;13:113-26.
5. Spencer PS, Ludolph A, Dwivedi MP, Roy DN, Hugon J, Schaumburg H, et al. Lathyrism: Evidence for the role of neuro-excitatory amino acid BOAA. *Lancet* 1986;2:1066-7.
6. Sleman WH. Rambles and recollections of an Indian official, Vol 1. London: Hatchard and Sons, Sleeman WH. Rambles 1944:1-3.
7. Tesfaye Gebreab, Zewdu Wolde-Gabriel, Maffi M, Zein Ahmed Zein, Ayele Tekle-Mariam, Fanta Haile. Neurolathyrism: A review and a report of epidemics. *Ethiop Med J* 1978;16:1-11.
8. Redda Tekle-Haimanot, Yemane Kidane, Elizabeth Wuhib, et al. The epidemiology of Lathyrism in North and Central Ethiopia. *Ethiop Med J* 1993;31:15-24.
9. Redda Tekle Haimanot. Lathyrism in Ethiopia. In: Spencer PS, ed. Grass-pea: Threat and promise. New York: Third World Medical Research Foundation, 1989:36-40.
10. Ferr-Luzzi G. Malatia da "Sebere" e lathyrismo in Eritrea. *Bulletino della Societa Italiana di Medicine and Igiene Tropical* 1947;7:483-93.
11. Rizzotti G. Lathyrism in Ethiopia. *Bulletino della Societa Italiana di Medicine and Igiene Tropical* 1952;33:493-500.
12. Redda Tekle-Haimanot, Yemane Kidane, Elizabeth Wuhib, et al. Lathyrism in rural north-western Ethiopia: a highly prevalent neurotoxic disorder. *Int J Epidemiol* 1990;19:664-72.
13. Haque A, Hossain M, Khan Dpharm JK, Kuo YH, Lambein F, De Reuck J. New findings and symptomatic treatment for neurolathyrism. *Paraplegia* 1994;32:193-5.
14. Rohlf F, Sokal J. Sample size for randomized clinical trials. In: Freeman RS, ed. Statistical Tables. New York: River Press 1981:40-65.
15. The United Kingdom Tizanidine Trial Group. A double blind, placebo controlled trial of tizanidine in the treatment of spasticity caused by multiple sclerosis. *Neurology* 1994;44:570-1.
16. Ashworth B. Preliminary trial of carisoprodol in multiple sclerosis. *Practitioner* 1964;192:540-2.

17. Setsuo T. Toxicologic studies on Mydocalm: intestinal absorption and metabolic fate of Mydocalm. *Pharmacology* 1972;6:149-57.

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Blood pressure variation among Ethiopians on the Simien Plateau

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Summary. This report presents information on determinants of blood pressure variation in a rural sample of 263 Ethiopian highlanders 14–86 years of age, resident at 3530 m on the Simien Plateau. Mean systolic and diastolic blood pressures for males and females were 108/75 and 106/73, respectively, and there were no age differences. These findings confirmed that men and women can have low normal blood pressure throughout adulthood. Blood pressures increased with increasing body mass index (BMI) among adult males, although the mean BMI of 19·1 kg/m^2 was low compared with US values. This illustrates that BMI variation may be associated with blood pressure variation in men even at low mean values of both. Blood pressure did not vary with adult haemoglobin concentration.

1. Introduction

An age-related increase in blood pressure is so widespread in Western industrial populations that it is considered a part of the usual ageing process and considered remarkable when a few individuals escape (Finch 1990). However, data from over 36 populations in which blood pressure does not change with age illustrate that it is not an inevitable concomitant of the passage of time (James and Baker 1995). These populations also offer a natural experimental situation in which to evaluate the independent influence of other sources of variation such as body mass index (BMI). Furthermore, many of these populations reside in tropical or high-altitude environments, and their physiological adaptations may influence blood pressure variation. The purpose of this report is to present information on determinants of blood pressure variation in a sample of 263 Ethiopian rural agropastoralists 14–86 years of age and resident at 3530 m on the Simien Plateau. Mean systolic and diastolic blood pressures for males and females were low, and did not increase with age or haemoglobin concentration. Blood pressure increased with BMI among adult males.

2. Materials and methods

2.1. *Population and sample*

Data were collected in December 1995 from 314 males and females 14 years of age and older, native to, and residing around, 3530 m in the Ambaras Region of the Simien Mountains National Park, North Gondar, Ethiopia. The rural population farms barley and herds sheep, goats and cattle for sale and consumption. The sample is about 18% of the resident population in the relevant age range in the two Peasant Associations from which the study sample was drawn.

2.2. *Measurements*

An Ethiopian physician (A.G.) collected from each participant a brief health history that focused on cardiopulmonary signs and symptoms. Blood pressure was taken with a sphygmomanometer and stethoscope by an American member of the

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research team (M.D.) after the study participant had been seated for a few minutes while other measurements were obtained. Systolic and diastolic blood pressures were recorded as the appearance (phase 1) and disappearance (phase V) of sounds. Three measurements were taken and averaged for analysis. If one measurement were more than 100 mmHg different from another, then a fourth measurement was made immediately, and the outlier was discarded. This was usually the individual's first experience with blood pressure measurement. Measurements were taken in an open-air field laboratory in the presence of other villagers. Measurements of height, weight and a venepuncture blood sample for immediate determination of haemoglobin concentration were also obtained.

2.3. Analysis

The analysis was limited to 263 healthy, non-pregnant villagers. Forty-two people were excluded because of acute or chronic illness, and seven were excluded because of pregnancy. The sample was homogeneous for many lifestyle characteristics. All drank locally brewed barley beer daily and were non-smokers, 260 drank coffee, 252 were full-time agropastoralists, 249 had no education. Table 1A describes the morphological characteristics of the study sample. Because height, weight and BMI increased during the teens, mean values of these variables are given separately for those under 21 years of age and for adults. Reported ages were approximate: 54% gave ages that ended in zero or five. However, reported ages were consistent with life cycle stage revealed by genealogies collected from study participants. For example, only those claiming ages of 50 years or older reported adult children and grandchildren, while those claiming ages in their 20s and 30s reported only young children.

BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure was classified as normal (systolic < 130 and diastolic < 85 mmHg), high normal (systolic 130–139 and diastolic 85–89), stage 1 hypertension (systolic 140–159 and diastolic 90–99), stage 2 hypertension (systolic 160–179 and diastolic 100–109), stage 3 hypertension (systolic 180–209 and diastolic 110–119) or stage 4 hypertension (systolic ≥ 210 and diastolic ≥ 120) (Joint National Committee V 1993). When systolic and diastolic pressures fell in different categories the higher category was used. Means and standard errors are reported. Correlation, partial correlation and multiple regression analyses describe the relationship between blood pressure and other variables. A significance level of 0·05 is used.

3. Results

Mean systolic and diastolic blood pressures for males and females were 109/75 and 106/73 mmHg, respectively (Table 1B). Figure 1 demonstrates that systolic and diastolic blood pressures remained stable across adulthood in this sample ($r_{\text{systolic-age}} = -0{\cdot}08$ and $r_{\text{diastolic-age}} = -0{\cdot}16$ for men; $r_{\text{systolic-age}} = +0{\cdot}08$ and $r_{\text{diastolic-age}} = 0$ for women, all $p > 0{\cdot}05$). The mean male systolic blood pressure was significantly higher than female ($t = 2{\cdot}1$, $p < 0{\cdot}05$). There were no sex differences in mean diastolic blood pressure ($t = 1{\cdot}5$, $p > 0{\cdot}05$); 95·7% of males and 97·6% of females had normal or high normal blood pressure (table 2). Figure 2 demonstrates that adult male blood pressures increased slightly with increasing BMI ($r_{\text{systolic-BMI}} = r_{\text{diastolic-BMI}} = +0{\cdot}25$, $p < 0{\cdot}05$ for men; $r_{\text{systolic-BMI}} = +0{\cdot}07$ and $r_{\text{diastolic-BMI}} = +0{\cdot}17$ for women, $p > 0{\cdot}05$). Figure 3 demonstrates that adult blood pressures did not vary with haemoglobin concentration in this high-altitude population ($r_{\text{systolic-hb}} = +0{\cdot}15$ and $r_{\text{diastolic-hb}} = +0{\cdot}12$ for men and $r_{\text{systolic-hb}} =$

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Table 1. Characteristics of Amharas, Ethiopia blood pressure sample.

	All males ($n = 139$), $\bar{X} \pm$ SEM	Males under 21 ($n = 23$)		Males 21+ ($n = 113$), $\bar{X} \pm$ SEM	All females ($n = 124$), $\bar{X} \pm$ SEM	Females under 21 ($n = 28$)		Females 21+ ($n = 96$) $\bar{X} \pm$ SEM
		Range	$\bar{X} \pm$ SEM			Range	$\bar{X} \pm$ SEM	
A: General								
Age (years)	38 ± 1·5	14–86	17 ± 0·4	42 ± 1·5	35 ± 1·3	14–80	17 ± 0·4	40 ± 1·3
Height (cm)†	162 ± 0·7	138–176	151 ± 1·9	164 ± 0·5	151 ± 0·5	136–165	148 ± 1·3	152 ± 0·5
Weight (kg)	49 ± 0·7	29–67	38 ± 1·7	51 ± 0·5	43 ± 0·5	30–66	39 ± 1·3	45 ± 0·6
BMI (kg/m^2)	18·6 ± 0·2	13·3–26·1	16·6 ± 0·4	19·1 ± 0·15	18·8 ± 0·2	14·0–26·4	17·6 ± 0·4	19·2 ± 0·2
Haemoglobin concentration (g/dl)†	15·9 ± 0·1	12·9–19·1	–	–	15·0 ± 0·1	12–18·4	–	–
Percentage oxygen saturation of arterial haemoglobin	95·7 ± 0·2	84–99	–	–	94·8 ± 0·2	83–99	–	–
B: Blood pressure								
Systolic (mmHg)	109 ± 1·1	79–134	–	–	106 ± 1·1	82–141	–	–
Diastolic (mmHg)	75 ± 0·8	55–101	–	–	73 ± 0·7	56–91	–	–
Pulse (f/min)	70 ± 0·9	44–104	–	–	83 ± 1·0	60–124	–	–

† Female height sample is 123 because height was not measurable in one woman. Haemoglobin concentration sample sizes (limited to iron-sufficient individuals) are 136 for males and 117 for females.

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$+0{\cdot}06$ and $r_{\text{diastolic-hb}} = +0{\cdot}14$ for women; all $p > 0{\cdot}05$). BMI did not vary with age among adults ($r = -0{\cdot}14$ for men and $r = -0{\cdot}18$ for women, $p > 0{\cdot}05$). Haemoglobin concentration decreased with age among men ($r = -0{\cdot}36$, $p < 0{\cdot}05$), but not women ($r = +0{\cdot}05$, $p > 0{\cdot}05$). Multiple-regression analyses confirmed that BMI is the only one of the three variables that explains significant variation in systolic and diastolic blood pressures (table 3). There were no significant interactions among the three variables (analyses not shown).

4. Discussion

Mean blood pressure was low, and there were no age differences in systolic or diastolic blood pressure in this cross-sectional sample of Ethiopian highlanders 14–86 years of age. Longitudinal studies will be required to determine whether blood pressure remains stable throughout adulthood in this population. These studies would yield accurate information on the passage of time (important in the absence of birth certificates verifying age), and consider possible alternative explanations to the apparent blood pressure stability. The possibility that an adult decrease in BMI accounts for the apparent adult blood pressure stability is discounted by the lack of association between adult age and BMI. The possibility that a secular trend towards higher blood pressure among young adults accounts for the apparent stability seems unlikely, in light of reported mean blood pressure of 121/80 for a sample of 22 males of unreported age studied in a nearby area during the mid-1960s (Harrison, Kuchemann, Moore, Boyce, Baju, Mourant, Godber, Glasgow, Kopec, Tills and Clegg 1969). A re-analysis of those data reported that blood pressure was negatively correlated with age, and suggested that this was due to selective elimination of blood pressures above average (Clegg, Jeffries and Harrison 1976). It is not possible to

Table 2. Blood pressure classification of Ambaras sample.

	Males		Females	
	n	Percentage	*n*	Percentage
Normal	116	83·5	115	92·0
High normal	17	12·1	7	5·6
Hypertension, stage 1	5	3·6	3	2·4
Hypertension, stage 2	1	0·7	0	0
Hypertension, stage 3	0	–	0	–
Hypertension, stage 4	0	–	0	–

Table 3. Sets of beta weights and multiple correlation coefficients of adult systolic and diastolic blood pressure.

	Multiple *R*	Age	BMI	Haemoglobin concentration
Male				
Systolic	+0·257*	−0·06	+0·20	+0·09
Diastolic	+0·286**	−0·14	+0·21	+0·02
Female				
Systolic	+0·126	+0·07	+0·10	+0·06
Diastolic	+0·234	−0·09	+0·15	+0·15

* $p = 0{\cdot}06$; ** $p < 0{\cdot}05$.

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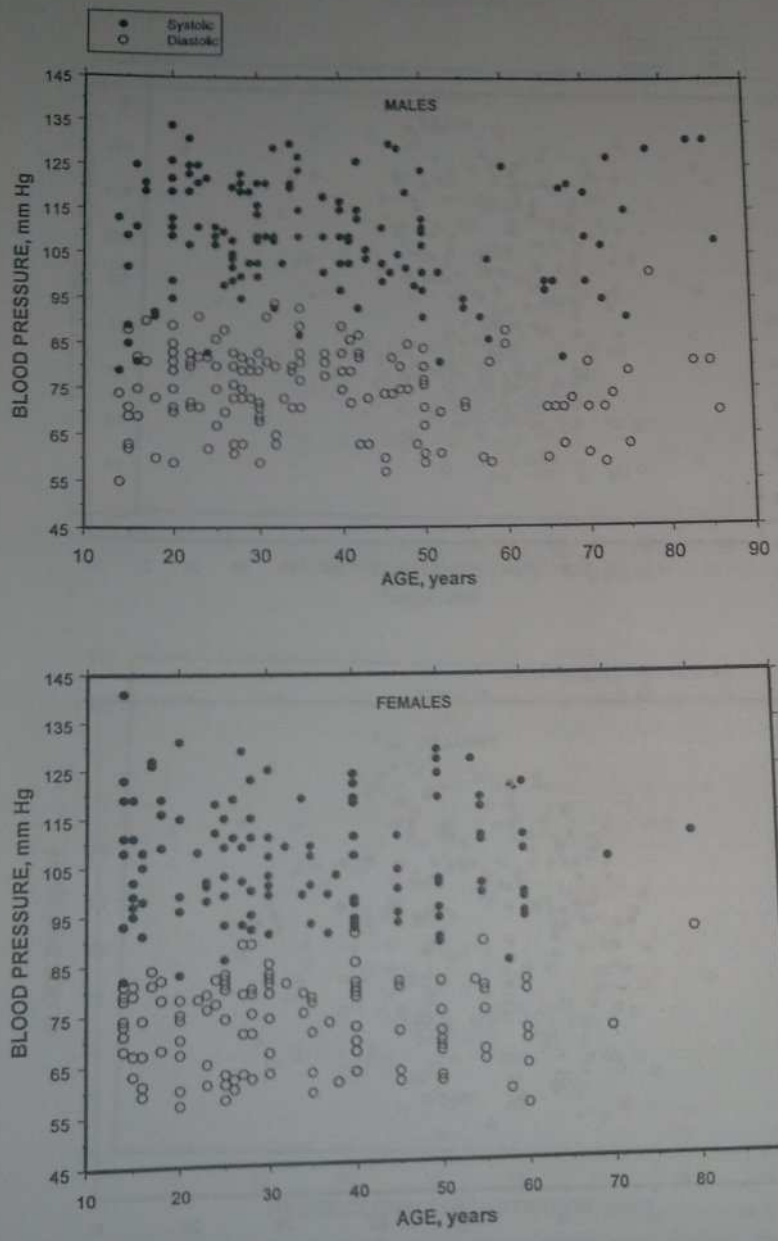


Figure 1. Scatterplot of male and female systolic and diastolic blood pressures with age.

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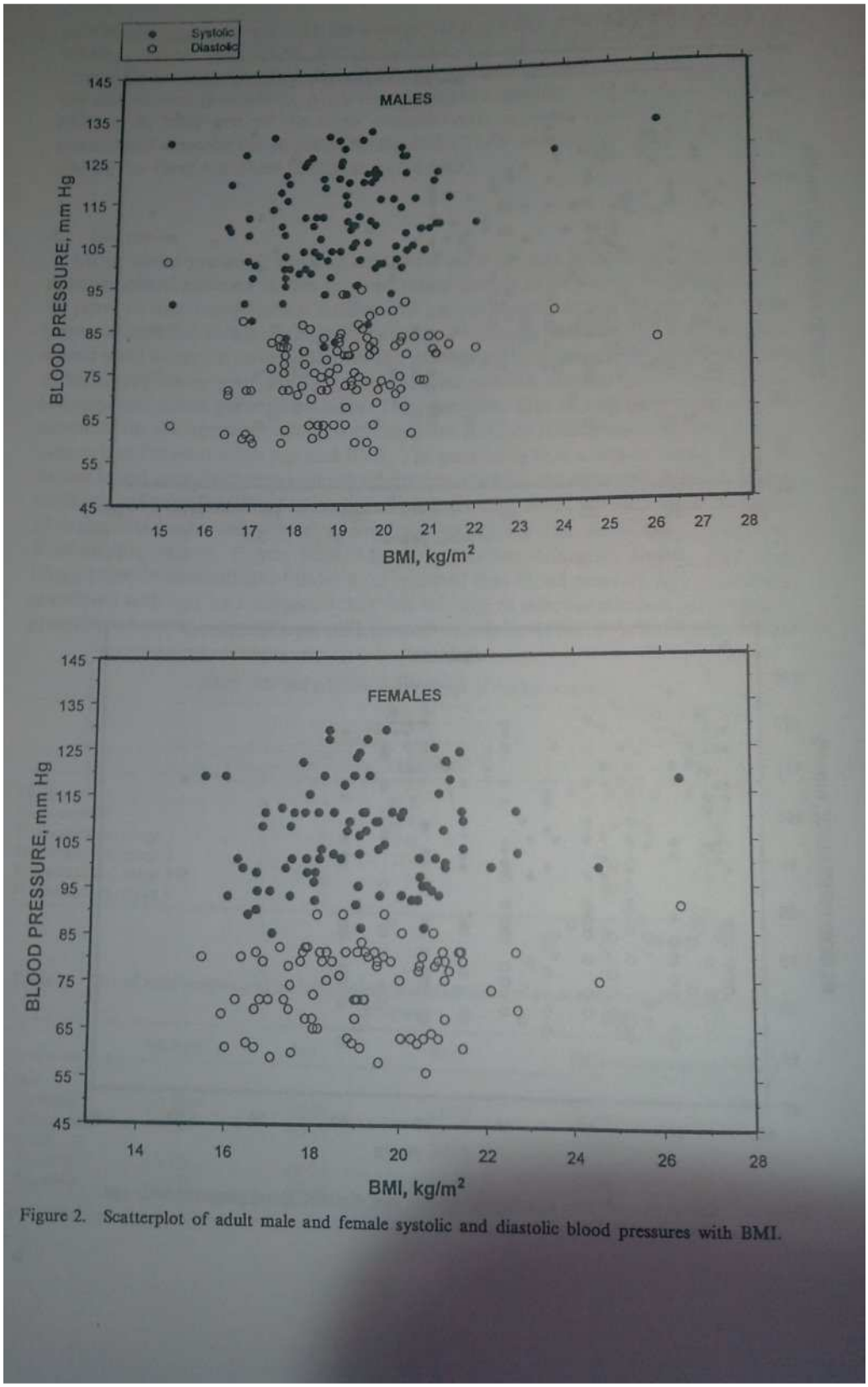


Figure 2. Scatterplot of adult male and female systolic and diastolic blood pressures with BMI.

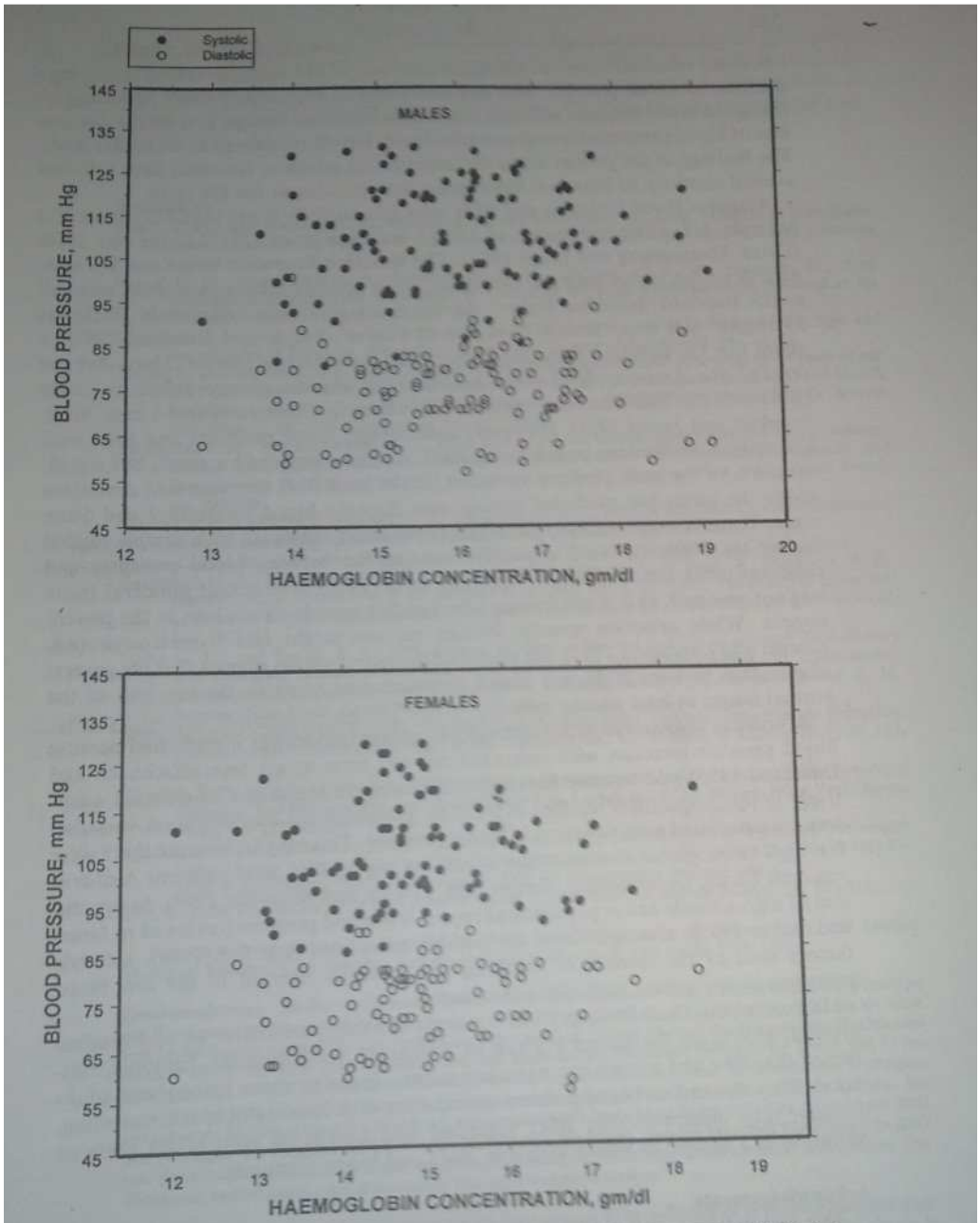


Figure 3. Scatterplot of adult male and female systolic and diastolic blood pressures with haemoglobin concentration.

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determine whether there is a substantive difference between those results indicating a decrease in blood pressure with age and those of the present study indicating no change in blood pressure with age, because information on age, and on the distribution of blood pressures throughout adulthood, was not presented in the earlier study. The findings of the present study do indicate that adults in Ambaras have sufficient arterial elasticity to have low blood pressures throughout the life cycle.

Ambaras blood pressures and BMI were substantially lower than US values. For example, US males had means of 129/81 and females of 123/77 in the late 1970s (Drizd, Dannenberg and Engel 1986). The systolic means were about one US standard deviation higher than the Ethiopian, while diastolic means were about one-half a US standard deviation higher. At the same time, US mean male BMI was 25·3 kg/m^2 and mean female BMI was 25·0 kg/m^2 (Najjar and Rowland 1987) or about one US standard deviation higher than the Ambaras sample. Thirty-five per cent of the Ambaras BMI were in the adult chronic energy deficiency range (BMI < 18·5). This has been reported elsewhere in Ethiopia (Ferro-Luzzi, Sette, Franklin and James 1992). However, even though blood pressures and BMI were low relative to Western populations, BMI variation explained a small, but significant, 6% of the male pressure variation. Males with BMI two standard deviations above the mean had predicted systolic and diastolic blood pressures 7 and 5 mm higher than the average. The re-analysis of the small Ethiopian male sample studied during the 1960s reported a positive relationship between blood pressures and ponderal index (stature/$weight^{1/3}$) (Clegg *et al.* 1976). The actual ponderal index was not reported, so it is not known whether that sample was as lean as the present sample. While attention usually focuses on overweight and hypertension (e.g. Krieger and Landsberg 1995), the present Ethiopian findings suggest that the general relationship between BMI and blood pressures also exists at the low end of the normal range, at least among men.

A possible association with haemoglobin concentration was investigated because blood pressure increases with increased red cell mass at sea level (Eschbach and Davidson 1995) and because haemoglobin concentration might be elevated adaptively in this population resident at 3530 m. Haemoglobin concentration variation was not associated with variation in blood pressure. This may be because there were few high haemoglobin concentration values among healthy, iron-sufficient Ambaras residents (Beall, Gebremedhin, Brittenham, Decker and Shamebo 1996). Many studies of high-altitude native populations report low blood pressure (reviewed in James and Baker 1995), although there are notable exceptions (e.g. Sun 1986). Lifestyle factors such as the absence of smoking are probably relevant to the low blood pressures of the present high-altitude sample.

In conclusion, these findings from a sample of Ethiopians native to, and resident around, 3530 m on the Simien Plateau in Ambaras, North Gondar, Ethiopia, confirmed that men and women can have low normal blood pressure throughout adulthood. They showed no blood pressure association with haemoglobin concentration. Finally, they illustrated that BMI variation was associated with blood pressure variation in a sample of males with low BMI and blood pressures.

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References

BEALL, C. M., BRITTENHAM, G. M., MACUAGA, F., and BARRAGAN, M., 1990, Variation in hemoglobin concentration among samples of high-altitude natives in the Andes and the Himalayas. *American Journal of Human Biology*, **2**, 639–651.

BEALL, C. M., GEBREMEDHIN, A., BRITTENHAM, G. M., DECKER, M. J., and SHAMEBO, M., 1996, Hemoglobin concentration of Amhara at 3530 m on the Simien Plateau. Paper delivered at the Ethiopian Medical Society Meetings, May.

CLEGG, E. J., JEFFRIES, D. L., and HARRISON, G. A., 1976, Determinants of blood pressure at high and low altitudes in Ethiopia. *Proceedings of the Royal Society of London* (B), **194**, 63–81.

DRIZD, T., DANNENBERG, A. L., and ENGEL, A., 1986, Blood pressure levels in persons 18–74 years of age in 1976–80, and trends in blood pressure from 1960 to 1980 in the United States. Vital and Health Statistics, series 11, no. 234. DHHS Publication No. (PHS)86-1684, Public Health Service (Washington, DC: US Government Printing Office).

ESCHBACH, J. W., and DAVIDSON, R. C., 1995, Red blood cell mass/erythropoetin and blood pressure: lessons from patients with renal disease. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 390–398.

FERRO-LUZZI, A., SETTE, S., FRANKLIN, M., and JAMES, W. P. T., 1992, A simplified approach of assessing adult chronic energy deficiency. *European Journal of Clinical Nutrition*, **46**, 173–186.

FINCH, C. E., 1990, *Longevity, Senescence, and the Genome* (Chicago: University of Chicago Press.

HARRISON, G. A., KUCHEMANN, C. F., MOORE, M. A. S., BOYCE, A. J., BAJU, T., MOURANT, A. E., GODBER, M. J., GLASGOW, B. G., KOPEC, A. C., and TILLS, D., 1969, The effects of altitudinal variation in Ethiopian populations. *Philosophical Transactions of the Royal Society of London* (B), **256**, 147–182.

JAMES, G. D., and BAKER, P. T., 1995, Human population biology and blood pressure: Evolutionary and ecological considerations and interpretations of population studies. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 115–126.

JOINT NATIONAL COMMITTEE V, 1993, The Fifth Report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC V), *Archives of Internal Medicine*, **153**, 154–208.

KRIEGER, D. R., and LANDSBERG, L., 1995, Obesity and hypertension. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd edn, edited by J. H. Laragh and B. M. Brenner (New York: Raven Press), pp. 2367–2388.

NAJJAR, M. F., and ROWLAND, M., 1987, Anthropometric reference data and prevalence of overweight, United States, 1976–80. Vital and Health Statistics, series 11, no. 238. DHHS Pub. No. (PHS) 87–1688. Public Health Service (Washington, DC: US Government Printing Office).

SUN, S., 1986, Epidemiology of hypertension on the Tibetan Platean. *Human Biology*, **58**, 507–515.

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Zusammenfassung. In der vorliegenden Studie werden Informationen über Determinanten der Variation des Blutdrucks bei einer ländlichen Stichprobe von 263 Bewohnern des äthiopischen Hochlandes im Alter von 14–86 Jahren vorgestellt. Die Probanden leben auf dem Simien Plateau des äthiopischen Hochlandes in einer Höhe von 3530 Meter. Systolischer und diastolischer Blutdruck betrugen im Mittel 109/75 bei Männern und 106/73 bei Frauen, Altersunterschiede lagen nicht vor. Diese Beobachtungen bestätigen, dass Männer und Frauen im gesamten Erwachsenenalter geringe Blutdruckwerte aufweisen können. Bei erwachsenen Männern stieg der Blutdruck mit ansteigendem Body Mass Index (BMI) an, obwohl der BMI mit 19·1 kg/m^2 im Vergleich zu US-Werten gering war. Dies belegt, dass bei Männern Variationen im BMI mit Variationen im Blutdruck sogar bei geringen Werten für beide Merkmale assoziiert sein können. Der Blutdruck variierte nicht mit der adulten Hämoglobinkonzentration.

Résumé. On présente des informations sur les déterminants de la variation de la pression sanguine dans un échantillon rural de 263 montagnards éthiopiens âgés de 14 à 86 ans, résidant à 3530 m d'altitude sur le plateau Simien. Les pressions systolique et diastolique moyennes pour les hommes et pour les femmes,

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étaient respectivement 109/75 et 106/73 et dépourvues de différences d'âge. Ces résultats confirment que ces hommes et ces femmes ont une pression sanguine normale basse tout au long de la vie adulte. Les pressions sanguines augmentent avec l'accroissement de l'indice de masse corporelle (IMC) chez les adultes masculins, bien que l'IMC moyen de 19·1 kg/m^2 soit faible comparé aux valeurs des Etats-Unis. Ceci montre que la variation d'IMC peut être associée à celle de la pression sanguine chez les hommes, même à valeur moyenne basse des deux caractéristiques. La pression sanguine ne variait pas avec la concentration en hémoglobine chez l'adulte.

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First, Malawi's strategy has not, as yet, involved DOT apart from the initial intensive phase treatment in hospital. Second, the Chinese strategy seems to have relied on a resource capacity (technical, financial, and human) that is orders of magnitude greater than that available in Malawi. Thus TB suspects were initially referred to the county radiographer for screening by fluoroscopy, and only those with suspicious fluoroscopic findings proceeded to sputum submission for smear microscopy. Furthermore, every dose of the drug regimen was supervised by a village doctor on an outpatient basis (drug regimen: 2 months of streptomycin, isoniazid, pyrazinamide and rifampicin twice a week followed by 4 months of isoniazid and rifampicin three times a week). Malawi's human resources are so restricted that its TB-control programme has only one qualified doctor on its permanent staff, and the cadre of clinicians (clinical officers and medical assistants) who might approximate to China's village doctors are already heavily committed and unlikely to be able to supervise treatment on an outpatient basis nationally without a substantial increase in their numbers. On the grounds of financial restraint, Malawi has been denied antituberculous regimens containing rifampicin throughout. Further, Malawi has been unable to maintain its national quota of two Odelka cameras for mass miniature radiography screening, and would be hard-pushed to guarantee the consumables required (eg, continuous electricity supply) at district level to allow fluoroscopy for all TB suspects. Third, the strategy used in Malawi showed cure rates of 75–80% in the mid-1980s, which fell to 60–70% in the 1990s (national TB programme reports, 1992–95). This fall in cure rates coincided with increasing HIV seroprevalence levels that are unheard of in China.

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1 China Tuberculosis Control Collaboration. Results of directly observed short-course chemotherapy in 112 842 Chinese patients with smear-positive tuberculosis. *Lancet* 1996; 347: 358–62.

Epilepsy in a focus of onchocerciasis in Burkina Faso

SIR—Reports have noted a possible association between onchocerciasis and epilepsy in western Uganda,[1-3] and a WHO expert committee on onchocerciasis control has suggested studies to clarify the relation between the two diseases.[4] Burkina Faso, was a hyperendemic area for onchocerciasis in the 1970s (prevalence more than 60%) but since the Onchocerciasis Control Programme (OCP), the prevalence and incidence has greatly decreased. Currently, onchocerciasis exists at a hypoendemic level.[5]

We studied the prevalence of epilepsy in a focus of onchocerciasis in collaboration with an ongoing survey carried out by the National Team Against Onchocerciasis in villages bordering the river Bougouriba. After doing a census of the village population for the diagnosis of onchocerciasis, we used OCP methodology (two skin-snips taken from iliac crests of each villager were examined after 30 min incubation in distilled water for the presence and number of *Onchocerca volvulus* microfilariae; a second microscopic reading was done 24 h later for all negative samples; and a control examination was performed by another technician on each sample). Adhering to the WHO protocol for the diagnosis of the main neurological diseases in developing countries,[5] all villagers aged 15 years and above were interviewed. Those people with at least two episodes of seizures were considered to be epileptic. These people were examined by a neurologist. Five villages were screened and 1424 subjects examined, of whom 1062 were 15 years old and above. Among these, positive skin-snip for onchocerciasis were found in 135 people. There were 16 epileptics identified, two of whom had a positive skin-snip for onchocerciasis.

	People aged 15 years or more	Positive skin-snip	Prevalence of onchocerciasis (%)	95% CI
Non-epileptics	1046	133	12·7	10·7–14·7
Epileptics	16	2	12·5	0·0–31·2

The prevalence of epilepsy in this area is 1·5% (95% CI 0·9–2·3%), which is little different from a non-endemic area for onchocerciasis surveyed earlier, where we found a prevalence of 1·06%. The prevalences of onchocerciasis in the epileptic and non-epileptic groups were not significantly different (p=0·67, Fisher's exact tests). In this study, no association between onchocerciasis and epilepsy was demonstrated.

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1 Ovuga E, Kipp W, Mungherera M, Kasoro S. Epilepsy and retarded growth in a hyperendemic focus on onchocerciasis in rural Western Uganda. *East Afr Med J* 1992; 69: 554–57.
2 Kilian AHD. Onchocerciasis and epilepsy. *Lancet* 1994; 343: 983.
3 Kipp W, Kasoro S, Burnham G. Onchocerciasis and epilepsy in Uganda. *Lancet* 1994; 343: 183–84.
4 WHO Expert Committee on Onchocerciasis. Onchocerciasis and its control. *WHO Tech Rep Ser* 1995: no 852.
5 Schoenberg BS. Clinical neuroepidemiology in developing countries: neurology with few neurologists. *Neuroepidemiology* 1987; 3: 143–53.

Safety of tamoxifen

SIR—I am concerned that Choo (Feb 17, p 458)[1] should echo the "unease" of unspecified critics who believe that tamoxifen deserves "a much fairer assessment" than it is apparently expected to receive at the hands of the International Agency for Research on Cancer (IARC) during the Monographs meeting (Feb 13–20) to assess whether tamoxifen might be carcinogenic. Who are these critics? Why should they be pressing for tamoxifen to be treated any differently from any of the 800 other drugs, chemicals, and exposures that have been evaluated for their carcinogenicity in the Monographs programme over the past 20 years? Who exactly, your readers may wonder, is exposed to "the danger that tamoxifen might be replaced uncritically by [an antioestrogen] that is thought to be safer but for which there is much less human data on toxicity and effectiveness"?

But let us take the item at face value. It is not enough to say that the carcinogenicity of tamoxifen should not be evaluated because it saves the lives of many women with breast cancer (which it obviously and mercifully does). No-one is seriously suggesting that tamoxifen should be withdrawn for the treatment of breast cancer even if it were adjudged to be a human carcinogen by IARC. Many drugs in the chemotherapeutic arsenal are classed as carcinogens by IARC, including azathioprine, chlorambucil, methyl-CCNU, cyclosporin, cyclophosphamide, melphalan, MOPP, and other combined chemotherapy regimens that include alkylating agents—not to speak of radiotherapy. These treatments will continue to be prescribed as long as doctors and their patients judge that the benefit is worth the risk—

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Cysticercosis and neurocysticercosis in Africa: current status

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Absence of developed medical and sanitary infrastructures in Africa explains the relative scarcity of reports of human cysticercosis and neurocysticercosis from this continent. Almost nonexistent in Muslim parts of Africa, this disease affects sub-Saharan African countries. The most common presenting clinical symptom of neurocysticercosis in Africa is seizures. The confirmation of diagnosis requires use of modern medical imaging techniques which are expensive and often not available in Africa. Further epidemiological, socio-cultural and clinical studies must be carried out in different communities in order to determine the exact magnitude of the problem, understand the mechanism of transmission of cysticercosis and propose control measures well adapted to local conditions.

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Introduction

Cysticercosis exists in Africa, but its importance is not well recognized due to a lack of diagnostic facilities. Diagnosis of the first case of neurocysticercosis in a country often follows the completion of training of the first neurologist there. Cysticercosis is almost nonexistent in Muslim parts of Africa, where pork is not eaten. It affects almost all sub-Saharan African countries where the necessary conditions for its development are united: poor sanitary conditions and the presence of free-roaming pigs around residential areas, plus their slaughtering without veterinary control. Each step in the life cycle of the parasite is influenced by the environment. Man is the only definitive host, and his feces infect pigs. The pigs have easy access to human wastes and hence to the eggs of *Taenia solium*. In tropical Africa eggs probably survive out of doors for a long time. Man acquires the adult form of *Taenia solium* by eating raw or insufficiently cooked pork infested with cysticerci. In most rural areas meat inspection is carried out improperly or not at all. Even in localities where slaughterhouses exist, illegal channels of meat marketing are not subject to sanitary control which constitutes a serious health hazard. In some ethnic groups, pork infected with cysticercosis is considered to have better flavour than healthy meat. Raw or insufficiently cooked pork is sometimes consumed to ensure virility. In some tribes of South Africa tapeworm proglottids are a component part of the "muti" used by native herbalists to expel intestinal worms (Heinz and McNab 1965).

Epidemiology

Most of the studies carried out in Africa have been skewed because of lack of random sampling or appropriate epidemiological methodology. There are very few population-based epidemiological studies on human cysticercosis. This explains the high variability of prevalence rates in the different countries.

The first case of human cysticercosis confirmed by autopsy was reported by Andrianjafy in 1910 from Madagascar. Vosgien in 1911 quoting Betten-

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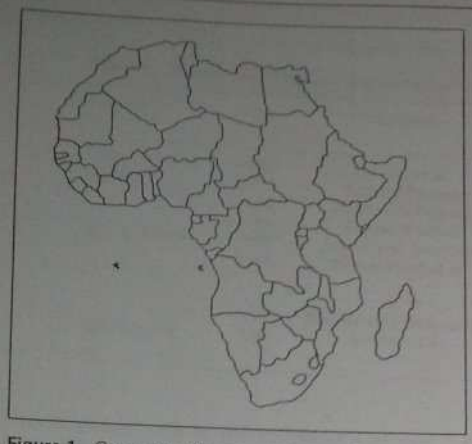


Figure 1 Countries with reported cases of human cysticercosis or neurocysticercosis.

court, noted cysticerci in an Angolan man who died of trypanosomiasis. Subsequently, in 1938, Gallais reported neurocysticercosis in a Benin patient with seizures.

Figure 1 shows the countries in which cases of human cysticercosis or neurocysticercosis have been reported.

In West Africa, some cases of human cysticercosis have been reported in several countries: Senegal (Collomb et al. 1961, 1964; Dumas et al. 1976), Benin (Avodé et al. 1994), Ivory Coast (Heroin et al. 1972; Giordano et al. 1976; Bullock 1980), Togo (Grunitzky et al. 1995), Ghana (Odamtten and Laing 1967), Burkina Faso (Kaboré, unpublished data). Cases have also been observed in Nigeria. In Senegal, the first focus of cysticercosis was reported by Collomb et al. (1964) during the 1960s in the Casamance region, where the population is Christian or animist. In Togo in 1994, out of 2604 consecutive patients presented for neurological consultation in the outpatient department of a teaching hospital, 38 had cysticercosis (Grunitzky et al. 1995); the diagnosis was based on the presence of subcutaneous cysticerci, CT scan and the serology (ELISA).

Table 1 shows the prevalence of human cysticercosis in Africa according to the published studies. The results of two large epidemiological surveys conducted in Togo and Benin are of interest. In Northern Togo, in the Kozah region, Dumas et al. (1989, 1990) carried out a population-based study in April 1987 in order to evaluate the prevalences of cysticercosis and epilepsy. This study included 5264 subjects over 15 years of age selected on the basis of random sampling. The prevalence rate for epilepsy was 16.7 per thousand (95% CI: 12.3–21.2 per thousand). The overall cysticercosis prevalence rate was 2.4%. However, serologies were performed on some of this sample (i.e. in epileptic subjects, their relatives and neighbors). In this sample the seroprevalence rate was high, 8.4% (95% CI: 6.5–10.7%), and the difference in prevalence between epileptic patients and non-epileptics was statistically significant ($p<0.001$).

In Benin, in the Savalou region, a population-based study was carried out in March 1993 by the same team using the same methodology (Adjien 1994). This study included 1443 subjects over five years of age. The prevalence rate of epilepsy was 15.2 per thousand (95% CI: 9.8–23.4 per thousand), which was comparable with the rate found in Togo. The overall cysticercosis seroprevalence was 3.9% (95% CI: 3.0–5.1%). The difference in the cysticercosis prevalence rate between epileptic patients and others was not statistically significant.

Two other studies were carried out in Benin by the same team. Adjidé (1994) performed a serological study for cysticercosis on a representative sample of 319 subjects in Vekky, a lakeside village situated near Cotonou in the Atlantique province, and found 11 positive cases (3·5%; 95% CI: 1·8%–6·3%). Houinato et al. (1994) tested with ELISA 2658 sera collected from all regions of Benin by cluster sampling. These sera constituted a representative sample of the national population. Forty-one subjects were seropositive for cysticercosis (1.5%; 95% CI: 1.1–2.1%). Two of the six Benin regions had a higher seroprevalence than the national rate: Atacora (3.8%) and Atlantique (3.2%). The regions in which the Muslim religion is dominant had a much lower seroprevalence: Borgou (0.3%). Ouémé (0.9%). The prevalence rate was higher in men (2.0%; 95% CI: 1.3–2.9%) than in women (1.1%; 95% IC: 0.6–1.8%).

In Central and East Africa, cases of human cysticercosis have been reported from Zaïre (Fain et al. 1956; Denisoff and Haenecour 1958; Lelo et al. 1992), Cameroun (Marty et al. 1985), Burundi (Aubry et al. 1990), Kenya (Ruberti et al. 1985), Rwanda (Kestelyn and Taelman 1985; Gascon et al. 1989). Cases have also been observed in Tanzania and Uganda. Human cysticercosis was found in 7% out of 300 autopsies carried out at Butare, Rwanda (Vanderick and Mbonyingabo 1972). A community-based study was carried out in Burundi by Nzisabira and collaborators (1992) in the Kayanza region in the North of Burundi, where pork consumption is high. Although this research was based on a small and non-representative sample ($n=98$), 25% of the epileptic patients were found to have cysticercosis. In this study the diagnosis of neurocysticercosis was based on the presence of anti-cysticerci antibodies in the CSF.

Table 1 Prevalence of human cysticercosis in Africa

Country	Year	Authors	Population studied	Prevalence (%)
Togo	1989	Dumas et al.	General population	2.4
Benin	1993	Adjien/Dumas	General population	3.9
	1994	Houinato/Dumas	General population	1.5
	1994	Adjidé/Dumas	General population	3.5
South Africa	1965	Heinz and MacNab	General population	8.5
	1987	Pammenter et al.	School children	
			Transkei	0.23
			KwaZulu	2.49
	1991	Shasha and Pammenter	School children	5.5
Madagascar	1993	Michel et al.	General population	18

In the Southern part of the continent, Gelfand reported cysticercosis in 1948 in 0.45% of 2148 autopsies conducted in Zimbabwe. In 1963, Proctor identified 71-cases of taeniasis in an autopsy survey of 7597 cases, most of which were also infected with cysticerci. Mason et al. (1992) found 12% positive results with an ELISA test for cysticercosis in 630 hospitalized patients. In South Africa, numerous cases have been reported (Becker and Jacobson 1951; De Villiers 1953; Gelfand and Jeffrey 1973; Thomson et al. 1984; Tuch and Saffer 1984; Joubert et al. 1985; Zini et al. 1990; Van As and Joubert 1991). Sacks and Berkowitz (1990) noted a rate of 7.4% by an ELISA test in hospitalized adult patients in Johannesburg. In Madagascar, Michel et al. (1993), using ELISA and Immunoblot reported a seroprevalence rate of 36% in 1132 neurological patients.

In South Africa, Heinz and MacNab (1965) reported a prevalence rate of 1.4% in the Zulu people and 20.4% in the people of the Transkei. Introduction of ELISA and CT scan allowed a better approach to prevalence studies. In an epidemiological study conducted by Pammenter et al. (1987) serological tests (ELISA) for cysticercosis were performed on groups of schoolchildren from two rural areas in Northern KwaZulu and Transkei. Of 736 Transkeian children 22 were serologically positive whereas only eight of 677 KwaZulu children were positive. These results, when corrected for the sensitivity and specificity of the test, indicate a cysticercosis prevalence rate of 2.49% in the Transkeian group and 0.23% in the KwaZulu group. The difference in the prevalence of cysticercosis between the two groups is in keeping with the meat-eating habits of the two communities. KwaZulu is primarily a cattle and meat-producing area, whereas in the Transkei many free-roaming pigs in addition to cattle are maintained. These observations concurred with the results of Verster (1966), which showed that porcine cysticercosis detected at abattoirs in the Transkei area was nearly seven times higher than in abattoirs close to the KwaZulu area. The differences in prevalence rates between the two studies of 1965 and 1987 could be a reflection of the improved specificity of modern tests or differences in the population groups studied, or alternatively, it could be a reflection of a true decrease in the prevalence of cysticercosis because public health standards had improved.

In 1991, Shasha and Pammenter, in another study in school children from two rural areas of Transkei, found an overall seropositivity rate of 5.5%. The rates for the two areas, which had different climatic conditions, did not differ. But the seropositivity rates differed between undernourished children (3.3%) and well-nourished children (7.3%). These authors stated that this discrepancy may be attributed to an impaired serum antibody response associated with protein-energy malnutrition. Based on these findings, they recommended that all undernourished children with clinical features of cysticercosis should have computed tomographic scanning, irrespective of the ELISA result for serum antibodies.

Andrianiandra et al. (1969) noted that 91 out of 34,137 patients with neurological symptoms living in Madagascar were suffering from cysticercosis. Michel et al. (1992, 1993) examined 1408 sera from healthy individuals living in all six provinces of Madagascar and demonstrated a very high seroprevalence rate of 18% by ELISA and immunoblot (EITB).

Clinical aspects

In Africa, the most common presenting clinical symptom is seizures, which have been reported in 30–60% of cases. All types of epilepsy can be observed, but the frequency of partial-complex seizures with secondary generalization is higher than in patients with idiopathic epilepsy. Different types of seizures could be seen in the same patient, reflecting multiple localizations of cysticerci in the brain.

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Table 2 Prevalence of cysticercosis in epileptic patients in Africa

Country	Year	Authors	No of epileptic patients	Prevalence (%)
Togo	1989	Dumas et al.	88	29.5
Togo	1995	Grunitzky et al.	305	10.8
Burundi	1992	Nzisabira et al.	98	40.8
South Africa	1962	Bird et al.	200	12.8
				5.5
	1965	Heinz and MacNab	200	15.5
	1966	Powell et al.	180	34.4
	1987	Naidoo et al.	77	30
	1987	Campbell and Farrell	106	50.9
	1991	Van As and Joubert	578	28

Table 2 shows the prevalence of cysticercosis in epileptic patients in Africa. In Togo, Dumas et al. (1989) have demonstrated cysticercosis in 29.5% of 88 epileptic patients identified in a population-based survey. In the epileptic patients there was a progressive increase in frequency of cysticercosis with age. The frequency rose from 15.3% for those in their twenties to 66.6% for those over 50 years of age. In Burundi evidence of cysticercosis was found in 40 of 98 epileptic patients (Nzisabira et al. 1992). Of these 25 patients had a positive cerebrospinal fluid (CSF) serology. In another hospital-based study in Madagascar Michel et al. (1992), reported a 36% serological positivity for cysticercosis out of 1132 consecutive hospitalized neurological patients. Neurocysticercosis was implicated as a cause of epilepsy in 28% of 578 epileptic patients in South Africa (Van As and Joubert 1991).

Apart from seizures, neurocysticercosis can present other symptoms. These include: syndrome of intracranial hypertension (ICH), psychiatric symptoms and focal neurological deficits. In a report from South Africa by Thomson et al. (1984) ICH was a presenting symptom in 24% of 61 children with cerebral cysticercosis. In this report headache and meningoencephalitis were present in 28 and 13% of patients respectively. Some patients with neurocysticercosis present multiple signs and symptoms. Avode et al. (1994) reported a patient from Benin with diffuse cysticercosis who had epilepsy, signs of intracranial hypertension, confusion, myositis and cysticerci subcutaneous nodules. Involvement of the spinal cord is rarely reported from Africa. There are very few reports of ocular cysticercosis.

Confirmation of the diagnosis of neurocysticercosis requires the use of modern medical imaging techniques which are expensive and not available in most African countries. In addition to confirming the diagnosis these modern medical imaging techniques are of great interest because they allow distinction of its different forms (i.e. the parenchymatous form, the ventricular form and the meningeal form).

In the absence of these tests the diagnosis depends on clinical examination, serology, and circumstantial evidence, notably the presence of cysticerci in other organs.

Subcutaneous cysts can be biopsied, and the diagnosis of subcutaneous cysticercosis can be confirmed by histopathological examinations. Differentiation from onchocerciasis might be a diagnostic problem in endemic areas. For example, in Togo (Dumas et al. 1989) out of 53 biopsies of subcutaneous nodules cysticerci were found only in 12 cases (22.7%), while onchocerci were found in 36 cases (67.9%). In the remaining five patients, the subcutaneous nodules were of a non-parasitic nature. The clinical examination had an excellent positive predictive value in the case of onchocerciasis (100%) compared with that of cysticercosis (only 45.8%).

In Togo (Dumas et al. 1989) in five of the 12 cases with subcutaneous cysts where the pathological examination showed cysticercosis, this finding was the only one confirming the diagnosis. This shows the necessity of doing different types of examinations to reach the final diagnosis. It is important to note that of the 36 cases having onchocerciasis (onchocercal subcutaneous nodules), three were epileptic, two of which had intracranial calcifications typical of cysticercosis. This demonstrates the possibility of double infection and again the need for doing multiple types of examinations. In the series from Togo, intracranial calcifications were noted in 22.2% of epileptic patients.

Serological tests, which have high sensitivity and specificity, are reasonable alternatives to modern neuro-imaging techniques for most African countries. The ELISA test has been used in many studies, but neither Western blot nor enzyme-linked immunoelectrotransfer blot (EITB) has been used in Africa except in Madagascar (Michel et al. 1992, 1993). In the inactive phase, however, serological

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results can be negative, and then medical imaging techniques become important.

Prevention and control

Taeniasis/cysticercosis in humans and pigs has been accepted as "potentially eradicable" for the following reasons (Pawlowski, 1990). Neurocysticercosis has a considerable impact on human health and its economic impact is certainly high, even if sufficient data are not available in this continent. Taeniasis/cysticercosis shows a definite epidemiological vulnerability, being the only source of cysticercosis for humans and pigs. In endemic foci, effective and practical intervention is available through the chemotherapy of human taeniasis, and the cost of such intervention is feasible as it uses existing veterinary and medical services and inexpensive medication. Any national or regional control program has to consider both the biological and the economic aspects of *T. solium* infections as well as the logistical complexity of control activities. For more rapid progress towards eradication and substantial reduction of the consequences of neurocysticercosis, WHO proposed a short-term control program directed at identifying foci and treating all diagnosed or suspected human cases of taeniasis. The delivery of drugs in an identified focus of human taeniasis would be a challenge to the organizational capabilities of decentralized primary health care services and would rely on the often insufficient cooperation between local veterinary and medical practitioners. To be effective over the long run, these chemotherapy control measures have to be supported by educational campaigns and significant improvements in personal hygiene and general sanitation within the disease-endemic area. An effort should also be made to increase the overall number of veterinary inspections. Proper animal husbandry practices should prevent pigs from having access to human feces. Unfortunately the practice of having free-roaming pigs feeding on domestic wastes and fecal matter, with minimal feeding and running costs, is a considerable source of income for small farmers. Despite the husbandry techniques that are utilized, the most important factor remains the access of pigs to human wastes: in Western Cameroon, Marty et al. (1986) reported that each family raises pigs in cages but the inhabitants frequently defecate inside the cages.

References

Adjien KC. Contribution à l'étude de la prévalence de la cysticercose et de la neurocysticercose dans la sous-préfecture de Savalou, Département du Zou au Bénin. Thèse Méd Cotonou, Benin, 1994, 107 p

Adjidé CC. Prévalence de la cysticercose au Bénin et dans la commune lacustre de Vekky. Diplôme d'Etudes Approfondies «Santé Publique et pays en développement», Paris 6, 1994, 76 p

Andriamiandra A, Cros J, Dodin A, et al. La cysticercose à Madagascar. Bull Soc Path Ex 1969;62:894–900

Andrianjafy A. Cysticercose humaine. Bull S S Mad 1910; 2:53–60

Aubry P, Ndayiragije A, Kamamfu G, et al. A propos de 2 cas de cysticercose au Burundi. Bull Soc Path Ex 1990; 83:288–9

Avodé DG, Bouteille B, Avimadje M, et al. Epilepsie, hypertension intracrânienne, syndrome confusionnel et cysticercose cutanée, à propos d'un cas observé en milieu hospitalier au Bénin. Bull Soc Path Ex 1994;87: 186–8

Becker BJP, Jacobson S. Infestation of the human brain with *Coenurus cerebralis*. Lancet 1951;2:1202–4

Bird AV, Heinz HJ, Klintworth G. Convulsive disorders in Bantou mine workers. Epilepsia 1962;3:175–87

Bullock A. La cysticercose cérébrale. A propos d'une observation clinique, électrique et anatomique. Thèse Méd Abidjan, Ivory Coast, 1980; 75p

Campbell GD, Farrel VJR. Brain scans, epilepsy and cerebral cysticercosis (correspondence). S Afr Med J 1987;72:885–6

Collomb H, Larivière M, Philippe Y. Cysticercose cérébrale (premier cas au Sénégal). Bull Soc Méd Afr Noire Lang Fr 1961;6:695–700

Collomb H, Larivière M, Philippe Y, et al. Foyer de cysticercose de la Baïse Casamance (Sénégal). Bull Mém Fac Méd Pharm Dakar 1964;12:148–55

Denisoff N, Haenecour F. Nouveau cas de cysticercose généralisée dans l'Est Congolais. Ann Soc Belge Méd Trop 1958;38:529–30

De Villiers PD. Cerebral cysticercosis: an aspect of the diagnosis. S Afr Med J 1953;27:1097–8

Dumas M, Grunitzky EK, Deniau M, et al. Epidemiological study of neurocysticercosis in Northern Togo (West Africa). Acta Leidensia 1989;57:191–6

Dumas M, Grunitzky EK, Belo M, et al. Cysticercose et neurocysticercose: enquête épidémiologique dans le nord Togo. Bull Soc Path Ex 1990;83:263–74

Dumas M, N'Diaye IP, Daumens JP, et al. Cysticercose cérébrale (deux nouveaux cas sénégalais). Bull Soc Méd Afr Noire Lang Fr 1976;21:203–11

Fain A, Duren P, Fels P. Cysticercose généralisée et plasmocytose médullaire (myélome) associés chez une femme de race Muhutu. Ann Soc Belge Méd Trop 1956; 36:239–46

Gallais P. Deux cas de cysticercose cérébrale avec manifestations épileptiques. Bull Soc Path Ex 1938;31: 915–9

Gascon J, Corachan M, Ramirez J. A propos de 5 cas de cysticercose au Rwanda. Méd Trop 1989;49:77–80

Gelfand M. Cysticercosis of the brain in the Africans of Rhodesia. E Afr Med J 1948;25:110–2

Gelfand M, Jeffrey C. Cerebral cysticercosis in Rhodesia. J Trop Med Hyg 1973;76:87–9

Giordano C, Vedrenne H, Daga-Ekribi A, et al. Cysticercose cérébrale à forme confusionnelle avec aspects périodiques à l'EEG, étude anatomo-clinique. Méd Afr Noire 1976;23:43–51

Grunitzky EK, Balogou AK, M'Bella M, et al. La cysticercose chez des malades neurologiques en milieu hospitalier à Lomé, Togo. Ann Méd Int 1995;146:419–22.

Heinz HJ, Macnab GM. Cysticercosis in the Bantu of Southern Africa. S Afr J Med Sci 1965;30:19–31

Heroin P, Loubière R, Doucet J, et al. Un cas de cysticercose sous-cutanée en Côte d'Ivoire. Rev Méd Côte d'Ivoire 1972;8:26
Houinato D, Bouteille B, Adjidé CC, et al. Séroprevalence de la cysticercose au Bénin. Second International Congress of Tropical Neurology 1994, September 21–23, Limoges, France
Joubert J, Joubert MJ, Lombaard CM. Neurocysticercosis—a comprehensive approach to medical treatment. S Afr Med J 1985;68:11–4
Kestelyn P, Taelman H. Effect of praziquantel in intraocular cysticercosis: a case report. Br J Ophthalmol 1985;69: 788–90
Lelo T, Malenga M, Ndoma K, et al. La cysticercose cérébrale à Kinshasa, à propos de deux observations. Afr J Neurol Sci 1992;11:36–7
Marty P, Herzog U, Marty-Jaussan, et al. 2 cas de cysticercose observés au Cameroun. Méd Trop 1985;45: 83–6
Marty P, Mary C, Pagliardini G, et al. Courte enquête sur la cysticercose et le taeniasis à *Taenia solium* dans un village de l'Ouest Cameroun. Méd Trop 1986;46:181–3
Mason P, Houston S, Gwanzura L. Neurocysticercosis experience with diagnosis by ELISA serology and computerized-tomography in Zimbabwe. Cent Afr J Med 1992;38:149–54
Michel P, Callies P, Raharison H, et al. La cysticercose à Madagascar: mise au point diagnostique et thérapeutique. Dakar Méd 1992;37:191–7
Michel P, Callies P, Raharison H, et al. Epidémiologie de la cysticercose à Madagascar. Bull Soc Path Ex 1993;86: 62–7
Naidoo DV, Pammenter MD, Moosa A, Van Dellen JR, Cosnett JE. Seventy black epileptics: cysticercosis, computed tomography, and electroencephalography. S Afr Med J 1987;72:837–8
Nzisabira L, Nsengiyumva G, Bouteille B, et al. La cysticercose dans la province de Kayanza (Burundi). Bull Soc Path Ex 1992;85:374–7
Odamtten SE, Laing WN. Cysticercosis of the brain. Ghana Med 1967;6:97–105
Pammenter MD, Rossouw EJ, Dingle CE. Serological detection of cysticercosis in two rural areas of South Africa. Trans Roy Soc Trop Med Hyg 1987;81:242–4
Pawlowski ZS. Perspectives on the control of *Taenia solium*. Parasitol Today 1990;6:371–3
Powell SJ, Proctor EM, Wilmot AJ, Macleod IN. Cysticercosis and epilepsy in Africans: a clinical and serological study. Ann Trop Med Parasitol 1966;60:152–8
Proctor NST. Problems associated with tapeworm infestation of the central nervous system. In: Tropical Neurology. Van Bogaert L, Käfer JP, Poch GF, eds., Lopez Libreros Editores, Buenos Aires, 1963;140
Ruberti RF, Mwingi SMG, Dekker N, et al. Epilepsy in the Kenyan African. Afr J Neurol Sci 1985;4:1–3
Sacks LV, Berkowitz I. Cysticercosis in an urban black South African community: prevalence and risk factors. Trop Gastro-enterol 1990;11:30–3
Shasha W, Pammenter MD. Sero-epidemiological studies of cysticercosis in school children from two rural areas of Transkei, South Africa. Ann Trop Méd Parasitol 1991; 85:349–55
Thomson AJ, De Villiers JC, Moosa A, et al. Cerebral cysticercosis in children in South Africa. Ann Trop Paediatr 1984;4:67–77
Tuch PS, Saffer D. Cerebral cysticercosis: a case report and review of the literature. S Afr Med J 1984;65:211–6
Van As AD, Joubert J. Neurocysticercosis in 578 black epileptic patients. S Afr Med J 1991;80:327–8
Verster A. Cysticercosis, hydatidosis and coenurosis in the republic of South Africa. J S Afr Vet Med Assoc 1966; 37:37–45
Vanderick FX, Moboryingabo P. La cysticercose humaine au Rwanda. Ann Soc Belge Méd Trop 1972;52:153–5
Vosgien Y. Le *Cysticercus cellulosae* chez l'homme et chez les animaux. Thèse Méd Paris, 1911; 273p
Zini D, Farrell VJR, Wadee AA. The relationship of antibody levels to the clinical spectrum of human neurocysticercosis. J Neurol Neurosurg Psychiatry 1990; 53:656–61

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Pleural tuberculosis in patients infected with HIV in Addis Ababa.

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Objective: To determine the prevalence of HIV-1 infection in patients with pleural tuberculosis and compare the clinical and radiological characteristics, effects and side effects of drugs, compliance to treatment and outcome by HIV status.

Design: A hospital based, prospective study.

Subjects: 90 adult patients with pleural tuberculosis. 120 surgical patients admitted for non-HIV related conditions served as controls.

Setting: Black Lion Hospital, a tertiary care referral hospital in Addis Ababa, Ethiopia.

Results: 22% of the patients with pleural tuberculosis versus 6.7% for the controls ($p<0.01$) were seropositive for HIV-1. Amongst patients with pleural tuberculosis, HIV seropositive patients were more likely to be male, and had longer duration of illness prior to coming to hospital ($p<0.05$). They had more disseminated disease ($p<0.01$), bilateral pleural effusion ($p<0.05$), adverse reaction to anti-tuberculosis drugs ($p<0.05$) and increased mortality ($p<0.05$). Hepatitis was the most common side effect observed. There was no difference in size of the effusion, clinical improvement, radiographic resolution or defaulter rate.

Conclusion: HIV associated pleural tuberculosis commonly occurs with other forms of extra pulmonary tuberculosis and responds well to treatment. Hepatitis is a frequent side effect and entails poor prognosis. We therefore recommend careful documentation of a previous history of liver disease and baseline investigation of liver function tests before initiation of treatment. The finding of bilateral pleural effusion may indicate the presence of underlying HIV infection.

Introduction

Both pulmonary and extra-pulmonary tuberculosis are increasing at an alarming rate in developing countries such as Ethiopia. The reasons for this are:

1. The HIV/AIDS epidemic.
2. Protracted starvation and subsequent malnutrition.
3. Poor tuberculosis control programmes.

Earlier on, extra-pulmonary tuberculosis was reported as the most common form of HIV-associated tuberculosis in North America.[1] However, as the AIDS epidemic started to unfold in Africa, despite the fact that HIV seroprevalence was higher in patients with extra pulmonary tuberculosis alone,[2,3] it became evident that the most frequent presentation was pulmonary disease.[4,5]

In Ethiopia, both pulmonary and extrapulmonary tuberculosis were diagnosed in 30% of 100 HIV infected patients between 1986 and 1989 and 35% of 636 AIDS patients in November 1990[6,7]. In both reports, there is no mention of pleural tuberculosis as a major manifestation of extrapulmonary tuberculosis. Even before the era of AIDS, the prevalence of pleural tuberculosis was reportedly low, between 3.8% to 9%.[8,9]

Over the past two years however, we have noted an increase in the number of patients with pleural tuberculosis referred to the chest unit of our hospital. We, therefore, prospectively studied the prevalence of HIV infection and compared the clinical and radiological presentation, efficacy and side effects of anti-tuberculosis drugs, compliance to treatment and outcome in HIV seropositive and HIV seronegative patients.

Materials and Methods

Between August 1990 and July 1991, 90 consecutive adult patients with pleural effusion referred to the chest unit of Black Lion Hospital and subsequently diagnosed as having pleural tuberculosis were included in the study. Black Lion Hospital is a tertiary case referral hospital in Addis Ababa, Ethiopia. One hundred and twenty age and sex matched surgical patients admitted for non-HIV related conditions served as controls to compare the HIV status. Serum samples were analyzed for HIV using the ELISA test and those positive were later confirmed by the Western Blot method.

All patients with pleural tuberculosis had a detailed clinical examination including data for involvement of other extrapulmonary sites, other AIDS related signs and symptoms and a postero-anterior and lateral chest X-ray before the start of the treatment. A diagnosis of tuberculosis was made on one or more characteristic findings on cytology, histopathology and bacteriology of the involvement organs and biological fluids. Patients were then randomly assigned to one of the

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following treatment regimens:
(i) 2SHRZ + 6HR;
(ii) 2SHE + 1OHE;
(iii) 2SHT + 1OHT.

They were then followed for clinical and radiographic response, adverse reaction to drugs, compliance to treatment and outcome at two, six and 12 months.

Significant differences in findings between HIV positive and HIV negative patients were analyzed using the chi-square test for proportions and the t test to compare the means. The Fisher exact test was employed wherever appropriate.

Results

Twenty patients (22%) with pleural tuberculosis versus eight patients (6.7%) for the controls were seropositive for HIV-1 ($p<0.01$).

Age and sex distribution.

Of the 90 patients, 46 were male and the male to female ratio was 2:1 for HIV seropositive patients and 1:1 for seronegative patients. The mean (SD) age for HIV seropositive and HIV seronegative patients was 29 (9.86) and 25.1 (8.84) years respectively. Among HIV seropositive patients the mean age for males was 33 years and for females 22 years ($p<0.01$). The mean age for HIV seronegative male and female patients was 24.8 and 25.4 years respectively.

Clinical and chest X-ray findings.

The clinical and chest X-ray presentation in HIV seropositive and seronegative patients is compared in Table I. The mean duration of illness prior to coming to hospital was longer in the seropositive patients than in the seronegative patients ($p<0.05$). In nine of the 20 seropositive patients, the disease has already disseminated to several other sites including the lymphnodes in eight, liver and spleen in five, peritoneum in four, pericardium in two and the meninges in one. Disseminated tuberculosis was diagnosed in only nine of the 70 seronegative patients ($p<0.01$).

Fifty five percent of the seropositive patients fulfilled a revised version of the WHO clinical case definition which excluded cough as a minor criteria. Four patients had chronic diarrhoea and profound weight loss at the time of presentation. Three patients each had oral thrush and HIV encephalopathy and a fourth patient had peripheral neuropathy. A tuberculin and energy skin test were not performed in our patients.

Twelve of the 20 seropositive patients had a moderate to large among of pleural effusion on a chest X-ray. Three time patients had massive pleural effusion with mediastinal shift and two patients had a mild amount of pleural fluid. These rates were not different for the seronegative patients, 60%, 10% and 18% respectively. Three of the 20 seropositive patients compared to only one of the 70 seronegative patients (1.4%) had bilateral pleural effusion ($p<0.05$). There was no other difference in the clinical presentation of the two groups of patients.

Adverse reactions and outcome.

The results of drug side effects and treatment outcome are shown in Table II. Of the 90 patients, 32 were started on 2SHRZ + 6HR, 30 on 2SHE + 1OHE and 28 patients on 2SHT + 1OHT. The corresponding number of HIV positive patients put on these regimen were 7,8 and five. Five patients in the seropositive group versus two patients (3%) in the seronegative group ($p<0.05$) developed adverse

Table I: Clinical and chest X-ray findings.

Clinical/C-X-ray characteristics	HIV +VE n=20	HIV -ve n=70 (%)	CI	p value
Duration of illness in days — mean (SD)	100.8	68.4 (59.2)		<0.05
Type of tuberculosis:				
Pleural alone	8	54 (77)	0.3 (0.14, 0.65)	<0.01
Pleuropulmonary	3	7 (10)	1.41 (0.5, 3.98)	0.68
Disseminated	9	9 (13)	3.27 (1.6, 6.68)	<0.01
Chest X-ray findings:				
Massive effusion	3	7 (10)	1.41 (0.5, 3.98)	0.68
Moderate to large	12	49 (59.6)	0.71 (0.33, 1.55)	0.56
Mild	2	13 (18)	0.56 (0.14, 2.18)	0.5
Bilateral	3	1 (1.4)	6.79 (1.87, 7.2)	<0.05

Table II: Drug side effects and outcome.

Side effects and outcome	HIV +ve patients: Total n of pts	Pts. with n	HIV -ve patients (n=70): Total n of pts	Pts. with n (%)	CI	p value
Side effects:	18		48			
Hepatitis		4		2 (4)-		
Dermatitis		2		0 (0)		
Total		5		2 (4)	3.24 (1.66, 6.34)	p<0.05
Defaulter rate	20	5	70	22 (31)	0.78 (0.31, 1.92)	p=0.78
Mortality	15	3	48	1 (2)	3.69 (1.73, 7.87)	p<0.05

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reactions to the anti-tuberculosis drugs. Four of the five patients had hepatitis while two had dermatitis. Of the side effects, 60% occurred in the thiazina group. One patient each in the ethambutol and rifampicin group developed hepatitis. The two patients on the rifampicine and ethambutol containing regimen who developed hepatitis also expired. Fatal Steven Johnson syndrome was not observed in our patients.

Five of the 20 seropositive patients and 22 of the 70 seronegative patients (31%) defaulted their treatment and therefore could not be followed up to 12 months. Amongst the patients who completed their treatment, three patients in the seropositive group versus one patient (2%) in the seronegative group expired ($p<0.05$). Two of the three patients who expired had disseminated tuberculosis and were on a standard regimen that included isoniazid and ethambutol. The third patient had chronic diarrhoea, oral thrush and HIV encephalopathy. He was on short course chemotherapy and developed hepatitis during the course of the treatment.

Discussion

The seroprevalence of HIV infection in our patients (22%) was lower than what has been reported for extrapulmonary tuberculosis from several African countries, which ranged from 17% to 77%.[3,10] In Abidjan, Cote d'Ivoire, amongst 751 patients with extrapulmonary tuberculosis 60% were seropositive for HIV-1.[3] In Lusaka, Zambia, 81% of patients with pleural tuberculosis were positive for HIV-1.[3] The seroprevalence for HIV infection among all types of tuberculosis both in a sanatorium and an urban general hospital in Ethiopia was 40% (Kassahun Temesgen, unpublished data and Bert Lindtjorn, personal communication).

Most of our seropositive patients were young (range 17 to 48 years) and 80% were below 40 years of age. The average for both sexes was 29 years. This indicates that sexual acts, as in most countries in Africa, are the most frequent mode of HIV transmission in Ethiopia.[7,11] Among HIV seropositive patients, female patients tended to be younger than males , a pattern also observed in several other places including Lusaka, Zambia.[3]

In 12 of the 20 patients, pleural tuberculosis occurred as either pleuropulmonary or disseminated disease. Most patients with disseminated tuberculosis had very advanced disease and two died while on treatment. The most common extrapulmonary manifestation was lymphadenopathy (40%). Lymphadenopathy is the most frequent form of extrapulmonary tuberculosis as reported from Central African Republic,[12] Zambia[3] and Kenya.[13] Hepatosplenomegaly, though not proved by biopsy, occurreed in five patients.

The amount of pleural fluid did not differ in the two groups of patients. However, more patients in the seropositive group had bilateral pleural effusion than in the seronegative group. In South African Blacks, amongst a total of 23 seropositive patients with pleural tuberculosis, the effusion was evenly distributed on th eleft and right.[14] Our finding suggests that the presence of bilateral pleural effusion may indicate the presence of underlying HIV infection. The clinical and radiological improvement was comparable in the two groups of patients. Complete resolution of the effusion was attained in 67% and 80% of the seropositive and seronegative patients respectively.

The most common addverse reaction observed in our patients was hepatitis. Previous reports have emphasized that fatal exfoliative dermatitis and milder forms of generalized rash are the most frequent side effects related to thiacetazone in HIV-associated tuberculosis. Very little has been reported about the frequent occurrence of hepatitis. In our clinical experience and in this study, hepatitis probably related isoniazid was the most frequent side effect and occurred early in the course of treatment. Two patients with hepatitis in our series expired. The presence of hepatitis appeared to entail poor prognosis. We, therefore, recommend that careful documentation of a previous history of liver disease and baseline investigation of liver function tests be done before initiation of treatment. Besides, future studies should look into this and the possible relationship of drug induced hepatitis with the acetylation status of patients.

The case fatality from tuberculosis in HIV infected African patients is higher than non-HIV infected individuals.[15–18] Besides, mortality and relapse rates have been shown to be higher in patients treated with standard chemotherapy than short course chemotherapy.[16,19] In our series, two patients on standard chemotherapy and one patient on short course chemotherapy expired. It will be difficult, however, to draw any reasonable association between mortality and drug regimen because the number of patients who died was too small. All the three patients who died had advanced immunosuppression and two developed hepatitis. Considering the fact that 11 of our patients fulfilled the clinical case definition for AIDS and 9 had disseminated tuberculosis, the case fatality (3/15) was probably lower than expected.

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References

1. Tuberculosis and acquired immunodeficiency syndrome. *MMWR* 1987;36:785–90, 795.
2. Kelly P, Burnham G, Radford C. HIV seropositivity in a rural Malawi hospital. *Trans R Soc Trop Med Hyg* 1990;84:725–7.
3. Elliott AM, Luo N, Tembo G, *et al*. Impact of HIV tuberculosis in Zambia: a cross sectional study *Br Med J* 1990;301:412–5.
4. DeCock KM, Gnaaore E, Adjorlolo G, *et al*. Risk of tuberculosis in patients with HIV-1 and HIV-II infection in Abidjan, Ivory Coast. *Br Med J* 1991;302:496–9.
5. DeCock KM, Selik RM, Soro B, Gayle H, Colebunders RL. AIDS surveillance in Africa: a reappraisal of case definition. *Br Med J* 1991;303:1185–8.
6. Tsega E. The demographic, social and clinical presentation of one hundred Ethiopian patients with Human Immunodeficiency Virus (HIV) infection. *Ethiop Med J* 1990;28:81–8.

Amir sultan (MD), 2014

7. Hailu N, Hailu K, Khodakovich L, Debrework Z, Bekele S. Profile of AIDS cases in Ethiopia. *Ethiop J Health Dev* 1990;4(2):213–7.
8. Getachew F, Bayu T. Analysis of adult tuberculosis admission to St Peter sanatorium, Addis Ababa. *Ethiop Med J* 1983;21(3):143–7.
9. Eyassu HG, Befekadu G, Nengistu H, *et al.* Analysis of admission to Gondar Hospital in northwestern Ethiopia. *Ethiop Med J* 1976;14(2):49–59.
10. Cathebras P, Vohito JA, Yete ML, *et al.* Tuberculose ét infection par le virus de l immunodeficience humaine en Republique Centrafricaine. *Med Trop* 1988;48:401–7.
11. Howlett WP, Nkya WM, Mmuni KA, Missalck WR. AIDS in northern Tanzania. III International Conference on AIDS and associated cancers in Africa Arusha, Tanzania. September 14–16, 1988; abstract TP21.
12. Lesbordes JL, Baquillon G, Georges MC, *et al.* La tuberculase au cours de l' infection par le virus de l' immunodeficience humanine (HIV) à Bangui (Republique Centrafricaine). *Medicine Tropicale* 1988;48:21–5.
13. Gilks CF, Brindle RJ, Otieno LS, *et al.* Extrapulmonary and disseminated tuberculosis in HIV-I seropositive patients. AIDS 1889;4:981–5.
14. Saks AM, Poser R. Tuberculosis in HIV positive patients in South Africa: a comparative radiological study with HIV negative patients. *Clin Radiol* 1992;46:387–90.
15. Raviglione MC, Narain JP, Koch A. HIV associated tuberculosis in developing countries: Clinical features, diagnosis and treatment. *Bull WHO* 1992;70(4):515–26.
16. Perriens JH, Colebunders RL, Karahunges C, *et al.* Increased mortality and tuberculosis treatment failure rate among human immunodeficiency virus (HIV) seropositive compared with HIV seronegative patients with pulmonary tuberculosis treated with "standard" Chemotherapy in Kinshasa, Zaire. *Am Rev Respir Dis* 1991;144:750–5.
17. Murray CJL, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. *Bull Int Union Tuberc Lung Dis* 1990;65:624.
18. Colebunders RL, Ryder RW, Nzibambi N, *et al.* HIV infection in patients with tuberculosis in Kinshasa, Zaire. *Am Rev Respir dis* 1989;139:1082–5.
19. Nunn P, Wasunna K, Galtha S, *et al.* Cohort study of HIV among tuberculosis patients in Nairobi, Kenya: Baseline features. VI International Conference on AIDS, San Francisco, California, 20–24 June 1990. Abstract Th. B486:243.

Fractures in children in south eastern Nigeria

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Objectives: To evaluate the pattern of fractures in children in the south eastern region of Nigeria, seeking to highlight the planning and designs of buildings, roads and playgrounds to prevent paediatric fractures.
Design: Retrospective review of paediatric patients with fractures who presented at the only tertiary referral centre in the region.
Setting: A university teaching hospital situated in the south eastern corner of Nigeria.
Subjects: 102 paediatric patients treated for fractures between January 1993 and December 1995.
Main outcome measures: Causes of fractures and their anatomical distribution.
Results: 71 boys and 31 girls were treated for fracturs. The commonest cause of fractures was road traffic accidents accounting for 50% of the patients, followed by falls and collapsing mud walls. Most of the fractures seen (58 patients) were of the green stick type. The femur was the commonly fractured bone (25 patients) follwed by the radius (22 patients) and humerus (20 patients). Road traffic accident victims presented earlier at the hospital than victims of other causes. Also lower limb fracture patients presented earlier than patients with upper limb fractures.
Conclusion: Paediatric fractures are amenable to conventional conservative therapy and open reduction became necessary in long neglected cases with associated malunion. Since the causes of fractures are largely preventable, public enlightenment campaigns, provision of pedestrian pathways, care on the part of school authorities and proper structural construction of the traditional houses may help reduce the number of cases of fracture in our environment.

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ISE REPORT

EXTRAMEDULLARY BLAST TRANSFORMATION IN A PATIENT WITH CHRONIC MYELOGENOUS LEUKAEMIA

Amha Gebremedhin, MD[1].

BSTRACT:
Xtramedullary blast transformation (crisis) of stable, chronic myelogenous ukaemia in a 71-year old male Ethiopian is presented. The difficulties encoun-red in the diagnosis and management of this rare but grave condition are iscussed and literature is reviewed.

INTRODUCTION

hronic Myelogenous Leukaemia (CML) is clonal myeloproliferative disorder of a ematopoietic stem cell origin. It may have biphasic or triphasic course. Characteristi-lly the chronic phase is followed by a lastic transformation, with some patients eveloping a distinct intermediate acceler-ted phase (1,2). The blast transformation f CML in most patients originates in the one marrow (2). Occasionally, however, he first manifestation of either the acceler-ted or blast crisis may be an extra-nedullary site (3).

Extramedullary disease (EMD) in CML enotes the presence of immature leukaemic ells at sites other than the bone-marrow, pleen or liver (4). The appearance of EMD n patients with CML implies a grave prog-osis (3,4).

This report describes a patient with CML n haematologic remission who developed unusual constellation of signs and symptoms of extramedullary blast crisis.

CASE PRESENTATION

MHG, a 71 year-old male Ethiopian, presented to the medical outpatient department (OPD) of Tikur Anbessa Hospital, in the month of August 1990. He complained of fatigue, low-grade fever and weight loss of two months; pain and swelling of the upper one-third of the right thigh of a month duration. The swelling started as a painful deeply-seated lump that gradually increased in size. He was anorexic and lost 8 Kilograms of his body weight in the preceding 2 months.

On examination at the time of admission, the patient appeared chronically ill and emaciated. The vital signs were normal. He had mild conjunctival pallor and the liver was barely felt below the right costal margin, with a total liver span of 12 cm. The

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spleen was 6 cm below the left costal margin. There was a 6×8 cm mass felt, just below the right inguinal ligament. It was hard, fixed to the underlying tissue and moderately tender. There were also two firm, mobile, non-tender subcutaneous nodules, measuring 1×2 and 2×2 cm each, respectively, on the upper part of the back just between the scapulae. The rest of the physical examination was normal.

Investigations showed a Haemoglobin (Hb) of 10gm/dL and a white blood cell (WBC) count of 175,000/mm^3, with a differential count of: Segmented Neutrophils 40%, Bands 10%, Metamyelocytes 15%, Myelocytes 10%, Myeloblasts 4%, Lymphocytes 15%, Basophils 3%, Eosinophils 1% and Monocytes 2%. The Platelet count was 230,000/mm^3. Bone marrow was suggestive of CML in chronic phase. The uric acid level was 12mg/dL and x-ray survey of the pelvic bones and the right femur showed periosteal reaction only. Fine needle aspiration from the mass at the right thigh showed sheets of granulocytes at different stages of development.

As a case of CML in chronic phase with extramedullary disease, the patient was started on busulfan and allopurinol treatment. His general condition gradually improved and the cell counts normalized. However, the thigh-mass and the subcutaneous nodules showed no significant change. He was discharged 6 weeks later to be followed at the haematology referral and follow-up clinic. During the one month follow-up period his WBC count remained below 14,000/mm^3, but the subcutaneous nodules were increasing in number and size. The patient had severe bone pain requiring strong analgesics.

Biopsy from two subcutaneous nodules showed sheets of immature leukaemic cells without granules diffusely infiltrating the subcutaneous fat. Unfortunately, cytochemical staining procedures were not done, because facilities were not available at that time. The patient complained of headache and later developed recurrent convulsive generalized seizure. As a case of CML with extramedullary blast crisis involving the subcutaneous tissue, bone and possibly the central nervous system, he was admitted to hospital for supportive treatment. His Hb was 11gm/dL, WBC count 21,800/mm^3 with a differential count of Polymorphs 35%, Bands 10%, Metamylocytes and Mylocytes 30%, Blasts 7%, Lymphocytes 12%, Basophils 4%, Eosinophils 1% and Monos 1%. The bone marrow was still consistent with stable, chronic phase CML. Lumbar puncture, to confirm central nervous system involvement, was not done due to signs of increased intracranial pressure. But the patient did not have other reasons that could have caused seizure. The patient died because of frequent generalized seizure 5 days after admission, despite supportive treatment, steroids and anti-convulsants.

DISCUSSION

Chronic myelogenous leukaemia is characterized by a prolonged, chronic stable phase that eventually proceeds to an accelerated phase and finally to a medullary blast crisis. The timing of the blast transformation is not predictable in an individual patient and it can occur any time in the course of the disease (1,2). Two types of marrow blast crisis have been recognized. About 67% of patients may transform to an acute non-lymphocytic leukaemia (ANLL) which is usually refractory to treatment. The remaining 33% will transform to acute lymphocytic leukaemia (ALL) which may have a

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SCUSSION

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Rarely, blast transformation in an extramedullary site may predate the onset of medullary blast crisis (3,7). Extramedullary blast crisis in patients with CML has been noted to occur in many sites, including lymph nodes, bone, skin, soft tissues and the central nervous system (4,7). This results from an infiltration into or proliferation within tissues by very immature leukaemic cells (8,9). As a result, patients may manifest with multiple sites of involvement at one point in time (9).

The patient presented here had predominantly subcutaneous tissue involvement. Though he complained of severe bone pain, x-ray survey of the bones did not show osteolytic lesions which are characteristic of this condition (10).

The occurrence of a seizure disorder in a patient without previous history of epilepsy also suggest central nervous system involvement, though this was not confirmed with certainty in this patient.

Although the bone marrow may still suggest chronic, stable CML at the time when patients manifest with extramedullary blast crisis, the development of blastic proliferation in an extramedullary site heralds the onset of medullary transformation in a short while (4,7). The interval between development of extramedullary disease and marrow blast crisis has been estimated to be from one to eight months (11). The patient reported here did not survive longer to develop medullary blast crisis.

In one study involving 73 patients, 27 patients (37%) developed extramedullary leukaemia during the course of their CML. Among these, 12 patients had extramedullary blast crisis either shortly before or simultaneously with the onset of bone-marrow or peripheral blood blast crisis. Of these, 4 patients had lymph node and 2 patients skin involvement. Four patients were diagnosed to have lytic bone lesions. The remaining two patients had neurologic manifestations: one had extradural accumulation of blast cells (granulocytic sarcoma) resulting in cord compression and the other patient had severe peripheral neuropathies secondary to nerve infiltration by leukaemic cells (7).

In another report, one patient developed myeloblastic involvement of the central nervous system 3 months before medullary blast transformation. This was confirmed by the demonstration in the cerebrospinal fluid (CSF) of myeloblasts containing the Philadelphia (Ph') chromosome (12). In a review of 180 adult Ethiopians with leukaemia, 104 (58%) patients had CML. None of these was reported to have extramedullary transformation of CML (13).

The diagnosis of extramedullary disease in patients with CML has been difficult and confusing until recently. Pathologists had difficulty in distinguishing lymphomatous involvement of tissues from a diffuse leukaemic infiltration, when utilizing fixed tissue sections alone (14). As a result, such cases were labelled to have CML with superimposed poorly differentiated malignant lymphoma (14,15). However, with the aid of tissue imprints, prepared immediately after biopsy and stained with Wright's stain, the confusing microscopic morphologic findings were clarified. The development and use of enzyme histochemical studies, electron microscopy, terminal deoxynucleotidal transferase (TdT) assays and other immunological studies supplemented with cytogenetic studies have facilitated highly the diagnosis of extramedullary disease in CML (3).

The distinction between extramedullary

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blast transformation of CML and a malignant lymphoma co-existing with CML is very important. Blast crisis in CML either in the bone marrow or initially restricted to a localized extramedullary site carries an ominous prognosis. Such patients generally fail to respond to conventional modalities of therapy and usually die within few months of the onset of blast transformation (4). The patient reported here was given supportive treatment only, because there were no other alternatives.

In summary, the development of extramedullary disease is a sign of poor prognosis in CML. Consequently, it warrants the early consideration of aggressive therapeutic modalities like bone marrow transplantation in eligible patients. Its distinction from other readily treatable conditions, such as lymphoma, is also emphasized.

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REFERENCES

1. Hughes TP, Goldman JM. Chronic myelogenous leukaemia. In: Hoffman R, Benz EJ, Shattil SJ, et al, eds. Haematology: Basic principles and practice. New York. Churchill Livingstone Inc. 1991; 854-869.
2. Kantarjian HM. Chronic myelogenous leukaemia: A concise update. *Blood* 1993;82:691-6.
3. Jacknow G, Frizzera G, Gajl-Peczalska K, et al. Extramedullary presentation of the blast crisis of chronic myelogenous leukaemia. *Br J Haematol* 1985;61:225-36.
4. Terjanian T, Kantarjian H, Keating M, et al. Clinical and prognostic features of patients with Philadelphia chromosome-positive chronic myelogenous leukaemia and extramedullary disease. *Cancer* 1987;59:297-300.
5. Koeffler HP, Golde DW: Chronic myelogenous leukaemia: New Concepts. *N Eng J Med* 1981;304:1201-9.
6. Rosenthal S, Canellos GP, Whang-Peng J, et al. Blast crisis of chronic granulocytic leukaemia: Morphologic varients and therapeutic implications. *Am J Med* 1977;63:542-547.
7. Rosenthal S, Canellos GP, DeVita VT, et al. Characteristics of blast crisis in chronic granulocytic leukaemia. *Blood* 1977;49:705-14.
8. Pascoe HR. Tumors composed of immature granulocytes occurring in the breast in chronic granulocytic leukaemia. *Cancer* 1970;25:697-704.
9. Neiman RS, Barcos M, Bernard C, et al. Granulocytic sarcoma: A clinicopathologic study of 61 biopsied cases. *Cancer* 1981;48:1426-37.
10. Chabner BA, Haskell CM, Canellos GP. Destructive bone lesions in chronic granulocytic leukaemia. *Medicine* 1969;48:401-5.
11. Woodson DL, Bennett DE, Sears DA. Extramedullary blastic transformation of

Amir sultan (MD), 2014

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A concise update. *Blood*

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Haematol 1985;61:225-36.
ical and prognostic features of
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aemia: New Concepts. *N Eng J*

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implications. *Am J Med*

istics of blast crisis in chronic

cytes occurring in the breast in
704.
sarcoma: A clinicopathologic

uctive bone lesions in chronic

ary blastic transformation of

chronic granulocytic leukaemia. *Arch Intern Med* 1974;134:523-6.
12. Kwaan HC, Pierre RV, Long DL. Meningeal involvement as first manifestation of acute myeloblastic transformation in chronic granulocytic leukaemia. *Blood* 1969;33:348-52.
13. Milkias Shamebo. Leukaemia in adult Ethiopians. *Ethiop Med J* 1990;28:31-7.
14. Duvall CP, Carbone PP, Bell WR, et al. Chronic myelocytic leukaemia with two philadelphia chromosomes and prominent peripheral lymphadenopathy. *Blood* 1967;29:652-66.
15. Garfinkel LS, Bennett DE. Extramedullary myeloblastic transformations in CML simulating a coexistent malignant lymphoma. *Am J Clin Pathol* 1969;51:638-45.

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EPIDEMIOLOGICAL FEATURES OF SEVERE PAEDIATRIC MALARIA IN NORTH WESTERN ETHIOPIA
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EPIDEMIOLOGICAL FEATURES OF SEVERE PAEDIATRIC MALARIA IN NORTH WESTERN ETHIOPIA

T. SEBOXA and R.W. SNOW

SUMMARY

Malaria remains a major public health challenge in sub-Saharan Africa, yet our knowledge of the epidemiology of malaria in terms of patterns of mortality and morbidity is limited. To examine the clinical and epidemiological presentation of severe life-threatening malaria in Humera, north western Ethiopia studies were conducted among the childhood population in the community, those presenting to out-patient facilities and those admitted to the district hospital. The overall *P.falciparum* parasite rate among children aged 0-9 years resident within the area was only 12% confirming the low level of endemicity in this area. *P. vivax* infections were present in 5% of children. Between July 1993 and June 1994 peak out-patient presentation with *Plasmodium falciparum* coincided with the rains with over 50% of cases occurring between August and October whilst *P. vivax* infections were predominant during the hot, dry months. Malaria was an important cause of paediatric admission to the local district hospital with an estimated 4.7% of the at-risk childhood community warranting intensive clinical management each year. Case fatality rates were high and the clinical spectrum of severe disease indicated a preponderance of cerebral malaria cases. In addition, respiratory distress was a feature in 12% of the malaria admissions. The suggestion that the co-existence of *Plasmodium falciparum* and *Plasmodium vivax* may serve to reduce the severe clinical consequences of *P. falciparum* malaria is not supported by these observations.

INTRODUCTION

The epidemiology of malaria across Ethiopia has been well described in terms of vector ecology and the region's ability to sustain varying degrees of endemicity. The ecology and topography of Ethiopia supports a wide range of *Plasmodium falciparum* transmission from the complete absence of transmission to holoendemic malaria(1-3). As with other areas within the Horn of Africa, communities are subject to both *P. falciparum* and *vivax* transmission(4). The possible epidemiological effects of cross-species plasmodial infection upon disease progression is receiving renewed interest(5). This interest has focused primarily among populations in the South Pacific and South-East Asia and there have been few descriptions of the disease-spectrum among African populations exposed to concomitant P. *falciparum* and *vivax* malaria. In this paper we have used a system of continuous paediatric ward surveillance to define the basic epidemiology of clinical malaria presenting to a rural district hospital from a well defined population in north western Ethiopia whilst describing the levels of both *P. falciparum* and *Vivax* transmission within the childhood populations.

MATERIALS AND METHODS

Study area: Humera district is located in the north western part of Ethiopia in Wolkayit District at the junction of the Ethiopian, Eritrean and Sudanese borders. The area is semi-arid, 700 m above sea level with an average annual rainfall of 560 mm mainly occurring between June and September. Humera town is located on the bank of the river Tekezie and is divided into five Kebeles (administrative units). The population of Humera town and its surroundings consists predominantly of the Tigrayan ethnic group and farm sorghum for home consumption and sesame as a cash crop. The community also comprises a significant number of merchants, administration workers, people displaced following the war and migrant labourers from the highlands of Ethiopia. The population of the five administrative units immediately surrounding Humera District Hospital (HDH) were selected for the purposes of these studies. According to the 1995 national household census the population aged 0-9 years for the study area was 9,750. The community has easy access to HDH located close to the hospital. HDH provides both in-patient and out-patient services free of charge to all paediatric patients. Five doctors provide round the clock cover for the 70 bedded inpatient services. In addition, two government-run dispensaries operate within the town and over-the-counter proprietary forms of drugs are available from several retail outlets.

Despite the lack of rigorous or recent malaria vector

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studies early survey reported the presence of *An.gambiae s.l., An. pharoensis* and *An.funestus (2)* within this region of Ethiopia. Cross sectional surveys conducted in 1991 within the study area indicated that 10.9% of children aged two to nine years were infected with either *P.falciparum* or *P. vivax* suggesting a *P.falciparum* hypoendemic transmission situation(6).

Cross sectional parasitological surveys: A cross sectional survey was conducted amongst children aged 0-9 years resident within the study area during October 1995. Following informed consent finger prick blood samples were obtained from children in their homes. Giemsa-stained thick blood smears were read using a 100 oil immersion magnification lens and parasite counts were made following the examination of 100 white cells.

Out-patient parasitological surveillance: A clinic-based survey at five laboratories, including the hospital laboratory, in Humera town was conducted between July 1993 and June 1994. These surveys were used to define the relative frequency of *Plasmodium falciparum* and *Plasmodium vivax* among out-patients who reported fever as a dominant morbid complaint.

Paediatric ward surveillance: Between 1st October 1994 and 31st September 1995 each child admitted to HDH aged between one and 120 months and resident in one of the five administrative units selected as part of this study, was examined by one of the hospital physicians. Demographic, historical and clinical details were all recorded on a pre-coded proforma. A finger prick blood sample was taken for the estimation of packed cell volumes and malaria parasitology. Packed cell volumes were determined from blood collected into microhaematocrit centrifuge tubes and spun at 12,000 rpm for five minutes. Giemsa-stained thick blood smears were read by the hospital technician per 100 white cells. Additional clinical and laboratory investigations were made as indicated including chest X ray and lumbar puncture. All laboratory and clinical details were recorded on the child's proforma. These data were reviewed by the investigating physicians and a final diagnosis for admission made within 48 hours of admission. A primary diagnosis of malaria was made following the exclusion of all other causes for the febrile illness and in the presence of detectable P.*falciparum* malaria on the admission blood film. These diagnoses and other details were reviewed each month by one of us (T.S.).

RESULTS

Parasitology

Cross sectional results: A total of 616 slides were taken from randomly selected children aged 0-9 years of age during October 1995. The *P.falciparum* parasite rate among this sample was 12%. The parasite rate was consistent across all the age groups with the exception of infants whose parasite rate was only 0.8% due possibly to the seasonality of infection risk in this area of Ethiopia. *P.vivax* infections were present in 5% of children: 3% were mixed infections with *P.falciparum*. *P.malariae* infection was uncommon(1%) and no P.*ovale* infections were noted.

Clinic surveys: A total of 3,475 blood smears were examined from five laboratories in Humera town between July 1993 and June 1994 obtained from febrile children aged 0-9 years. Peak patient examination occurred between March and April(26%) and 32% between the months of August and October. A total of 715(21%) of parasitological examinations revealed evidence of P.*falciparum* whilst 4.5% showed evidence of *P.vivax* infection. Table 1 shows the monthly *P.falciparum* and *P.vivax* positivity rates demonstrating a predominance of *P.vivax* during the hot, dry months (March - April) which was reversed during the cooler wet months in favour of *P.falciparum* (August - October).

Table 1

Seasonal distribution of P.falciparum and P.vivax infections among febrile outpatient attenders aged 0-9 years living in Humera Town between July 1993 and June 1994.

Month	Numbers examined	*P.falciparum* infections	*P. vivax* infections
January	142	14(10%)	7(5%)
February	300	15(5%)	5(2%)
March	450	23(5%)	35(8%)
April	460	23(5%)	46(10%)
May	320	30(9%)	4(1%)
June	160	9(7%)	2(1%)
July	191	53(28%)	6(3%)
August	385	161(42%)	15(4%)
September	281	108(38%)	7(3%)
October	450	208(46%)	14(3%)
November	227	58(26%)	10(4%)
December	109	13(12%)	7(6%)

Clinical spectrum of severe disease: Of the 705 paediatric admissions to HDH aged one month to ten years, 458(65%) were clinically regarded as having a primary diagnosis of malaria. Given the national census estimates for this community the crude risk of admission to hospital with malaria was 47 per 1000 children aged 0-9 years per annum. The mean age for the malaria admissions was 40.7 months(95% CI:37.8 43.5). Malaria was a common cause for admission throughout the year with a slight rise during the months of August, September, October and November. Due to the war there has been no systematic rainfall measurements in the area since 1988 however an eight year monthly averages for the period 1980 through to 1988 show that 77% of rainfall occurs between June and August. The case-fatality rate among the paediatric malaria admissions was high: 99(21.6%) children died whilst on the ward. The spectrum of disease presenting to the hospital included 135 children who were defined as having cerebral malaria(unable to localise to a painful stimulus); 40 children who had packed cell volume of less than 15%; 55 children with signs of respiratory distress manifested as either Kussmaul's breathing or ataxic ventilation, with increased inspiratory effort. Seven children had signs of renal failure with a progressively declining urine output for three to seven days and with associated Kussmaul's breathing, three of these seven patients died with anuria. Eighty seven children were hypoglycemic (blood.glucose level < 40mg/dl) and 23 children had signs of spontaneous bleeding from gums, nose, and gastrointestinal tract. 156

DISCUSSION

Cross sectional estimates of both *P. falciparum* and P. *vivax* malaria infection among the childhood population at Humera in north western Ethiopia were similar to recent parasitological findings among populations in Santo, Vanuatu, in the Pacific(5) and a Karen refugee populations on the Thai-Burmese border(7). In both the Karen and Vanuatu populations the incidence of severe paediatric falciparum disease was relatively low. Admission to the local hospital in Santo, Vanuatu with severe malaria was extremely uncommon and for two complete years no child was seen with cerebral malaria (K.Maitland, personal communication). Among the Karen population, 193 person-years of observation of children aged 4-15 years resulted in eight severe disease events warranting hospitalisation although none of these showed any signs of organ failure and none was in coma(7). These observations and the reviewed findings of multiple species infection of murine simean and avian plasmodial(8,9) have led some to argue that early exposure to *P.Vivax* may confer protection against the severe manifestations of falciparum malaria. In Vanuatu this has been used as an argument in favour of selection for alpha- thalassaemia which shows an increased susceptibility to *P.vivax* infection(8). Although we did not complete any haematological examinations among the Humera population, studies among the Tigrayeans have shown the apparent absence of alpha - thalassaemia. However, regarding the Duffy blood types in Ethiopia, Mathews and Armstrong in their extensive study have found that among the Hamito Semites (from which the population of Humera derive) Duffy positivity was 70%(10).

By contrast to the Asian and Pacific populations the Tigrayan childhood population we have described experience exceptionally high morbidity rates. Malaria was an important cause of paediatric admission to the local district hospital with an estimated 4.7% of children below the age of 10 years warranting intensive clinical management each year. Case-fatality rates were high and the clinical spectrum of severe disease indicated a preponderance of cerebral malaria cases. In addition we noted that respiratory distress was a feature in 12% of malaria admissions. Unlike children exposed to meso-hyperendemic P.f*alciparum* transmission(11) the clinical spectrum of disease included children in renal and circulatory failure both conditions more commonly associated with severe malaria among non-immune adults(12). To this end we cannot entirely exclude the possibility that older children who succumbed to severe malaria were not completely immunologically naive, having migrated into the area from non-endemic areas. However, the majority of the resident childhood population of Humera have been resident from birth and those who have migrated from the Ethiopian highlands form a minority group. Nevertheless, severe malaria among adults is a common feature of admission to HDH (YT, unpublished data) and at Gondar 250 km away in the same region malaria amongst adults is a major cause of mortality at the district hospital(13-17).

The area is of hypo-mesoendemicity for *P. falciparum* transmission and the severe malaria patients we have described have an age and clinical spectrum consistent with low intensity *P. falciparum* transmission(18). Over 25% of severe malaria admissions were above five years of age. Although we were unable to record rainfall during the course of the studies, rain in this area does follow an acutely seasonal pattern. Nevertheless the perennial distribution of malaria admissions suggests that transmission occurs throughout the year and is likely to be a result of the proximity of both the Tekezie River and the presence of the May-Kadra plantation site with its perennial swampy ecology. There were however important differences in the relative frequency of *P. Vivax* to *P.falciparum* infections among clinical cases treated both as ambulatory out-patient and severe inpatients. This seasonal pattern has been described amongst other populations where both plasmodial species co-exist(8,7,19-20).

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REFERENCES

1. Covell, G. Malaria in Ethiopia. *J. trop. Med.+Hyg.*, 1957; 60: 7-16.
2. O'Connor, C.T. The distribution of anopheline mosquitoes in Ethiopia. *Mosq. News*, 1967; 27: 42-54.
3. Chand, D. Malaria problem in Ethiopia. *Ethiop. Med. J.* 1965, 1: 27-34.
4. Mekuria, Y. and Wolde Tsadik, G. Malaria survey in North and North Eastern Ethiopia. *Ethiop. Med. J.* 1970; 8: 201-206.
5. Maitland, K., Williams, T.N., Newbold, C.I. *Plasmodium vivax* and *P.falciparum*: biological interactions and the possibility of cross-species immunity. *Parasit. Today*, 1997; 13: 227-231.
6. Wezam, A. Malaria survey at Humera, North Western Ethiopia. *Ethiop. Med. J.* 1994; 32: 41-45.
7. Luxemburger, C., Thwai, K.L., White, N.J., Webster, H.K., Kyle, D.E., Maelankirri, L., Chongsuphajaisiddhi, T., Norsten, F. The epidemiology of malaria in a Karen population on the Western border of Thailand. *Trans. roy. Soc. trop. Med. Hyg.* 1996; 90: 105-111.
8. Williams, T.N., Maitland, K., Bennet, S., Ganczakowski, M., Peto, T.E.A., Newbold, C.I., Bowden, D.K., Weatherall, D.J., Clegg, J.B. High incidence of malaria in a-thalassaemic children. *Nature*. 1996; 383: 522-525.

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9. Maitland, K., Williams, T.N., Bennett, S., Newbold, C.I., Peto, T.E.A., Viji, J., Timothy, R., Clegg, J.B., Weatherall, D.J., Bowden, D.K. The interaction of *P.falciparum* and *P.vivax* in children on Espiritu Santo, Vanuatu, South West Pacific. *Trans. roy. Soc. Trop. Med. Hyg.*, 1996; **90**: 614-620.
10. Mathews, H.M., and Armstrong, J.C. Duffy Blood Types and *Vivax* Malaria in Ethiopia. *Amer. J. Trop. Med. Hyg.* 1981; **30**: 299-303.
11. Marsh, K., Forster, D., Waruiru, C., Mwangi, I., Winstanley, M., Marsh, V., Newton, C., Winstanley, P.A., Warn P.A., Peshu, N,, Pasvol, G., Snow, R.W. Life-threatening malaria in African children: Clinical spectrum and simplified prognostic criteria. *N. Engl. J. Med.* 1995; **332**: 1399-1404.
12. Warrell, D.A., Molyneux, M.E., Beales, P.F. Severe and complicated malaria, 2nd Edition. *Trans. roy Soc. Trop. Med. Hyg. 84*, supplement 1990; 2: 1-65.
13. Molineaux, L., Plorde, I., Dasnoy, J. Analysis of Medical Admissions to the Gondar Hospital 1963-1965. *Ethiop. Med. J.* 1966; **5**:47.
14. Habte Gabar, E., Glirma, B., Meherete, M., Mehari, A., Tekle, E., Belachew, K., Amera, B. Analysis of admissions to Gondar hospital in North Western Ethiopia 1971-1972. *Ethiop. Med. J.* 1976; **14**: 49.
15. Mengistu, M., Maru, M., Ahmed, Z. Malaria in Gondar, Ethiopia, 1975-1978: A review of 435 cases with special reference on cerebral malaria. *Ethiop. Med. J.* 1979; 5:47.
16. Seboxa, T., Teklemariam, N., Engelman, N.L., Wendekam, R. A review of the malaria situation in Gondar College of Medical Science Hospital with special attention to cerebral malaria. *IX. Proc. Med. Res.Conf: Gon. Med. Sci. Gondar.* 1987; 108.
17. Endeshaw, Y. and Assefa, D. Cerebral malaria. Factors affecting out come of treatment in a suboptimal Clinical setting. *J. Trop. Med. Hyg.* 1990; 93:44-47.
18. Snow, R.W., Omumbo, J.A., Lowe, B., Molyneux, C.S., Obeiro, J., Palmer, A., Weber, M.W., Pinder, M., Nahlen, B., Obonyo, C., Gupta, S., Marsh, K. Relation between severe malaria morbidity in children and levels of *Plasmodium falciparum* transmission in Africa. *Lancet.* 1997; **349**:1650-1654.
19. Balfour, M.C. Malaria studies in Greece. *Amer. J. Trop. Med.*, 1935; **15**: 301-329.
20. Earle, W.C. Epidemiology of malaria in Peurto Rico. *Peurt. Ric. J. Pub. Hyg. Trop. Med.* 1939; 15:3-27.

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Variable major lipoprotein is a principal TNF-inducing fact of louse-borne relapsing fever

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Massive release of tumor necrosis factor is responsible for the potentially fatal Jarisch-Herxheimer reaction that follows antibiotic treatment of relapsing fever due to *Borrelia recurrentis*. We have undertaken the quantitative purification of the components of *B. recurrentis* that stimulate human monocytes to produce tumor necrosis factor. We show that the predominant factor inducing tumor necrosis factor is a variable lipoprotein homologous to the variable major protein of *B. hermsii*. We found antibodies to different forms of variable major protein in two patients with louse-borne relapsing fever. The three purified variable major proteins studied here differ in their ability to induce tumor necrosis factor production, which may partly explain the variable clinical severity of borrelial infection. These results may be of considerable relevance for the pathogenesis of Lyme disease and other forms of human borreliosis.

Borreliae are spirochetal pathogens that can provoke episodes of a severe inflammatory reaction in humans. Relapsing fevers are caused by several species. Most species (for example, *Borrelia hermsii*) are transmitted to the human host by soft ticks of the genus *Ornithodoros*, but one, *B. recurrentis*, is transmitted by the human body louse. Although the clinical features of louse-borne and tick-borne relapsing fever are very similar, the former tends to be more severe[1]. *B. burgdorferi*, transmitted to humans by *Ixodes* ticks, is the agent of Lyme disease. Because of the high fatality of relapsing fever and the chronic debilitating nature of Lyme disease, there is considerable interest in how borreliae cause inflammation. An important clue comes from the clinical observation that antibiotic treatment can cause a transient but profound deterioration of symptoms, known as the Jarisch-Herxheimer reaction[2–4]. Recent clinical studies of relapsing fever have demonstrated that this reaction is caused by the release of tumor necrosis factor (TNF), a potent pro-inflammatory cytokine. A sharp rise in fever and cardiorespiratory deterioration are immediately preceded by a corresponding rise in circulating TNF levels[5], and these clinical effects can be suppressed by administration of anti-TNF antibodies[6].

Systemic TNF production provoked by bacterial lysis is, therefore, probably central to the inflammatory process, yet borreliae do not express classical TNF-inducing factors such as lipopolysaccharide[7] (LPS). TNF-inducing activity has been reported for many borrelial proteins with an N-terminal lipid modification[8,9] that is thought to be structurally similar to the cysteine-linked tripalmitate moiety of *E. coli* lipoproteins[8,10–11]. It is uncertain how this relates to pathogenesis, as the *B. burgdorferi* genome encodes potentially over a hundred proteins that have a signal sequence for this type of lipid modification[12]. Moreover, the lipid-modified proteins that have been reported to induce TNF are not necessarily expressed in human host, and other important TNF-inducing struct may exist that are as yet unknown. Using a quantitative chemical approach, we have identified here the variable m protein (vmp) of *B. recurrentis* to be the predominant TN ducing component.

Purification of the TNF-inducing activity

Starting with crude lysates of *B. recurrentis* isolate A1 (ref. we undertook the purification of the factors that stimu human peripheral blood mononuclear cells (PBMCs) an monocytic cell line (MonoMac6) to release TNF. The prin TNF-inducing activity could not be extracted from the lysate with aqueous buffers or organic solvents such as a ture of chloroform:methanol (2:1), but it was soluble in 0 sodium dodecylsulfate (SDS)(Fig. 1*a*). The TNF-inducing a ity of the SDS-soluble fraction exceeded that of the crude lysate, indicating that this detergent may extract the ac molecule from inhibitory factors or optimize its presenta for monocyte stimulation. At the highest concentration u SDS did not induce TNF, nor did it substantially affect th sponse to the positive control (purified *E. coli* LPS) (Fig. Similar results were obtained with PBMCs and MonoM cells. Further work used MonoMac6 cells, as this system of greater 'throughput' and consistency of response. SDS extr of *B. recurrentis* and *E. coli* LPS differ in their serum and Cl requirements for expression of activity and in their sensitiv to polymixin B (Fig. 1*c*). This indicates that the TNF-induc factor is not structurally related to LPS. The TNF-inducing tivity of *B. recurrentis* extracts was greatly reduced by treatm with lipases or mild base hydrolysis, but was unaffected treatment with Proteinase K (Fig. 1*d*). These results indi that a lipid is an essential component, although the acti

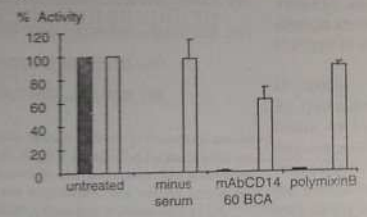
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a

	Bio-activity (TNF pg/ml)	
	PBMC	MM6
Whole cell lysate	2020	970
Aqueous extract	171	200
MCW extract	717	0
SDS soluble extract	5234	2575
Insoluble material	231	0

b

	Bio-activity (TNF pg/ml)			
	PBMC		MM6	
LPS (ng/ml)	- SDS	+ SDS	- SDS	+ SDS
10	2924	2854	1095	1301
2	3155	2271	402	511
0.4	2733	1021	116	130
0	9	9	47	18



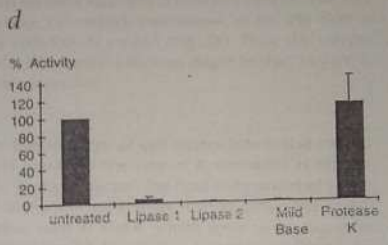
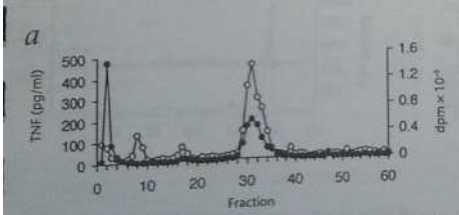


Fig. 1 Extraction and properties of the TNF-inducing activity. *a*, Whole-cell lysates of *B. recurrentis* (isolate A1) were progressively extracted with HEPES-buffered saline to extract cytosolic protein, then with methanol:chloroform: water (MCW; 8:4:3) to extract most of the lipids, then with 0.1% SDS in HEPES-buffered saline, leaving an insoluble residue. A representative experiment is shown for extracts assayed on PBMCs (at a dilution of 1:12,500) as well as on MonoMac6 cells (at a dilution of 1:2,500). *b*, The effect of SDS at a concentration of 0.0005% on the response of PBMCs and MonoMac6 cells to stimulation with LPS. A representative experiment is shown for PBMCs, as well as MonoMac6 cells. *c*, The effect of treatments on the TNF-inducing activity of *E. coli* LPS (■) and SDS extracts of *B. recurrentis* isolate A1 (□). Values are an average with standard error ($n = 4$). *d*, The effect of treatments on the TNF-inducing activity of SDS extracts of *B. recurrentis* isolate A1. Lipase 1 is from pig pancreas; lipase 2 is from *Rhizopus arrhizus*. Values are an average with range ($n = 2$), except Proteinase K data, which is an average with standard error ($n = 3$). Analysis by SDS–PAGE confirmed that after Proteinase K treatment, all proteins had been degraded, as no bands were detected (data not shown).

cannot be extracted by organic solvents. Thus, the TNF-inducing activity probably is not a simple lipid but is a more complex structure, possibly a lipoprotein. Therefore, bacterial cultures were metabolically labelled with ^{3}H-palmitate before further purification of TNF-inducing activity. More than 90% of the radioactivity was soluble in organic solvents and 5% was incorporated into the SDS-soluble fraction. When the SDS extract was fractionated by preparative polyacrylamide gel electrophoresis, it was found to contain a single main peak of TNF-inducing activity coincident with a discrete peak of radioactivity (Fig. 2*a*). Analysis of the active fractions on conventional SDS–PAGE demonstrated a single Coomassie-staining and radiolabelled band of 42 kDa, whose intensity correlated with the level of TNF-inducing activity in the different fractions (Fig. 2*b*). These results identified the principal TNF-inducing factor as a 42-kDa protein with a lipid modification that is essential for activity.



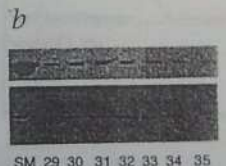


Fig. 2 Purification of the TNF-inducing factor. *a*, Preparative gel electrophoresis of the SDS-solubilized membrane protein after *in vivo* labelling with ^{3}H-palmitate. Fractions were assayed for TNF-inducing activity on MonoMac6 cells at a 1:1,000 dilution, and radioactivity was determined by scintillation counting. O, TNF-inducing activity; ●, radioactivity. dpm, disintegrations per minute. *b*, Conventional SDS–PAGE analysis of the TNF-inducing fractions (numbers 29–35) from preparative electrophoresis, detected by autoradiography (upper panel) and Coomassie blue staining (lower panel). SM, starting material.

The TNF-inducing molecule is vmp

The nature of this 42-kDa lipoprotein was unknown, as *B. recurrentis* has only recently been cultured *in vitro*[14]. To identify the protein component, we subjected the lipoprotein to tryptic digestion, and three peptides obtained by reverse-phase microbore HPLC were sequenced by Edman degradation. Degenerate oligonucleotide primers were designed using this sequence information and the codon bias of *B. hermsii*. Using these primers and *B. recurrentis* DNA in a polymerase chain reaction, a 715-bp fragment was amplified. The fragment was sequenced, and a BLAST search of GenEMBL database using the deduced amino-acid sequence showed that it shares 73% identity with vmp12 of *B. hermsii* (Fig. 3). This provides the first formal evidence, to our knowledge, of a vmp-like sequence in *B. recurrentis*.

Vmp is one of the most abundant proteins in *B. recurrentis* thus, a contaminant might be responsible for the TNF-inducing activity that co-purifies with the 42-kDa lipoprotein. Further purification by reverse-phase HPLC demonstrated a main peak of ultraviolet absorbance that was coincident with the main peak of TNF-inducing activity (Fig. 4*a*). Analysis of this peak by SDS–PAGE showed that it contained a single band of 42 kDa whose intensity correlated with the level of TNF-inducing activity. These observations confirmed that the TNF-inducing activity could not be dissociated from the 42-kDa protein of isolate A1. *B. recurrentis* isolates express a single vmp of broadly different molecular weights[13]. Therefore, to confirm that the TNF-inducing factor is vmp, we repeated this purification scheme for

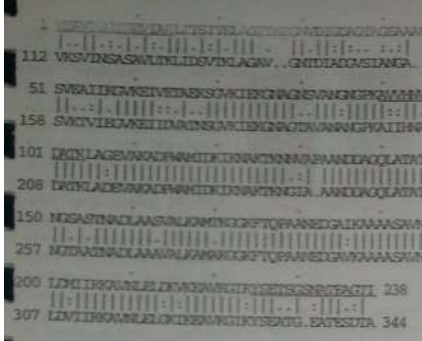


Fig. 3 Alignment of partial peptide sequence from *B. recurrentis* isolate A1 with that of vmp12 of *B. hermsii*. Gaps (represented by dots) were introduced by Bestfit program to maximize the alignment. The sequences of the microsequenced peptides are underlined. The vmpA1 partial sequence has been deposited with EMBL (accession number AJ224157).

two additional Ethiopian isolates, A9 and A18. The size of the TNF-inducing factor differed between isolates (Fig. 4*b*). A18 had a single main peak of TNF-inducing activity corresponding to a protein of 23 kDa, whereas A9 had biphasic active peak of around 37 kDa. For each, the size of the purified TNF-inducing factor corresponded to that of the major membrane protein[13] (Fig. 4*c*), confirming that vmp is indeed the active molecule.

Vmps are expressed in the host

To determine if these TNF-inducing factors are expressed in the human host, we analyzed sera from two patients who had recently recovered from *B. recurrentis* infection and from two control subjects. Western blotting demonstrated that IgG from European and Ethiopian serum control subjects did not cross-react with vmps. In contrast, IgG from both patients' sera cross-reacted with the three vmps. One patient's serum cross-reacted mainly with vmpA1 and A18; the other, mainly with A9 (Fig. 5*a*). This indicates that vmps are expressed during natural infection and are immunogenic in the two patients studied. Differences in the intensity of the detection indicate that the vmps are antigenically variable.

Vmps vary in their potency

During the purification process, we observed differences in the level of TNF induced by the three vmps. We investigated this more rigorously by assaying the vmps across a range of protein concentrations on both MonoMac6 cells and PBMCs. The TNF-inducing activity of vmpA9 was found to be 2% that of vmpA18, and 10% that of vmpA1 (Fig. 5*b*). Thus, the variable clinical severity of borrelial infection might be due, in part, to the type of vmp expressed.

Discussion

By systematic fractionation of cell lysates into active components, we have identified the vmp of *B. recurrentis* as the predominant TNF-inducing factor. The lipid component of vmp is essential for TNF-inducing activity. Unlike other TNF-inducing lipoproteins, such as OspA, which seem to be expressed only in the tick vector[15–18], this TNF-inducing lipoprotein is expressed in the human host. Vmp was first identified in *B. hermsii* as a structure that undergoes multiphasic antigenic variation[19–21]. Another role for vmp in mammalian infection has been demonstrated in a murine model of *B. turicatae* infection, in which differential expression of vmp variants altered the pattern of disease[22]. Recent experimental studies of *B. burgdorferi* have associated infectivity in mice with the presence of a plasmid encoding vmp-like structures, raising the possibility that these structures are important in establishing the infection[23]. Our finding of differences in the potency of vmp in induction of TNF indicates that an important consequence of the variation in vmp-like structures may be to modulate the host inflammatory response. It will be important to determine whether strain differences in TNF induction arise from variation in the lipid moiety itself or from modulation of activity by differences in the polypeptide sequence. Better understanding of these structure–function relationships may help to explain the clinical heterogeneity of borrelial diseases, and raises the possibility of new therapeutic approaches for these debilitating inflammatory conditions.

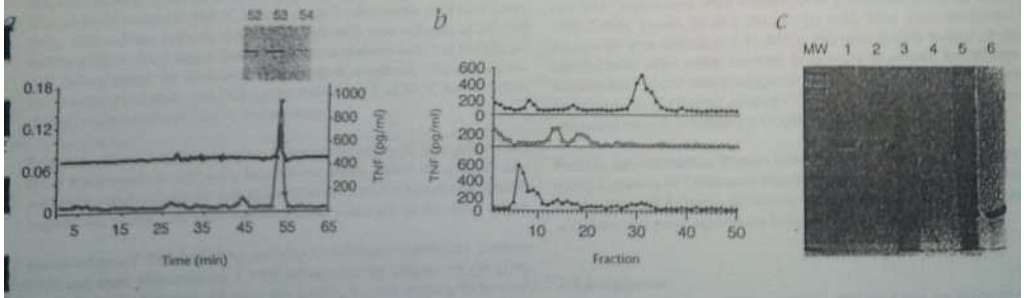


Fig. 4 Vmp is the TNF-inducing molecule. *a*, Purification by reverse-phase HPLC of the TNF-inducing activity from isolate A1. Fractions were assayed on MonoMac6 cells (at a 1:1,000 dilution). Inset, The main peak of TNF-inducing activity was analyzed by SDS–PAGE followed by Coomassie blue staining. —, A214; ⊖, TNF-inducing activity. *b*, Preparative electrophoresis of SDS-solubilized protein extracts from *B. recurrentis* isolates A1, A9 and A18. Fractions were assayed on MonoMac6 cells at a 1:1,000 dilution (A1 and 18) or 1:200 dilution (A9). ●, A1; ○, A9; ▲, A18. *c*, Conventional SDS–PAGE analysis with Coomassie blue-staining of protein extracts from isolates A1, A9 and A18 (lanes 1, 3 and 5) and of the corresponding TNF-inducing fractions purified by preparative electrophoresis and concentrated using Centriplus 10 concentrator device (lanes 2, 4 and 6). Molecular weight markers (MW, top to bottom): 200, 115, 97, 67, 45, 31, 21 and 14 kDa.

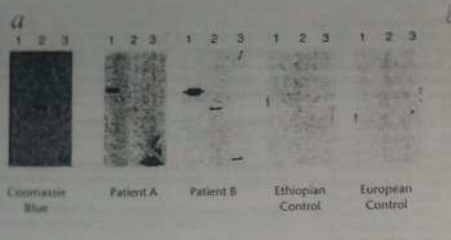


Fig. 3. Vmps are expressed in the host and elicit different TNF responses in vitro. **a**, Purified vmps were transferred onto nitrocellulose after separation by SDS–PAGE. The blots were probed with sera from two Ethiopian patients (A and B) who were treated 10 days previously for relapsing fever due to *B. recurrentis*. Control blots (right) were probed with sera from Ethiopian and European donors. Vmps were also analyzed by Coomassie blue staining of a polyacrylamide gel. Lanes 1, 2 and 3: vmpA1, vmpA9 and vmpA18. **b**, Results of three experiments on MonoMac6 cells (mean and standard error) expressed as a percentage of the amount of TNF induced by 30 nM vmp from isolate A1, which averaged 3543 ± 345 pg/ml. Experiments on PBMCs gave similar results. ◇, vmpA1; □, vmpA9; ○, vmpA18.

Methods

Extraction of the TNF-inducing activity from *B.recurrentis*. *B. recurrentis* isolate A1 was grown in BSKII medium supplemented with 6% rabbit serum[14]. When the culture reached late logarithmic phase, cells were harvested, washed in HEPES buffered saline (HeBS; 25 mM HEPES, pH7.5, 150 mM NaCl) and sonicated in 1 ml HeBS. The lysate was centrifuged for 15 min at 16,000*g*. The HeBS-insoluble fraction was extracted with methanol:chloroform:water (8:4:3); the organic phase was dried down and resuspended in 500 µl of HeBS, 0.1% SDS. The remaining pellet was extracted with 0.1% SDS in HeBS.

TNF-inducing assay. MonoMac6 cells were grown as described[24]. PBMCs were isolated as described[15]. Cell stimulation with serial dilutions of stimuli and TNF ELISA was done as described[15].

Properties of TNF-inducing activity. MonoMac6 cells were stimulated for 6 hours with either an SDS extract diluted to a protein concentration of 600 ng/ml or with 10 ng/ml *E. coli* LPS. To determine serum dependence, cells were washed three times with serum-free medium before 'plating out' with and without 5% FCS. To assess CD14 dependence, cells were stimulated in the presence or absence of 60BCA culture supernatant at a dilution of 1:20. 60BCA hybridoma cells were grown as recommended by the supplier (ATCC). To determine polymixin B sensitivity, cells were stimulated in the presence or absence of 10 Units/ml polymixin B. To determine lipase sensitivity, SDS-soluble extracts were treated at 37 °C for 16 hours with either 1000 Units/ml pig pancreas lipase or 10000 Units/ml *Rhizopus arrhizus* lipase in 50 mM HEPES buffer, pH 7.5 plus 10 mM $CaCl_2$. Digests were heated at 100 °C before assay. To determine mild base sensitivity, SDS-soluble extracts were incubated with nine volumes of 50 mM NaOH at 56 °C for 3 hours. Samples were neutralized with 1 M HEPES, pH 7.5, before assay. To determine Proteinase K sensitivity, SDS-soluble extracts were incubated with 100 µg/ml Proteinase K at 37 °C for 16 hours. Digests were heated at 100 °C before assay.

Palmitic acid metabolic labelling. *B. recurrentis* isolate A1 was grown in 15 ml of BSKII medium until early logarithmic phase, before the addition of 4 mCi of [9,10(n)-^{3}H]palmitic acid (Amersham). When the culture reached late logarithmic phase, cells were harvested and processed as described above.

Purification of TNF-inducing activity. Solubilized membrane proteins (350 µg) were separated on a 12% polyacrylamide preparative gel (Prep cell 491; inner diameter, 28 mm; gel length, 5.5 cm; BioRad, Richmond, California) according to manufacturer's instructions. Fractions (5 ml each) were collected and assayed for TNF-inducing activity on MonoMac6 cells and for radioactivity by scintillation counting. Active fractions were analyzed by SDS–polyacrylamide slab gels. Gels were either processed for fluorography or stained with Coomassie blue.

Cloning of vmpA1. Active fractions were concentrated using a Centriplus 10 concentrator device (Amicon, Houston, Texas), then precipitated with 10% TCA. The pellet was processed for trypsin digestion as described[27]. The digestion products were fractionated by reverse-phase HPLC, and three peptides were chosen for microsequencing on a ABI Procise 49. (Applied Biosystem, Norwalk, Connecticut). *B. recurrentis* DNA (ref. 27) and primers derived from peptide sequences (forward primer: GTWGARGTWATWCAAGGWAC; reverse primer, TGWATAGTWC CWGCXTCAGTWGC) were used to obtain a 715-bp PCR product, which was cloned into pCR T vector (Invitrogen, Carlsbad, California) and sequenced using BigDye Terminator (Applied Biosystems, Norwalk, Connecticut). The deduced amino-acid sequence was used to BLAST search GenEMBL. Peptide sequences were aligned using Bestfit. All computer programs were from the Wisconsin Genetics Computer Group, Madison, Wisconsin.

Reverse-phase HPLC. A portion (20%) of the most active fraction from a preparative electrophoresis separation was applied at a flow rate of 1 ml/min to a Vydac 214TP column equilibrated with 95% water, 5% acetonitrile, 0.1% TFA (Solvent A). The column was washed for 10 min with this solvent, and then developed with a 2%-per-min gradient of solvent B (5% water, 95% acetonitrile, 0.1% TFA) to 100% solvent B. The column was washed for a further 10 min with 100% solvent B. One-minute fractions were collected, dried under vacuum and reconstituted in HeBS/0.1% SDS before assay on MonoMac6 cells.

Immunoblot analysis. Purified vmps (100 ng) were separated by 12% SDS–PAGE gels and transferred to nitrocellulose membrane. Membranes were stained with Ponceau S to confirm that similar amounts of each protein were transferred. After incubation in phosphate-buffered saline (PBS) with 0.05% Tween 20 and 5% non-fat milk, blots were incubated for 2 hours with sera diluted at 1:15,000 in PBS with 0.05% Tween 20 and 2% non-fat milk. After being washed, blots were incubated for 30 min with horseradish peroxidase-conjugated rabbit antiserum to human immunoglobulin G (Sigma), diluted 1:3,000. Bands were detected using the enhanced chemiluminescence kit (Amersham).

Protein determination. Protein concentration was determined by densitometry scanning of Coomassie blue-stained bands on a polyacrylamide gel and by microBCA (Pierce, Rockford, Illinois). BSA was used as the standard and there was good agreement between the two methods.

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1. Johnson Jr., W.D. in *Principles and Practice of Infectious Diseases, 4th ed.* (ed. Mandell, G.L., Bennett, J.E. & Dolin, R.) 2141–2143 (Churchill Livingstone, New York, 1994).

2. Hyams, A.D. Clinical pathology of the Jarisch-Herxheimer reaction. *J. Infect. Dis.* 133, 696–704 (1976).
3. Luger, S.W. *et al.* Comparison of cefuroxime axetil and doxycycline in treatment of patients with early Lyme disease associated with erythema migrans. *Antimicrob. Agents Chemother.* 39, 661–667 (1995).
4. Wright, D.J. Reaction following treatment of murine borreliosis and Shwartzman type reacion with borrelial sonicates. *Parasite Immunol.* 2, 201–221 (1980).
5. Negussie, Y. *et al.* Detection of plasma tumor necrosis factor, interleukins 6, and 8 during the Jarisch-Herxheimer Reaction of relapsing fever. *J. Exp. Med.* 175, 1207–1212 (1992).
6. Fekade, D. *et al.* Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor alpha. *N. Engl. J. Med.* 335, 311–315 (1996).
7. Takayama, K., Rothenberg, R.J. & Barbour, A.G. Absence of lipopolysaccharide in the Lyme disease spirochete, Borrelia burgdorferi. *Infect. Immun.* 55, 2311–2313 (1987).
8. Radolf, J.D. *et al.* Lipoproteins of Borrelia burgdorferi and Treponema pallidum activate cachectin/tumor necrosis factor synthesis. Analysis using a CAT reporter construct. *J. Immunol.* 147, 1968–1974 (1991).
9. Weis, J.J., Ma, Y. & Erdile, L.F. Biological activities of native and recombinant Borrelia burgdorferi outer surface protein A: dependence on lipid modification. *Infect. Immun.* 62, 4632–4636 (1994).
10. Bouchon, B., Klein, M., Bischoff, R., Van Dorsselaer, A. & Roitsch, C. Analysis of the lipidated recombinant outer surface protein A from Borrelia burgdorferi by mass spectrometry. *Anal. Biochem.* 246, 52–61 (1997).
11. Braun, V. Covalent lipoprotein from the outer membrane of Escherichia coli. *Biochim. Biophys. Acta* 415, 335–377 (1975).
12. Fraser, C.M. *et al.* Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. *Nature* 390, 580–586 (1997).
13. Cutler, S.J. *et al.* Borrelia recurrentis characterization and comparison with relapsing-fever, Lyme-associated, and other Borrelia spp. *Int. J. Syst. Bacteriol.* 47, 958–968 (1997).
14. Cutler, S.J. *et al.* Successful *in-vitro* cultivation of Borrelia recurrentis. *Lancet* 343, 242 (1994).
15. de Silva, A.M.D. & Fikrig, E. Borrelia burgdorferi genes selectively expres ticks and mammals. *Parasitol. Today* 13, 267–270 (1997).
16. de Silva, A.M. & Fikrig, E. Arthropod- and host-specific gene expressi Borrelia burgdorferi. *J. Clin. Invest.* 99, 377–379 (1997).
17. Schwan, T.G., Piesman, J., Golde, W.T., Dolan, M.C. & Rosa, P.A. Induct an outer surface protein on Borrelia burgdorferi during tick feeding. *Pro Acad. Sci. USA* 92, 2909–2913 (1995).
18. Montgomery, R.R., Malawista, S.E., Feen, K.J. & Bockenstedt, L.K. demonstration of antigenic substitution of Borrelia burgdorferi *ex vivo*: ration of the paradox of the early immune response to outer surface prot and C in Lyme disease. *J. Exp. Med.* 183, 261–269 (1996).
19. Barbour, A.G., Tessier, S.L. & Stoenner, H.G. Variable major proteins of Bo hermsii. *J. Exp. Med.* 156, 1312–1324 (1982).
20. Plasterk, R.H., Simon, M.I. & Barbour, A.G. Transposition of structural ge an expression sequence on a linear plasmid causes antigenic variation i bacterium Borrelia hermsii. *Nature* 318, 257–263 (1985).
21. Barbour, A.G. Antigenic variation of a relapsing fever Borrelia species. *Rev. Microbiol.* 44, 155–171 (1990).
22. Cadavid, D., Thomas, D.D., Crawley, R. & Barbour, A.G. Variability of a b ial surface protein and disease expression in a possible mouse model o temic Lyme borreliosis. *J. Exp. Med.* 179, 631–642 (1994).
23. Zhang, J.R., Hardham, J.M., Barbour, A.G. & Norris, S.J. Antigenic variat Lyme disease borreliae by promiscuous recombination of VMP-like sequ cassettes. *Cell* 89, 275–285 (1997).
24. Pradines Figueres, A. & Raetz, C.R. Processing and secretion of tumor ne factor alpha in endotoxin-treated Mono Mac 6 cells are dependent on ph myristate acetate. *J. Biol. Chem.* 267, 23261–23268 (1992).
25. Allan, R.J., Rowe, A. & Kwiatkowski, D. Plasmodium falciparum varies in its ity to induce tumor necrosis factor. *Infect. Immun.* 61, 4772–4776 (1993).
26. Matsudaira, P. in *A Practical Guide to Protein Purification for Microseque* (Academic Press, London, 1993).
27. Barbour, A.G. & Garon, C.F. Linear plasmids of the bacterium Borrelia burg feri have covalently closed ends. *Science* 237, 409–411 (1987).

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CLINICAL PROFILE OF ETHIOPIAN PATIENTS WITH BREAST CANCER
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CLINICAL PROFILE OF ETHIOPIAN PATIENTS WITH BREAST CANCER

A. GEBREMEDHIN and M. SHAMEBO

ABSTRACT

This prospective study was designed to obtain information on demographic characteristics, clinical profile and problems related to early diagnosis and treatment of breast cancer in 72 Ethiopian patients. There were 62 females and 10 males, the female to male ratio being 6.2:1. The age range of the females was 21-82 (mean 4 1. 8±12.8) years and that of the males' was 38-75 (mean 52.1±12.2) years. The time interval between the onset of breast-related symptoms to diagnosis varied from 2-108 (median 12) months. Infiltrating ductal and lobular carcinoma histologic types accounted for 85% and 11%, respectively, in 62 cases who had surgical biopsies. Surgery was performed in 46 cases out of whom only 21 cases received adjuvant treatment. Eighteen females refused mastectomy at some point before they came to our clinic with metastatic disease. After a median follow-up duration of 36 (range 2-120) months, 29 cases were alive, 24 died and 19 were lost to follow-up. The cause of death in 17 subjects (71%) was rapidly refilling pleural effusion and superimposed infection. Both females and males had similar clinical characteristics, except that, the males were older by 10 years. Moreover, the females in this series developed breast cancer at a younger age (72% were premenopausal) and 76% had advanced disease (Stages III and IV) at presentation, similar to females from other African countries. We suggest that the attitude of Ethiopian females towards breast cancer has to change through continuous but targeted public education.

INTRODUCTION

Breast cancer is a major public health problem for women living in the western world. In the United States, it was estimated to account for 32% of all cancers diagnosed in females in 1994(1). The incidence of breast cancer varies widely among different populations. In general, low incidence rates and mortality from breast cancer have been reported in most African and Asian countries(2). In most sub-Saharan African countries, it is only exceeded by cervical cancer as the most common malignancy in females(3). Although epidemiological and/or clinical studies on breast cancer have not been conducted in Ethiopia, an analysis of pathological material from 2 pathology centers in the country reported that breast cancer was the second most common malignancy in females after cancer of the uterine cervix(4).

Male breast cancer is, however, rare in caucasians accounting for less than 1% of all breast cancers in contrast to reports from Africa with incidence rates ranging from 2.4 % to 15%(5,6).

The objectives of this study were to describe the demographic, clinical characteristics and problems related to early diagnosis and treatment of breast cancer in patients attending a referral clinic in a teaching hospital and to compare these with studies of similar nature from Africa and elsewhere.

MATERIALS AND METHODS

The study was conducted at the hematology referral clinic of Tikur Anbessa Hospital (TAH), the main teaching hospital in Addis Ababa, Ethiopia, between January 1992 to December 1997. The study group consisted of 72 consecutive, biopsy proven cases of breast cancer. Fifty-seven subjects were referred from the medical and surgical departments of TAH. The remaining 15 cases came back from abroad to finish their adjuvant chemotherapy and to continue follow-up in our center. These patients came after a median follow-up duration of 6 (range 2-60) months abroad.

Data was collected according to a preset protocol that contained information on the following variables:

Age, sex, date of diagnosis, presenting complaints and duration, family history of breast cancer, oral contraceptive use, traditional medicine use, size and description of the primary breast lump, clinical stage, histologic type, type of therapy offered and response, duration of the follow-up period and outcome of the patients under consideration.

Treatment modalities offered included:

Surgery: modified or radical mastectomy ± oophorectomy or lumpectomy. Medical;adjuvant or palliative treatment with chemotherapy ± radiotherapy ± tamoxifen. The Chemotherapy regimens consisted of: CMF (Cyclophosphamide + Methotrexate + 5-Fluorocuracil),CAF (Cyclophosphamide + Adriamycin + 5-FU), or CA (Cyclophosphamide + Adriamycin).

The choice of chemotherapeutic regimens depended upon availability and the doses were those suggested by Henderson *et*

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al.(7). Response criteria for systemic treatment were those of the Eastern Cooperative Oncology Group Recommendations(8).

RESULTS

During the 6-year study period, 62 females and 10 males with breast cancer were seen in our clinic, making a female to male ratio of 6.2:1. The age of the females ranged from 21-82(mean 41.8±13.3) years. 39 females (75%) were below the age of 51 years. The age range was 38-75 (mean 52.1±12.6) years. Table 1 shows the age and sex distribution.

Table 1

Age and Sex distribution of patients with breast cancer

Age group (years)	Sex		Total No.	%
	Female	Male		
21-30	10	–	10	13 9
31-40	27	2	29	40.3
41-50	10	3	13	18.0
51-60	9	2	11	15.3
61+	6	3	9	12.5
Total	62	10	72	100

The median time-lag between development of breast-related symptoms to diagnosis was 12 (range 2–108) months. The most common presenting complaints were: breast lump (92%), ulceration and retraction (40%) and nipple discharge (18%). Five females came with breast-unrelated symptoms; 3 with shortness of breath due to pleural effusion and 2 due to back pain and weakness of the lower limbs. Family history of breast cancer was strongly positive in 2 females; in the first, mother and sister and in the second mother and aunt died of breast cancer at a young age. Ten females gave a history of oral contraceptive use, with duration ranging from 6-96 (median 24) months. Twenty-four cases (22 females & 2 males) admitted that they had used traditional medicine.

Eighteen females who had refused mastectomy presented to our clinic with advanced disease after a median duration of 18 (range 8-60) months. The size of the primary breast lump in its largest diameter was 6. 5 (range 1.5-14) cm. . At initial presentation, 17 cases (24%) had stage II, 29 (40%) stage III and 26 (36%) stage IV disease.

Table 2 shows the histologic types among which the infiltrating ductal and lobular carcinoma accounted for 85% and 11%, respectively. While 62 cases had surgical biopsies, ten cases had fine needle aspiration cytology (FNAC) only.

Table 3 shows the treatment modalities offered to the patients Radical mastectomy and lumpectomy were performed in 44 and 2 cases respectively. In 2 females oophorectomy was performed additionally. Of the 46 subjects, it was only in 21 that an adjuvant treatment was considered immediately after surgery. As a result, 15 were referred abroad (11 to Kenya, 2 to the USA, one to South Africa and another one to the UK) and the remaining 6 cases were treated in our center. After a median follow-up period of 36 (range 3-120) months, 15 of the 21 patients remained alive, with a median disease free survival of 24 (range 3-120) months. Four died and 2 were lost to follow-up. For the remaining 25 cases, no further treatment was offered or suggested after surgery. They presented again with local recurrence or distant metastasis after a median duration of 18(5-70) months. Forty patients were given medical treatment with palliative intent (Table 3). The main treatment modality in 33 cases was chemotherapy. Of 24 cases who completed treatment, 6 achieved complete response ranging from 6-28 (median 12) months, 14 had partial response (median duration 6 months) and 4 patients had stable disease (median duration 4 months). The remaining 9 patients did not complete treatment because they died during treatment or were lost to follow-up.

Table 2

Histologic diagnosis in patients with breast cancer

Histologic	Sex		Total No	%
	Female	Male		
Infiltrating Duuctal Carcinoma	46	7	53	73.6
Infiltrating Lobular Carcinoma	6	1	7	9 7
Colloid (infiltrating)	2	-	2	2.8
Fine Needle Aspiration Cytologic Diagnosis only	8	2	10	13.9
Total	62	10	72	100

Table 3

Treatment modalities offered to patients with Breast Cancer

Modality of treatment	Sex		Total No	%
	Female	Male		
RM only	17	4	21	29 2
RM + Oophorectomy	2	-	2	3.2
Lumpectomy only	2	-	2	3 2
RM ± CTx ± RTx ±Tam (adjuvant therapy)	18	3	21	29.2
Palliative CTx ±RTx ±Tam	32	1	33	45.8
Palliative RTx only	3	0	3	4.2
PalliativeTamonly	4	-	4	5 5
Received no treatment	9	2	11	15.3

*Abbreviations: RM: Radical mastectomy
CTX: Chemotherapy
RTX: Radiotherapy
Tam: Tamoxifen

Eleven subjects were not given active treatment either because of poor performance status or there was no means of treating them.

After a median follow-up duration of 36 (range 2-120) months, 29 cases were alive, 24 died and 19 were lost to follow-up (most are believed to have died). The causes of death included recurrent pleural effusion complicated by respiratory embarrassment and superimposed infections in 17 cases, intractable seizure in 2, cord-compression and

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its complications in 2 and liver failure and spontaneous peritonitis in 3 patients.

DISCUSSION

Over the 6-year study period 72 cases of breast cancer were seen in our clinic. This does not by any means reflect the state of affairs of breast cancer in this hospital or the country. We believe more patients are seen and operated yearly but they are not considered for additional treatment or perhaps they come with advanced disease and are left alone to die at home. Although population-based studies on neoplastic diseases in general and breast cancer in particular have not been conducted in Ethiopia, we believe that breast cancer is a common malignancy in females . In an analysis of cancer specimens from 2 pathology centers in Ethiopia, Loutfi and Pickering have reported that breast cancer was the most common malignancy after cancer of the cervix in females(4). This is true with regard to the disease in females in most sub-Saharan countries(9).

Studies from Africa have consistently reported that African females with breast cancer are at least a decade younger than their counterparts in the West at diagnosis(10,11). Although the number of females reported here is small, they are even younger (mean age 41.8±12.8) than those reported from other African countries. Why African females develop breast cancer at a younger age is not clear. Some investigators attribute this to the wide base of the age pyramid in African populations(10). Interestingly the risk for breast cancer in young women in the United States is slightly but consistently higher in blacks(12). It is possible that genetic, reproductive and environmental factors also play a role(12,13).

Perhaps, the most undesirable aspect of breast cancer in Africans is that it is already advanced at presentation. This is not an unexpected finding since patients come late in the course of their illness with large, fungating tumors. Forty-seven (76%) of the 62 females in this series had advanced disease at presentation. This is in keeping with those reported from Zaire and Nigeria(10,11). The main histological tumor type reported in African females with breast cancer is infiltrating ductal carcinoma, accounting for 70-90% of cases(9,13,14). This is similar to reports in Caucasian females(15). Forty-six of 54 females (85%) who have had surgical biopsies had infiltrating ductal carcinoma in our series.

The high frequency of a more malignant histologic type reported in African females similar to black females in the USA could, therefore be due to the more advanced nature and large tumor size at presentation in these patients(11,12,14). In fact, tumor size and clinical stage are the two most important prognostic factors in the natural history of breast cancer(16). Moreover, black women in South Africa and the United States have been found to have a high proportion of oestrogen receptor negative tumors, worsening the prognosis of breast cancer in black females(12,17).

Breast cancer occurs rarely in men, accounting for less then 1% of the rate in women in the West(5). In the case of Africa, its incidence has been reported to range from 2.4%-15%(6). In our series, there were 10 males, accounting for 14% of the total and they were a decade older than the females. Although population differences could account for the higher incidence of male breast cancer in Africa, the exact reasons for this are not known. Some investigators think that this simply reflects the relative rarity of breast cancer in black women compared to Caucasians(6). Abnormalities of oestrogen metabolism resulting in gynaecomastia are cited by others as putative causes(18). Gynaecomastia was not evident in the males reported in this series.

In Ethiopia, early detection of breast cancer is hampered by many factors. The most important one is that patients seek treatment late in the course of their illness mainly due to lack of access to modern medical care. Consequently, they are left to the mercy of traditional healers. It is also likely and more so in this study that, these young females may not seek early medical care because of fear of mastectomy. Eighteen females (29%) refused mastectomy and 24 cases (33%) have resorted to traditional treatment. Even if patients come early to hospitals, the health care delivery system with limited resources is not well prepared and sometimes not enthusiastic to provide the necessary service and information. A typical example is the case of the 25 cases reported here who had undergone mastectomy or lumpectomy. They were not told about possibilities of further treatment after surgery until they came again with advanced disease.

It is not possible to infer meaningful conclusions from the results of medical treatment given to our patients, because the regimens were not uniform and the combinations incomplete and erratic. The reason for this is that, chemotherapeutic agents are not available in our hospital on a regular basis. As a result, only some rich or desperate patients could import them from abroad and were able to complete their treatment. It is high time that the one tertiary-care center we have in this country be equipped with the means to treat patients with cancer in general and breast cancer in particular. At the same time the attitude of Ethiopian females towards breast cancer has to change through targeted and continuous public education.

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REFERENCES

1. Boring, C.C., Squires, T.S., Tong, T. and Montgomery, S. Cancer statistics, 1994. CA: A Cancer Journal for Clinicians. 1994; 44:7-26.

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Doll, R., Payne, P. and Waterhouse, J. Cancer incidence in 5 continents. Berlin: Springer-Verlag, 1966.
Parkin, D.M. (ed). Cancer occurrence in developing countries. Oxford University Press, New York, 1996.
Loutfi, A. and Pickering, J.L. The distribution of cancer specimens from two pathology centers in Ethiopia. *Ethiop. Med. J.* 1992; 30:13-17.
Ewertz, M., Holmberg, L., Karjalainen, S., *et al.* Incidence of male breast cancer in Scandinavia: 1943-1982. *Int. J. Cancer.* 1989; 43:27-31.
Hassen, I. and Mabogunje, O. Cancer of the male breast in Zaria, Nigeria. *East Afr. Med. J.* 1995; 72:457-458.
Henderson, I.C., Harris, S.R., Kinne, D.W. and Hellman, S. Cancer of the breast. In: DeVita, Hellman, S., Rosenberg, S.A., (eds). *Cancer: Principles and practice of Oncology.* 3rd. ed. Philadelphia, J.B. Lippincott, 1989;1197-1268.
Hayward, J.L., Rubens, R.D., Carbone, P.P., *et al.* Assessment of response to therapy in advanced breast cancer. *Br. J. Cancer.* 1977; 35:292-298.
Amir, H., Kitinya, J.N., Parkin, D.M. A comparative study of carcinoma of the breast in an African population. *East Afr. Med. J.* 1994; 71:215-218.
Kenda Ngala, J.F., Chirimwami, B., Veyi, T. Clinico-pathological analysis of carcinoma of the breast in an African population. *Arch. Surg.* 1988; 12:972-974.
Chiedozi, L.C. Breast cancer in Nigeria. *Cancer* 1985; 55:653-657.
Moormeier, J. Breast cancer in black women: A review. *Ann. Intern. Med* 1996; 124:897-905.
Bjerregaard, B. and Kungu, A. Breast cancer in Kenya: a histopathologic and epidemiologic study. *East Afr. Med. J.* 1992; 69:22-25.
Mulhuphei, M.N. Carcinoma of the breast at Ga-Rankuwa Hospital Histopathology Laboratory (South Africa). *Cent. Afr. J. Med.* 1994; 40:254-257.
Burstein, N.A. Pathology of breast cancer. In: D'Orsi C.J., Wilson, R.E. (eds). *Carcinoma of the breast: Diagnosis and treatment.* Little, Brown and Company, Boston, 1983; 59-82.
Tabar, L., Fagerberg, G., Day, N.E., *et al.* Breast cancer treatment and natural history: New insights from results of screening. *Lancet* 1992; 339:412-414.
Savage, N., Levin, J., De Moor, N.G., Lange, M. Cytosolic oestrogen receptor content of breast cancer tissues in blacks and whites. *S. Afr. Med. J.* 1981; 59:623-4.
Bezwoda, W.R., Hesdeffer, C., Dansey, R., *et al.* Breast cancer in men: clinical features hormone receptor status and response to therapy. Cancer 1987; 60:1337-40.

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CUSHING'S SYNDROME: A TEN YEAR EXPERIENCE AT TIKUR ANBASSA HOSPITAL

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ABSTRACT: *A retrospective analysis of Cushing's syndrome from the retrieved records of 24 out of a total of 27 referred cases over a period of 10 years (January 1985-January 1995) showed confirmation in 16 cases. Fourteen (88%) were females, 9 (56%) were in their third decade. Duration of symptoms before admission was one year or less in eleven (69%) patients. Among symptoms weakness, fatigue and weight gain and among signs moon face, truncal obesity and buffalo hump were found in all patients. Amenorrhea occurred in all the women of childbearing age. Basal urinary 17-hydroxycorticoid (17-OHCS) values were elevated in 9/12 patients and low dose dexamethasone suppression test favoured Cushing's syndrome in 8/9 patients. Definitive treatment consisted of bilateral adrenalectomy in 7 and transfrontal pituitary adenomectomy in 3. Two patients declined surgery; 4 patients were lost to follow up before definitive treatment. Two patients who had bilateral adrenalectomy and two who had transfrontal adenomectomy died subsequently while three remained in good health on corticosteroid replacement therapy. Three of the 7 patients who had bilateral adrenalectomy developed Nelson's syndrome. It is concluded that Cushing's disease is the most frequent cause of Cushing's syndrome in our series. The patients presented with the well-known clinical manifestations of hypercortisolism. However, our laboratory facilities require considerable improvement to serve as a reliable adjunct to clinical evaluation. Since Cushing's syndrome, whatever the cause, is eventually fatal if left untreated, surgical intervention is mandatory after confirmation of the cause with appropriate laboratory tests.*

INTRODUCTION

Cushing's syndrome covers all causes of hypercortisolism including abnormalities of the hypothalamus, pituitary gland, adrenals, ectopic secretion of Adrenocorticotrophic

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hormone (ACTH) and exogenous steroid administration (1-4). The typical clinical manifestations of Cushing's syndrome are the result of the metabolic effects of excess cortisol and also excess sex hormones (mainly androgens), produced by the adrenal cortex. The central obesity, producing the characteristic moon face, buffalo hump and truncal obesity, is the result of the peculiar effect of hypercortisolism on the body fat distribution. The other clinical manifestations, which are found in variable combinations in cases of Cushing's syndrome are the result of chronic hypercortisolism on protein, glucose and sodium metabolism (3-6).

Observation at our clinic has indicated that Cushing's syndrome is not uncommon. However, a review of analyses of admissions from a number of hospitals in this country over the past three decades reveals that it is not mentioned in the list of endocrine diseases encountered at those centers (7). We therefore felt it worthwhile to draw attention to the occurrence of this problem in Ethiopia based on our experience in its diagnosis and management.

PATIENTS AND METHODS

This report is a retrospective analysis of the findings in 16 patients with confirmed Cushing's syndrome among 27 suspected cases referred to the Endocrine Clinic of the Tikur Anbassa Hospital over a period of 10 years starting from January 1985. The records of 3 patients were lost. Cushing's syndrome was diagnosed in 16 of the 24 cases whose records could be retrieved for analysis.

Diagnosis of Cushing's syndrome was based on:

(1) The presence of the well-known symptoms and signs of hypercortisolism and;

(2) Abnormality of one or more of the following tests which are the only available hormone determinations: basal urinary 24-hour 17-OHCS and/or 17-KS with, whenever possible, confirmatory dexamethasone suppression and/or 8:00 a.m. and 11:00 p.m. serum cortisol values. In addition one or more of the following findings in a patient with typical Cushing's syndrome favoured a pituitary adenoma as the underlying cause: a history of hyperpigmentation, evidence of temporal hair recession, facial hirsutism, radiological evidence of sellar enlargement.

The serum cortisol was determined by enzyme immunoassay; the urinary 17-OHCS and 17-KS by Porter Silber chromogen and quantitative colorimetric techniques respectively (8) at the National Research Institute of Health, Addis Ababa, Ethiopia.

In the assessment of Treatment Outcome, remission was defined as complete disappearance of the signs and symptoms of hypercortisolism. Recurrence is reappearance of signs and symptoms of hypercortisolism after a period of remission.

RESULTS

There were 14 (87%) females and 2 (13%) males with a median age of 24 years (range 16-55 years). Four patients were referred from the General Medical Outpatients Unit of the Tikur Anbassa Hospital and the other 12 from different parts of Ethiopia. The mean duration of symptoms at the time of diagnosis was 22 months (range 3-72 months).

As shown in Table 1 the classical symptoms and signs of Cushing's syndrome are well illustrated in this small series. Fourteen patients had hypertension, 6 mild and 8 moderate to severe. Seven patients had

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diabetes diagnosed on clinical and fasting blood glucose criteria. Generalised itching occurred in 8 (47%) patients 2 of whom had strongylojdiasis. Thirteen of the total of 14 women were in child bearing age and they all had amenorrhea.

Table 1. Symptoms and signs in 14 females and 2 males with Cushing's Syndrome, Tikur Anbassa Hospital, Addis Ababa, January 1985-January 1995.

Symptoms and signs	No. of patients	%
Weight gain	16	100
Weakness & fatigue	16	100
amenorrhoea	13/13*	100
headache	15	94
low back pain	10	63
generalised itching	8	47
moon face	16	100
truncal obesity	16	100
buffalo hump	16	100
acne	3	19
ecchymoses	4	25
purple striae	3	81
fungal & bacterial skin infections	7	44
hypertension	14	88
diabetes mellitus	7	44

*total of women in child bearing age.

Low dose dexamethasone suppression test (LDDST) showed failure of suppressions in 8 of the 9 patients tested. One patient was found to have normal suppression but she had the classical clinical picture of Cushing's syndrome and was subsequently inadvertently subjected to the high dose dexamethasone suppression test (HDDST) which showed failure of suppression. Repetition of LDDS test would have been more appropriate in this case with classical clinical findings. This patient had an elevated urinary 17-KS level. Bilateral adrenalectomy was performed and the histopathology revealed bilateral adenomatous hyperplasia.

Table 2. Correlation of histopathology with the pattern of hormonal abnormalities in 10 cases of Cushing's syndrome, Tikur Anbassa Hospital, Addis Ababa, January 1985-January 1995.

Pathologic Finding	No of SP.	U.17-KS (N=12)	U.17OHCS (N=11)	S.Cort. (N=9)	LDDST* (N=9)	HDDST* (N=2)
Pitutary adenoma (2-Basophilic 1-Chromophobe)	3	1	3	2	3	1
adrenal hyperplasia	3	1	2	1	3	0
adenomatous hyperplasia	3	1	2	1	1	1
adrenal adenoma	1	0	0	1	1	0
Total abnormal	10	3	7	5	8	2

N=number of tests
*=LDDST and HDDST: low and high dose dexamethasone suppression test
U=urine
SP=specimen
Cort=cortisol

Chest X-Ray done in 14 patients showed osteoporosis in one patient, osteoporosis and rib fracture in one other. Thoracolumbar spinal X-Ray showed generalised osteoporosis in 7 of 10 patients, skull X-Ray showed ballooned sella turcica with erosion of the dorsum sellae in 3 of 11 patients. Intravenous pyelography performed in 6 patients for indirect evidence of adrenocortical enlargement was negative in all. Ultrasound of the suprarenals done in 11 patients was also negative. On the basis of the clinical findings and laboratory results, Cushing's disease was diagnosed in 9 patients preoperatively, 3 patients who had X-ray evidence of seller abnormality and another 6 patients who had mild hyperpigmentation in the absence of clinical evidence of ectopic ACTH production.

Initial Care and Definitive Treatment: The time interval between the first visit to the referral clinic and surgical intervention in 9 patients was 3.8 months (range 1.5 to 11). Mean duration of hospital stay was 4 months (range 2 to 7 months). The patients with moderate and severe hypertension were put on methyldopa. Among the 7 patients who had diabetes mellitus 2 required oral agent, one required insulin and 4 responded to dietary control. Two patients declined

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surgery while another 4 patients were lost to follow-up before surgery.

Five patients had total bilateral adrenalectomy by the anterior abdominal approach, 2 patients had total adrenalectomy on one side and subtotal on the other side because of technical difficulties and 3 patients had transfrontal pituitary adenomectomy. Postoperatively hypertension resolved in 5 of the 14 patients in whom it had been found and diabetes in 6 of the 7 patients.

Histopathology of Adrenocortical and Pituitary Tissues: Among 7 patients who had bilateral adrenalectomy 3 had bilateral hyperplasia with a mean weight of the right adrenal of 6.2g (range 4.7 - 9.0g) and of the left of 6.7g (range 5.4 - 8.0g). Another 3 patients had bilateral adenomatous hyperplasia and one had unilateral adrenal adenoma with atrophy of the contralateral gland. Among 3 patients who had pituitary adenomectomy 2 had basophil adenoma and the third had chromophobe adenoma.

Immediate and Subsequent Postoperative Course: All 5 patients who had total bilateral adrenalectomy had complete remission. Of the 2 patients who had total removal of one gland and subtotal of the other because of technical difficulties, one failed to have complete remission and the other developed a recurrence. The latter declined a second operation and died at home 2 years later of undetermined cause. Among the 5 who had complete remission 3 developed Nelson's syndrome. One of the latter 3 was subsequently admitted with urosepsis and was responding to antibiotic therapy when she suddenly collapsed and died. Postmortem lumbar puncture showed haemorrhagic cerebro-spinal fluid presumed to be secondary to pituitary apoplexy. The other two patients had pituitary adenomectomy and continued on replacement therapy with prednisone. One of them died at home of unknown cause 5 months later while the other is doing well. Three patients remained in good health 3 to 10 years after surgery on prednisone 5 mg in the morning and 2.5 mg in the evening with advice to include a generous amount of salt in their diet.

Among the 3 patients who had transfrontal pituitary adenomectomy 2 obtained complete remission. However, one of the latter, a male, had mild postoperative diabetes insipidus and repeated episodes of adrenocortical insufficiency during adjustment of prednisone dosage. He was subsequently discharged after stabilisation of his condition but died at home 5 months later of unknown cause. The third patient, a female with chromophobe adenoma, developed pneumonia and died in the early post-operative period. Postmortem lumbar puncture showed haemorrhagic cerebrospinal fluid.

DISCUSSION

The results show that pituitary adenoma accounted for 9 (56%) of the 16 confirmed cases of Cushing's syndrome in this series. One case of adrenocortical adenoma and 3 of nodular adrenocortical hyperplasia were also seen. The preponderance of Cushing's disease is commensurate with the generally recognised pattern of the aetiology of Cushing's syndrome (9). However, the frequency of macro and micronodular hyperplasia, 3 in a total of 16 cases, seen in such a small series is unexpected. There were no cases due to ectopic hormone production in contrast to the findings such as those of Ziegler *et al* (9,28) from centers in developed countries. However, this is not surprising because the majority of our patients were in the third and fourth decades of life (mean age 24 years, range 16 to 55) when malignancies responsible for ectopic ACTH

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production are uncommon.

The patients had overt classical manifestations of Cushing's syndrome. Thus they all had a history of weight gain, weakness and fatigue and all females in childbearing age had amenorrhea. Physical signs of moon face, truncal obesity and buffalo hump were also found in all patients while purple striae were found in 13 (81%) of them.

Concerning diagnostic tests, our facilities are limited to measurements of 24-hour urinary 17-OHCS and 17-KS, dexamethasone suppression tests, serum cortisol and simple radiological and ultrasound investigations. Furthermore, because of the high costs, repetition of the tests(s) was seldom undertaken when the initial one was inconclusive or at variance with the clinical findings. Such was the case of the patient in whom a normal LDDS was reported in the face of classical clinical findings of Cushing's syndrome (paragraph 3 under result) who latter had non-suppressibility on HDDS test and histopathologically demonstrated adenomatous hyperplasia of the adrenals. The most likely explanation for the normal LDDS result in that case was laboratory error. We did not find intravenous pylography and ultrasound of much value in the investigation of the adrenal glands.

This situation is far removed from that in developed countries where more advanced producers enable identification of the specific cause with a high degree of accuracy (3). For these reasons and taking into consideration the fact that most cases had overt clinical evidence of Cushing's syndrome by the time they were seen at our clinical, diagnosis was based principally on careful clinical evaluation.

Regarding definitive treatment, whatever the aetiology, bilateral adrenalectomy was the only one available at our hospital until 3 years ago. But, transfrontal pituitary adenomectomy has become available more recently. Long term postoperative care consists of replacement therapy with prednisone and advice to the patient not to stop treatment in case of a superimposed illness but report as quickly as possible to the nearest doctor or hospital for additional care. In the case of patients with Cushing's disease, it is hoped that removal of the pituitary adenoma would in future solve the problem of lifelong replacement therapy for most of them. The frequency of Nelson's syndrome after bilateral adrenalectomy in our Cushing's disease cases, 3 (43%) out of 7, further emphasises the need for pituitary adenomectomy as the treatment of choice for this condition.

Concerning the problem of Cushing's syndrome in general, it is difficult to comment on the true frequency even in a hospital setting, because accessibility of health care for the population is still limited and the focus of health care is on communicable diseases. In addition, screening tests for Cushing's syndrome are expensive and not easily available. Under these circumstances, it is possible that the underlying cause can sometimes remain undetected for a long time in patients in whom hypertension or non-insulin-dependent diabetes first attracts the physician's attention.

Despite these limitations the results show that Cushing's syndrome is not uncommon at the referral clinic level in a country with a predominantly young population. Therefore, awareness of this condition is warranted, particularly in the evaluation of young females with obesity, hypertension and diabetes. Among such women, a history of oligomenorrhoea or amenorrhea should reinforce the suspicion of Cushing's syndrome indicating the need to do appropriate

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screening tests.

Cushing's syndrome due to any cause is eventually fatal unless appropriately treated. Common causes of death are intercurrent infections and the complications of diabetes, hypertension and atherosclerosis. Medical treatment with metyrapone, amino glutethimide or mitotane may temporarily mitigate the metabolic disturbances related to hypercortisolism but the definitive treatment is surgery except in the case of iatrogenic Cushing's (10-12).

In conclusion, this report shows that Cushing's syndrome is not uncommon at the level of a tertiary referral center in Ethiopia. Patients generally present with the classical manifestations of the condition. Since the disease is debilitating and eventually fatal if untreated, careful evaluation of the patient with appropriate utilisation of existing investigative facilities and surgical treatment (directed at the underlying cause whenever possible) are warranted to relieve debilitating symptoms and to save the life of the patient.

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REFERENCES

1. McCullagh EP. Cushing's syndrome. *Cleve Clin J Med* 1969;28:235-54.
2. O'Riordan DS, Farley DR, Young WF, Grant CS, Van Heerden JV. Longterm outcome of bilateral adrenalectomy in patients with Cushing's syndrome. *Surgery* 1994;116:1088-94.
3. Sheller LR. Cushing's syndrome. *Cleve Clin J Med* 1988;55:329-37.
4. Gold EM. The Cushing's syndrome. Cushing's view of diagnosis and treatment. *Ann Intern Med* 1979;90:829-44.
5. Smals AGH, Pieters GFFM, Haelst UJGV, Kloppenburg PWC. Macronodular adrenocortical hyperplasia in long standing Cushing's diseases. *J Clin Endocrinol Metab* 1984;58:25-31.
6. Larsen JL, Cathey WJ, Odell WD. Primary adrenocortical nodular dysplasia, a distinct subtype of Cushing's syndrome. *Am J Med* 1986;80:976-83.
7. Molineaux L, Plorde J, Dasnoy J. Analysis of Medical admissions to the Gondar Hospital. *Ethiop Med J* 1966;5:47-65.
8. Henry JB, Kreigh AF. Endocrine measurement. In: Davidson I, Henry JB. Clinical diagnosis by laboratory methodology. 14th ed., WB Saunders Company, Philadelphia. 1969;601-40.
9. Zeiger MA, Fraker DL, Pass HI et al. Effective reversibility of the signs and symptoms of hypercortisolism by bilatral adrnalectomy. *Surgery* 1993;114:1138-43.
10. Orth DN, Liddle GN. Results of treatment of 108 patients with Cushing's syndrome. *N Engl J Med* 1971;285:243-7.

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URINE GLUCOSE TESTING: ANOTHER LOOK AT ITS RELEVANCE WHEN BLOOD GLUCOSE MONITORING IS UNAFFORDABLE

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TRACT: *The reliability of urine glucose testing to monitor diabetic control investigated in patients attending the Diabetic Clinic of the Tikur Anbassa ital in Addis Ababa between October 1994 and January 1995 with the aim of sing it for those with a normal renal threshold who cannot afford the cost of blood glucose monitoring. Clinically important fasting blood glucose values taken as those >180mg/dl and important urine glucose values as those 25% by Clinitest. Capillary blood glucose was determined by visual and metre ings. Urine was tested for glucose by the standard Clinitest method. There 265 patients, 126 IDDM and 139 NIDDM. Urine glucose corresponded factorily with FBG in 80% of the patients. The sensitivity, specificity, positive negative predictive values of urine glucose results by Clinitest in comparison FBG by metre determination were 71%, 90%, 90% and 70% and by visual rmination 71%, 86%, 80%, 79% respectively. On the basis of these results onclude that urine glucose testing by Clinitest provides reliable information in e than 70% of our diabetic patients the majority of whom cannot afford the cost ome blood glucose monitoring.*

INTRODUCTION

o the early 80's urine glucose tests the only means available to diabetic nts to monitor their metabolic control. t control of the diabetes was not attain- with this method because glucose d not appear in the urine before the d glucose has reached a level of g/dl (10mmol/l) in patients with a al renal threshold. Other drawbacks to tilisation of urine glucose tests for monitoring diabetic control include the variability of the renal threshold in a significant number of individuals due to congenital or acquired factors and differences in urine glucose level related to variations in urine concentration (1-5) and the nonspecificity of most semiquantitative methods for urine glucose measurement (6,7). These problems gave impetus to the development of simplified methods for home blood glucose measurement. As a result home blood glucose monitoring has largely replaced

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urine glucose determination in developed countries. The recent demonstration of the relation between glycaemic control and the chronic complications notably in the diabetes control and complications trial (DCCT) (8) has further strengthened the case for tight glycaemic control and the importance of frequent home blood glucose monitoring for this purpose.

However, home blood glucose monitoring is not affordable in most third world countries. In Ethiopia the current cost of one Glucostix strip (Ames, Miles Limited) is 5.00 Birr (US$ 0.79) and of a Glucometer II from the same company is 400.00 Birr (US$ 63.49) while the cost of a Clinitest tablet (Hoechst Co.) is 0.85 Birr (US$ 0.13) and of the matching kit is 5.00 Birr. Therefore, if urine testing is abandoned as unreliable for monitoring diabetic control, the only alternative in many third world countries would be periodic or intermittent measurements of blood glucose in clinics or laboratories. This would seriously limit patient participation in diabetes management.

An earlier study of the correlation of blood and urine glucose levels in adult diabetic patients at this hospital concluded that urine glucose testing lacked overall reliability to be used for monitoring diabetic control (9). However, we felt that such a conclusion fails to give due consideration to the significant proportion of patients in whom a reliable correlation between blood and urine glucose levels may be demonstrable. The benefit of urine glucose testing in such patients is obvious in a situation where blood glucose monitoring and its goal of tight control are beyond their reach. Therefore, this study is a reexamination of the correlation of blood and urine glucose levels based on the premise that demonstrating of a reliable correlation between them in a significant proportion of our patients would warrant encouragement of urine glucose testing to contribute to patient participation in diabetes management.

PATIENTS AND METHODS

Every other patient was included from the weekly diabetic clinic of the Tikur Anbassa Hospital to reach the minimum target of 250 which was considered representative of the approximately 2,000 patients seen yearly at the clinic. The study was conducted between October 1994 and January 1995. Males with prostatism, females with pregnancy and all those with nephropathy (urine positive for protein) and neuropathy (peripheral neuropathy compatible with diabetic neuropathy on routine neurological examination other than isolated Achilles tendon reflex in patients over 60 years of age) were excluded.

Classification of diabetes was according to WHO criteria (10). Strips and metres used for the blood glucose determinations were of two types: Accu-Chek Advantage, Boehringer Mannheim Corporation, Indianapolis, USA and One Touch, Lifescan Inc, Johnson & Johnson Company, Milpitas, California, USA, both types donated to our unit through Professor P. Berhanu (see in Acknowledgments). Clinitest tablets and kit were donated by Hoechst Ethiopia Ltd, Addis Ababa. In each case, routine calibration of the metre was performed according to the instructions provided with the equipment. Blood glucose values shown by metre reading from capillary blood by finger prick generally fall within 15-20% of the laboratory value from venous blood (4,11).

Urine glucose and blood glucose determinations: Second void urine obtained 30 minutes after the first voiding was tested for

glucose using Clinitest (4,7,9,10). This semiquantitative method enables recognition of glycosuria ranging from 0.25% to 2%. Blood glucose was determined in capillary blood obtained by finger prick visually and by metre simultaneously with the urine glucose test. The tests were performed by Specialist Nurses at the Diabetic Clinic.

Sensitivity, specificity, positive and negative predictive values were calculated using appropriate statistical methods.

RESULTS

A total of 265 patients, 136 males and 129 females, were included in the study. There were 126 IDDM and 139 NIDDM cases of whom 21 (17%) patients were above 40 years of age. Only 13 patients (5%) were doing home blood glucose monitoring while 43 (16%) were doing only urine glucose tests. Information was not recorded in 2 patients. Fifty-four (82%) of 78 patients said they could not afford to buy reagents to do home blood glucose monitoring while 24 (18%) were not doing urine glucose tests because they had not been advised to do so or had not been given a prescription for the material required.

Correlation between capillary blood glucose by meter reading and visual reading with urine glucose by Clinitest: Tables 1 and 2 show the correlation between capillary fasting blood glucose (FBG) by metre reading with urine glucose results by Clinitest in IDDM and NIDDM respectively. Tables 3 and 4 show the details of the correlation between FBG by metre reading and urine glucose by Clinitest in IDDM and NIDDM respectively, at various levels of FBG and urine glucose. Urine glucose levels corresponded reliably with FBG levels by metre reading in 102 (81%) of 126 IDDM patients and in 109 (78%) of 139 NIDDM patients, i.e. in 211 (80%) of the total of 265 patients. Failure of correspondence between FBG levels and urine glucose was found in 24 (10%) IDDM and 30 (11%) NIDDM, 54 (20%) patients in all.

In comparing FBG by visual reading with urine glucose findings, there was reliable correlation in 110 (87%) of 126 IDDM and 101 (72%) of 139 NIDDM patients, i.e. in 211 (80%) of the total of 265 patients. There was failure of correspondence between FBG and urine glucose findings in 16 (13%) IDDM and 38 (27%) NIDDM patients, 54 (20%) patients in all.

Sensitivity, specificity, positive and negative predictive values of urine glucose testing in comparison with FBG measurements by metre and visual reading were 71%, 90%, 90%, 70% and 71%, 86%, 80%, 79% respectively.

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Table 1. Correlation of FBG by metre reading with urine sugar by Clinitest in IDDM a NIDDM patients, Tikur Anbassa Hospital, October 1994 - January 1995.

Diabetes Type	FBG mg/dl	Glycosuria quantity (%)	No. of pts.	%
IDDM (n=126)				
	> 180	nil	17	6
		0.25-2	58	22
	≤ 180	nil	44	17
		0.25-2	7	3
NIDDM (n=139)	> 180	nil	27	10
		0.25-2	47	18
	≤ 180	nil	62	23
		0.25-2	3	0

Table 2. Correlation of FBG by visual method with urine glucose by Clinitest in IDDM a NIDDM patients, Tikur Anbassa Hospital, October 1994 - January 1995.

Diabetes Type	FBG mg/dl	Glycosuria quantity (%)	No. of pts.	%
IDDM (n=126)				
	> 180	nil	13	5
		0.25-2	50	18
	≤ 180	nil	60	23
		0.25-2	3	1
NIDDM (n=139)	> 180	nil	21	8
		0.25-2	35	13
	≤ 180	nil	66	24
		0.25-2	17	6

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Table 3. Correlation between capillary blood glucose by metre with urine glucose by Clinitest in IDDM patients, Tikur Anbassa Hospital, October 1994 - January 1995.

	Blood Glucose in mg/dl									
	-130		140-180		181-250		>250			
Urine sug	n	%	n	%	n	%	n	%	Total	
neg	35	28	9	7	16	13	1	1	61	48
1/4%(tr.)	0	-	0	-	6	5	0	-	6	5
1/2%(+)	0	-	1	1	1	1	1	1	3	2
3/4%(++)	0	-	0	-	0	-	0	-	0	-
1%(+++)	0	-	3	2	4	3	2	2	9	7
2%(++++)	1	1	2	2	17	14	27	21	47	37
Total	36	29	15	12	44	35	31	25	126	100

Table 4. Correlation between capillary blood glucose by metre with urine glucose by Clinitest in NIDDM patients, Tikur Anbassa Hospital, October 1994 - January 1995.

	Blood Glucose in mg/dl									
	-130		140-180		181-250		>250			
Urine sug	n	%	n	%	n	%	n	%	Total	
neg	35	25	27	19	21	15	5	4	88	63
1/4%(tr.)	0	-	1	1	5	4	1	1	7	5
1/2%(+)	0	-	1	1	2	1	0	-	3	2
3/4%(++)	0	-	0	-	0	-	0	-	0	-
1%(+++)	0	-	0	-	7	5	1	-	8	6
2%(++++)	2	1	1	1	14	10	16	12	33	24
Total	37	26	30	22	49	36	23	17	134	100

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DISCUSSION

The results show that there is a reasonable correspondence between the blood glucose and urine glucose levels in over 70% of the patients included in the study on the basis of the sensitivity, specificity, positive and negative predictive values. As stated earlier, the ideal aim of achieving normoglycaemia is presently unattainable for the majority of our patients because of unfavourable socio-economic conditions. Many are illiterate or barely literate, dietary control is difficult and home blood glucose monitoring is unaffordable. The realistic objectives of diabetes management in such a socio-economic context cannot extend beyond relief of acute symptoms, restoration of a sense of well being and minimisation of the risks of acute complications. These objectives can be attained in the majority of the patients who have a normal renal threshold, with semiquantitative home urine glucose monitoring augmented with periodic blood glucose measurements at the clinic.

Therefore, determination of the relation between urine glucose and blood glucose levels should be among the routine tasks in the initial evaluation of new diabetic patients in order to determine the suitability of urine glucose testing in the management of their condition. This procedure is in fact relevant even in developed countries, where home blood glucose monitoring is now the rule, in the case of patients unable, for economic or other reasons, or unwilling to adopt blood glucose testing (12). Utilisation of urine glucose testing in the management of the individual diabetic patient of course implies consideration of all pertinent factors including conditions of acquired and congenital high and low renal thresholds.

Patients eligible for urine glucose testing should be properly instructed in the technique and made adequately aware of the implications of changes in the level of glycosuria as part of their diabetes education. Tests should be performed in a consistent manner and the results recorded chronologically to contribute to diabetic control. In such patients a change in the level of glycosuria would be indicative of a corresponding alteration in the blood glucose level signalling improvement or deterioration of control or impending hypoglycaemia depending on the direction of change. In each case appropriate action should take all pertinent factors into consideration including current insulin dose, the temporal pattern of the change in urine glucose level, possible related alterations in time and quantity of food taken, and intensity and duration of physical activity. For instance, progression of urine glucose from a high level towards trace or negative over days should not necessarily warrant an immediate reduction in insulin dose because it may only mean a decline of blood glucose to just below 180mg/dl which is still far away from hypoglycaemia. So, in urine glucose monitoring, just as in home blood glucose monitoring, appropriate care should emphasize patient education and adaptation of the basic principles of diabetic care to the specific conditions of the individual patient.

The results of this study show that urine glucose testing has a place in the management of diabetes for patients who are unable to afford the cost of home blood glucose monitoring, the majority of whom have a normal renal threshold. Any procurable semiquantitative reagent can be used for this purpose although we used only Clinitest in this study. However, we would like to emphasize that this does not imply that means should not be continually sought to bring blood glucose monitoring within the reach of our patients in view of the benefits

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of tight diabetic control in preventing or retarding development of the chronic complications of diabetes which has amply been demonstrated by the DCCT study (8) and our wish is that all our patients would achieve that level of control. However, at the present time complex constraints in our situation show that can only be attempted in a very small minority of our patients.

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REFERENCES

1. Caired FI. Estimation of long-term control of diabetes. The relation of blood sugar levels to glycosuria. *Diabetes* 1967;16:502-3.
2. Walford S, Allison SP. Self monitoring of blood glucose, improvement of diabetic control. *Lancet* 1978;1:732.
3. Davis SH, Holman RR, Phillips M, Turner RC. Home blood sampling for plasma glucose assay in control of diabetes. *Br Med J* 1978;11:596-8.
4. Webb DJ, Lovesay JM, Ellis A, Knight AH. Blood glucose monitors: a laboratory and patient assessment. *Br Med J* 1980;208:362-4.
5. Feldman JM, Lebovitz FL. Tests of glycosuria. Analysis of factors that cause misleading results. *Diabetes* 1973;22:115-21.
6. Morris LR, McGee JA, Kitabchi AE, Tennessee M. Correlation between plasma and urine glucose in diabetes. *Ann Intern Med* 1981;94:469-71.
7. Harding P, Higgs M. Urine analysis in assessment of diabetic control. *Lancet* 1975;1:864-5.
8. The DCCT research group. The effect of intense treatment of diabetes on the development and progression of the longterm complications of IDDM. *N Engl J Med* 1993;329:977-86.
9. Mengistu M, Abebe Y. Relation between urine and blood glucose in adult Ethiopian diabetics. *Ethiop Med J* 1988;26:15-20.
10. WHO Expert Committee on Diabetes Mellitus. 1985. WHO Tech Rep Ser 727.
11. Toft A, Campbell I, Seth J. Diagnosis and management of endocrine diseases. Blackwell Scientific Publications. I[st] publication. Oxford, London, Edinburgh, Boston, Melbourne. 1981;46-8.
12. Peters AL. Should urine testing be declared obsolete. *Int Diab M* 1994;6:1-3.

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Original Article

1998

Insulin storage by diabetic patients in Ethiopia

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Abstract

A total of 140 insulin-treated diabetic patients attending the clinic of the Tikur Anbessa Hospital in Ethiopia were interviewed for information on how they store their insulin. Mean age was 31±10 y and most (60%) were males. Mean duration of diabetes was 8±5 y. Only 27% of patients stored their insulin in a refrigerator, the rest used clay pots filled with water and sand (18%), empty tins (17%), cupboards (17%), floors (16%) and glasses (4%). Mean HbA_{1c} was 11.1±2.3% (range 6.5–17.4%). The educational status, the income and the glycosylated haemoglobin were not statistically different among the different groups, whereas hyperglycaemic symptoms were significantly less in patients who stored their insulin in the refrigerator ($p<0.05$).

Introduction

Many studies, including the recent multicentre diabetes control and complications trial (DCCT) conducted in America, have demonstrated the relation of glycaemic control to the development of chronic complications.[1] The quality of glycaemic control depends on many factors, one of which is the potency of insulin in insulin-requiring patients. To maintain the potency of insulin the recommendation is that it should be stored in a refrigerator at a temperature of 4–8°C. However, many patients in Africa are not able to afford the cost of a refrigerator. Reports from several countries show access to a refrigerator among diabetic patients is in the range of 32–77%.[2–4] Those without access to a refrigerator used a variety of other places to store their insulin. In this paper we report on a survey of insulin storage methods among a sample of Ethiopian insulin-requiring patients.

Patients and methods

We interviewed 140 consecutive insulin treated diabetic patients attending the Diabetic Centre at the Tikur Anbessa Teaching Hospital, which is one of the two main diabetes referral hospitals in Ethiopia. Patients were asked the sites where they stored their insulin. The educational level, duration of diabetes and income of the patients were also recorded. Urine protein, fasting blood glucose (FBG) and glycosylated haemoglobin (HbA_{1c}) were determined for each patient. The local reference range for HbA_{1c} is 4.0–6.5%, and the assay was a microcolumn (Biorad) method. Educational status was determined as follows:

(a) Illiterate
(b) Elementary school (grade 1–6)
(c) Secondary school (grade 7–12)
(d) College
(e) Post-college

Income was assessed based on the total income of the family in terms of Ethiopian Birr (6.66 Birr currently equal US$1).

Hyperglycaemic symptoms were defined as the presence of one or more of polyuria, polydipsia or polyphagia.

Results

Of the 140 patients, 84 (60%) were males and 56 (40%) females. The mean age was 31±10 y. Mean duration of diabetes was 8±5 y (range 1–27 y). Since diagnosis 20% of the patients had interrupted their insulin for some days for various reasons, but mainly because of lack of insulin (60%). Only 26% of the patients stored their insulin in a refrigerator (Table 1). The educational status and the income of the patients were not statistically different in the various groups. The HbA_{1c} and the FBG were not statistically different, whereas the hyperglycaemic symptoms were significantly less common in those patients who keep their insulin in the refrigerator ($p<0.05$).

Discussion

The majority of our patients (74%) are not able to store their insulin in a refrigerator. Similar reports have indicated that the majority (69%) in Soweto, South Africa,[3] 77% in Tripoli,[4] and 68% in Nigeria[5] use refrigerators. Socio-

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Table 1: *Glycosylated haemoglobin, fasting blood glucose and hyperglycaemic symptoms among the 140 patients in relation to their insulin storage sites.*

Insulin storage sites	No (%)	HbA_{1c} (%)	FBG (mmol/L)	Hyperglycaemic symptoms
Refrigerator	37 (26%)	11.2±2.4	11.4±5.0	2 (5%)
Clay Pots	25 (18%)	10.9±1.8	9.9±3.5	9 (3%)
Empty tins	24 (17%)	11.2±2.7	10.0±5.0	11 (5%)
Cupboards	24 (17%)	10.9±2.3	11.1±4.5	11 (46%)
On floor	22 (16%)	10.9 ± 2.1	9.1±5.8	5 (23%)
Glasses	5 (4%)	12.6± 2.7	10.5±5.7	4 (80%)
Others	3 (2%)	8.7±1.8	12.0±7.6	1 (33%)

Note: FBG and HbA_{1c} levels were not statistically different between refrigerator and non-refrigerator users. There was however a difference ($p<0.05$) in hyperglycaemic symptoms between the 2 groups.

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economic conditions are worse in Ethiopia. The mean monthly income of the patients is 126.6 Birr, equivalent to US$19. The advice for patients without access to a refrigerator is to keep their insulin in a cool place away from direct sunlight. The use of clay pots is quite popular.[2,5] However, the effectiveness of this traditional method is not known. Our study showed that the HbA_{1c} was not statistically different between patients who used refrigerators and those who used other means (Table 1). The same was also true with the fasting blood glucose values. However, the frequency of hyperglycaemic symptoms were significantly lower in those patients who used refrigerators.

The comparison of glycaemic levels between refrigerator users and alternative storage users based on a single FBG value determined at our clinic on the interview day, is not a reliable indicator for the temporal pattern of the glycaemic profile. On the other hand HbA_{1c} values in both refrigerator users and alternative storage users were similar. The quality of glycaemic control is generally poor, regardless of socio-economic status and access to a refrigerator. The reasons for the prevailing poor control in our patients are multiple and complex. Therefore, it is difficult to draw firm conclusions from these findings regarding the effect of the methods of storage on insulin potency and the contribution of insulin potency to the quality of diabetic control. A more reliable method of testing insulin potency stored in various locations would be in the laboratory in vitro or in experimental animals.

REFERENCES

1. The DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. *New Eng J Med* 1993; 329: 977–86.
2. Harries AD, Muher D. Home insulin storage by patients with diabetes mellitus in Blantyre, Malawi. *Int Diab Digest* 1994; 5: 104.
3. Gill G, Ngubane T. Insulin storage by diabetic patients in Soweto, South Africa. *Int Diab Digest* 1995; 6: 89.
4. Bosseri S. Insulin storage by diabetic patients in Tripoli, Libya. *Int Diab Digest* 1996; 7: 44.
5. Famuyiwa OO. Important considerations in the care of diabetic patients in a developing country (Nigeria). *Diabetic Med* 1990; 7: 927–30.

Original Article

The clinical profile of Nigerians with long-standing diabetes mellitus

Dr F S Wokoma

Abstract

The clinical profile of a cohort of 60 Nigerian adults with long-standing diabetes mellitus was studied with a view to determining their overall clinical status, well-being and quality of life. They comprised 32 males and 28 females (sex ratio (1.1:1) with a mean age of 51±10 years. They had been diabetic for periods ranging from 5–30 y. Fifty-six (93%) had noninsulin-dependent diabetes (NIDDM), the remaining 4 (7%) being IDDM, and possible malnutrition related diabetes (MRDM). Results indicate that despite grossly suboptimal diabetic care NIDDM patients fared well. NIDDM patients have remained alive for a mean of 20±8 years (range 5–30 years) after diagnosis, with a long-term 3-year cumulative mean fasting blood glucose level of 8.9±0.8 mmol/L. Many had good quality of life. The commonest complications were non-disabling peripheral neuropathy. Longer survival rates and a higher quality of life status are achievable in Africa, given a motivating diabetic care team and a motivated diabetic population.

Introduction

Diabetes mellitus, a hitherto infrequently diagnosed disease in Africa has in recent times assumed increased magnitude[1] and importance. The diabetes scenario in Nigeria as in most African populations is characterized by a poor quality of care. Insulin and oral hypoglycaemic agents are in short supply, and are often not affordable to the bulk of diabetic patients. Organized diabetic clinics are few. In spite of these deficiencies, however, it is not infrequent to encounter some diabetic Nigerians with long-standing diabetes, living a relatively 'normal life' with minimal long-term complications. This study is thus a formal attempt to evaluate the long-term performance of a cohort of Nigerian diabetic population.

Patients and methods

The clinical data of 60 diabetic adult Nigerian patients with diabetes duration of a minimum of 5 years were evaluated. The patients were selected from a pool of 175 registered patients attending the medical clinic of the Department of Medicine, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. The required data for analysis were obtained from a combination of clinical case records and personal interview by the investigator. The diagnosis of diabetes and classification of types of diabetes were done in accordance with internationally recommended criteria.[2,3] Results are presented as mean±sd (standard deviation). Student's t-tests are used for comparison of paired variables.

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Results

1. Patient characteristics

The 60 patients comprised 34% of registered diabetic patients during the period of study.

The patients' characteristics are shown in Table 1. The bulk of the patients (80%) derived from the lower socio-economic classes. Only 6 (10%) of the patients were clearly obese (BMI>30.0). The mean duration of diabetes for the NIDDM patients was 20±8 y (range 5–30 y), and for the IDDM 7±3 y (range 10–19 y).

2. Treatment

All the patients received dietary educa-

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IMPOTENCE IN ETHIOPIAN DIABETIC MEN
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IMPOTENCE IN ETHIOPIAN DIABETIC MEN

B. SEYOUM

SUMMARY

Prevalence of impotence was assessed among 292 consecutive diabetic men attending the Tikur Anbessa Hospital, diabetic clinic. The mean age was 41.4±15.5 years (range 18-86 years). One hundred and forty nine (51.6%) were type I and 143 (49%) were type II patients. The mean duration of diabetes was 9.9±6.7 years and 37.7% had known long term diabetic complications. The overall prevalence of impotence was 48.7%. The mean duration of impotence was 3.5±3.4 years. In the majority of the cases, impotence started after diagnosis of diabetes mellitus. Many of the patients (79.1%) had never complained to physicians and 59.2% of the patients did not know that impotence is a complication of diabetes mellitus. All except 10 patients (7.5%) had libido. Impotence is significantly higher in type II as compared to type I patients (94/143 versus 40/132, p<0.001) and in patients with complications than without (76/104 versus 54/159, p<0.001). The mean duration of diabetes mellitus is significantly higher in patients with impotence than without impotence (12.3 years versus 8.1 years, p<0.001). We conclude that impotence is a common and significant problem in our diabetic men and we recommend further study to assess its social and psychiatric impact.

INTRODUCTION

Impotence is a common problem in diabetic patients. It is particularly distressing as it is usually irreversible while libido is commonly preserved(1,2). Impotence has been reported to occur in 49 - 75% of uncategorised diabetic men(2,5). It occurs more frequently and at an earlier age in the diabetic population than in the general population as a whole. Morever, impotence may even be the presenting symptom of diabetes in unrecognised patients(6-7).

Although impotence is a common problem in diabetic patients, there are no reports on it in Africa. This study was undertaken to assess the prevalence of impotence among the diabetic male patients and to have a baseline knowledge on this problem in Ethiopia.

MATERIALS AND METHODS

A total of 292 consecutive male diabetic patients 18 years and above attending the diabetic referral clinic of the Tikur Anbessa teaching hospital were included. All patients were interviewed and examined privately. The age, marital status, type of diabetes mellitus, duration of diabetes, treatment, presence of chronic complications were filled in prepared questionnaire by the author. Then, patients were asked for the presence of impotence, its duration and mode of onset and sexual interest. Information on morning erection was also sought. Physical examination was done on each patient to assess whether the genitalia, mainly the penis and the testis, were normal.

For the purpose of this study impotence was defined as difficulty in obtaining or maintaining an erection or difficulty in both obtaining and maintaining an erection leading to inability to engage in sexual intercourse. Those patients with congenital or acquired abnormalities of the genitalia and who are not able to perform sex were excluded.

RESULTS

A total of 292 consecutive diabetic men attending the Tikur Anbessa hospital diabetes centre were interviewed. The mean age was 41.4±15.5 years (range 18 - 86 years). One hundred and forty nine (51%) were type I and 143 (49%) were type II patients. The mean duration of diabetes was 9.9±6.7 years. One hundred ninety nine patients (68.2%) were married and the rest were either single, divorced or widower. The majority, 197 (67.5%) were on insulin, 83 (28.4%) on oral hypoglycaemic agents, six (2.1%) on insulin in combination with oral agents and six (2.1%) on diet.

Twenty patients were taking drugs which could produce impotence. Chronic complications such as retinopathy, nephropathy or neuropathy were found in 37.7% of the patients. Autonomic neuropathy was not assessed. No patient had peripheral vascular diseases.

The overall prevalence of impotence was 48.7%. Of the 292 patients interviewed, 17 patients were excluded during the analysis because they were young men who had never engaged in sexual intercourse, therefore, analysis was done on 275 patients. The mean duration of impotence was 3.5±3.4 years (1-20 years). In the majority, impotence started after the diagnosis of diabetes mellitus. However, in five patients (3.7%) impotence occurred before the diagnosis of diabetes mellitus. In 97.8% of the patients, the onset of impotence

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was gradual. Many of the patients, 106 (79.1%) had never complained to physicians mainly because of shyness. One hundred and seventy three (59.2%) patients did not know that impotence is a complication in diabetes mellitus. Almost all of the patients with impotence 124 (92.5%) had retained their libido.

Table 1

Characteristic of potent and impotent patients

Characteristic	Impotent (134)	Not impotent (141)	P value
Mean age	49.9±14.1	35.8±12.7	P<0.001
Duration of DM	12.3±6.7	8.1±6.1	P<0.001
Type of DM I/II	40/94	92/49	P<0.001
Treatment of :			
DM dietary	1	5	)
oral agents	54	29	)P<0.01
insulin	76	104	)
combination	3	3	)
Use of other drugs	10	10	P<0.05
Complication rate	76	32	P<0.001
Knowledge about impotence resulting from DM	60	59	P>0.05

The mean age and the mean duration of diabetes mellitus were significantly higher in patients with impotence than without impotence (49.9±14.1 versus 35.8±12.7; p<0.001 and 12.3±4.7 versus 8.1 ±6.1; p<0.001 respectively). Impotence was significantly higher in type II as compared to type I patients (94/143 versus 40/132, p<0.001). The rate of chronic complications of diabetes was significantly higher in patients with impotence. However, the knowledge of impotence being a complication of diabetes was similar in the two groups (Table 1).

DISCUSSION

Erectile impotence which is one of the commonest complications is present to a significant extent in diabetic patients(2-5). In keeping with these reports, the overall prevalence of impotence in our study was 48.7%. The ability to obtain a penile erection is an important male function. Any change in such ability will cause tremendous concern to the man and his partner, not only because of its effect on the ability to have sexual intercourse, but also on self esteem and self confidence in general. It is a much hidden complication, as relatively few sufferers complain of their problems to doctors. In our study, it is only 20.9% of those patients with impotence who had complained to their physicians about this problem. Probably this figure might have been exaggerated as many of our patients (59.2%) do not know that impotence is a complication of diabetes mellitus. As a result they might not have mentioned this problem to their physician. Because of impotence, many patients have broken family bonds. For this reason, the diabetic patient with impotence requires careful and sympathetic evaluation and guidance. It is worthwhile to directly but tactfully occasionally ask male diabetic patients, whether they are experiencing any problems with sexual relations.

Great care has been taken to exclude other causes of impotence particularly the psychogenic or non-organic causes and drug related causes. Psychic impotence is episodic, occurs suddenly, libido is low, depression and anxiety often accompany psychogenic impotence and usually does not prevent morning erections. All the diabetic men with impotence in our study were unable to experience morning erection and typically the onset of impotence was gradual, progressing over a period of six months to a year with a progressive decrease in firmness of the erection and finally, irreversible total absence of the erectile response. However, similar to other reports(1,2), the majority (92.5%) of the impotent men had intact libido. None of the patients had peripheral vascular disease. Some authors found that impotence does not correlate with the duration of diabetes mellitus(2,5,8) whereas we found that it correlated significantly with the duration of diabetes. The mean duration of diabetes mellitus in the impotent men reported here was 12.3±6.7 years as compared to 8.1±6.1 years in diabetics without impotence (Table 1).

Some authors(6,7) have reported, impotence to be the initial clinical manifestation of diabetes mellitus, and have suggested that when a man complains of premature impotence, diabetes mellitus must be considered as a possible cause. In our series we found that in 3.7% of our patients, impotence occurred before the diagnosis of diabetes mellitus. It has been also reported that prevalence of impotence increases with ageing(5,8). This was clearly shown in our study, in that as age increases prevalence of impotence also increased.

As seen in Table 1, patients were using different modes of treatment for their diabetes, namely: diet, oral agents, insulin and combination of oral agents and insulin. However, the mode of treatment of diabetes did not correlate with the presence or absence of impotence.

Different drugs are implicated to produce impotence(2), the most common ones are hypotensive drugs, antidepressants and oestrogen. We found 20 patients using drugs which could produce impotence of which, ten were among those patients with impotence and the rest were non-impotent men. There was no statistical difference. The drugs were discontinued in those patients with impotence but they continued to have impotence.

The prevalence of chronic complications, namely, peripheral neuropathy, nephropathy and retinopathy was similar in both groups. No patient was found to have peripheral vascular disease. Autonomic neuropathy,

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though relevant to assess in relation to impotence, was not assessed in our patients as there is no adequate and consistent standard.

In conclusion, impotence is a common and significant problem in Ethiopian diabetic men. We recommend the introduction of new therapeutic modes of intravenous self injection with vasoactive drugs and vacuum tumescence devices to be a part of the therapeutic armamentarium for erectile dysfunction and further studies to assess the social and psychiatric impact of impotence.

REFERENCES

1. Ellenberg, M. Social function in diabetic patients. *Ann. Intern. Med.* 1980; 92:331-33.
2. Ellenberg, M. Impotence in diabetes: the neurologic factor. *Ann. Intern. Med.* 1971; 75:213-19.
3. Nathan, D., Singer, D.E. and Godine, J.E. et al. Insulin dependent diabetes in older patients. *Amer. J. Med.* 1986; 81:837-42.
4. McCullock, D.K., Young, R.J. and Prescott, I.W. et al. The natural history of impotence in diabetic men. *Diabetilogia.* 1984; 26:437-40.
5. Kolondy, R.C., Kahn, C.E., Goldstein, H.H. and Barnett, D.M. Sexual dysfunction in diabetic men. *Diabetes.* 1974; 23:306-9.
6. Kaiswer, F.E. and Karenman, S.G. Impotence in diabetic men. *Ann. J. Med.* (suppl. 5A) 1988; 85:147-52.
7. McCullocck, D.K., Campbell, al. and Wu, F.C. et al. The prevalence of diabetic impotence. *Diabetologi.* 1980; 18:279-82.
8. Rubin, A. and Babbotti, D. Impotence and diabetes mellitus. *J. Amer. Med. Ass.* 1958; 168:498-500.

Antibody levels in Ethiopian children five years after vaccination with two different doses of hepatitis B vaccine: Is there a need for booster vaccine?

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E Tsega, J Horton, E Nordenfelt, et al. Antibody levels in Ethiopian children five years after vaccination with two different doses of hepatitis B vaccine: Is there a need for booster vaccine? Can J Gastroenterol 1998;12(1):57-60. It was hypothesized that, following effective initial vaccination, a booster dose of hepatitis B vaccine will not be necessary in areas of hyperendemicity for hepatitis B virus (HBV) infection. A total of 314 Ethiopian children, ranging from two to 14 years old, were alternatively vaccinated with 10 and 20 µg hepatitis B vaccine doses, using the initial, one- and six-month schedule. Five years later, 210 of the vaccinees were retested for anti-HBV surface antibody titres. Both 10 and 20 µg doses of hepatitis B rDNA yeast vaccine were equally immunogenic and protective against HBV infection for at least five years despite marked reduction of mean antibody levels and geometric mean titres, with 11% of the vaccinees showing antibodies below the protective level. For firm further recommendations a longer follow-up period of vaccinees is suggested.

Key Words: *Booster vaccine, Doses, Ethiopian children, Hepatitis B vaccine*

Taux d'anticorps chez des enfants éthiopiens cinq ans après une vaccination au moyen de deux doses de vaccin contre l'hépatite B : le rappel est-il nécessaire?

RÉSUMÉ : Selon l'hypothèse, après une vaccination initiale efficace, une dose de rappel de vaccin contre l'hépatite B ne sera pas nécessaire dans les régions d'hyperendémie d'infections au virus de l'hépatite B. En tout, 314 enfants éthiopiens de 2 à 14 ans ont été vaccinés au moyen de doses de vaccin contre l'hépatite B de 10 et de 20 µg à six mois d'intervalle. Cinq ans plus tard, 210 des patients vaccinés ont été testés de nouveau pour un dosage des anticorps anti-HBV de surface. Les deux doses de 10 et de 20 µg de vaccin à levure contre l'hépatite (BrDNA) se sont révélées tout aussi immunogènes et protectrices contre l'infection au HBV pendant au moins cinq ans, malgré la réduction marquée des taux moyens d'anticorps et des moyennes géométriques, 11 % des sujets vaccinés ayant présenté des anticorps sous le taux de protection. Pour une recommandation plus ferme, il faudrait exercer un suivi plus long auprès des sujets vaccinés.

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Ethiopia is one of the hyperendemic areas for hepatitis B virus (HBV) infection. The hepatitis B virus surface antigen (HBsAg) carrier srate is 8% to 12%, and the sequelae of HBV infection, chronic hepatitis, cirrhosis and hepatocellular carcinoma account for high morbidity and mortality (1-3).

Unlike in Southeast Asia, in Ethiopia vertical transmission is uncommon, but horizontal transmission occurs early in life within the family setting, and infection continues to rise gradually throughout adult life (4). Thus, although mass vaccination of newborns is an urgent need, the high cost of the available vaccines in third world countries prohibits implementation of such a program. To reduce the cost of the vaccine, the safety and immunogenicity of a rDNA HBV vaccine (Engerix B, SmithKline Beecham) were compared by administering 10 µg (group A) and 20 µg (group B) doses to two comparable (age and sex) groups of children between two and 14 years of age. Both doses, using the initial, one- and six-month schedule, were shown to be equally immunogenic, with seroconversion rates of 97% to 100% and anti-HBs geometric mean titres (GMTs) of 3421 to 6336 IU/L (5) a month after the third dose of vaccine. At the time of the vaccination trial, it was hypothesized that a booster injection of the vaccine after five to 10 years might not be needed because the repeated exposure to HBV in the endemic area could give rise to anamnestic response (ie, production, in response to an antigenic stimulus, of an antibody that has been produced in the host on some previous occasion) and, hence, an indefinite period of protection.

Therefore, determination of anti-HBs levels, five years after the initial vaccination, was planned in groups who received 10 µg (group A) and 20 µg (group B) of the vaccine, respectively.

SUBJECTS AND METHODS

Of 380 children (two to 14 years old) screened for HBsAg, antihepatitis B core (HBc) antigen and anti-HBs, 314 were found to be negative. These noninfected and nonimmune Ethiopian children (156 boys and 158 girls) were vaccinated with rDNA yeast-derived hepatitis B vaccine using the initial, one- and six-month schedule between October 1987 and March 1988. They did not have acute or chronic disease or a history of allergy to any vaccine preparation. Alternate subjects received either 10 or 20 µg of the vaccine intramuscularly into the deltoid muscle (5). The anti-HBs antibody titres and the GMT were determined a month after the first, second and third doses.

Parents of the vaccinees were advised to report to the hospital where they were vaccinated if their child developed jaundice during the ensuing five years. Five years later, during April and May 1993, blood specimens were collected to determine anti-HBs antibody levels. Results were obtained from 210 subjects (67% of the total 314 vaccinees), despite repeated attempts to contact all of the original vaccinees.

Anti-HBs antibody titres were determined by using the radioimmunoassay method (commercial kits from Abbott Laboratories), as in the authors' original study (5). The χ^2 test was used for comparison where applicable, and the geometric mean and its 95% CI were used to describe the average of individual titres.

TABLE 1
Levels of antihepatitis B virus surface (anti-HBs) antigen and geometric mean titre (GMT) after one month and after five years of vaccination

	Anti-HBs titre level (IU/L)	GMT (IU/L)
10 µg group (n=108)		
One month after vaccination	13,458.7	3651.5
Five years after vaccination	1193.6	192.6
20 µg group (n=102)		
One month after vaccination	16,834.7	4569.8
Five years after vaccination	843.6	261.2

TABLE 2
Distribution of antihepatitis B virus surface (anti-HBs) antigen titres in recipients of two different doses of hepatitis B vaccine one month and five years after vaccination

	Levels of titres (IU/L)				
	<10	10-100	101-1000	1001-10,000	>10,000
10 µg group (n=108)					
One month after vaccination	0	2	26	40	40
Five years after vaccination	16	25	37	27	3
20 µg group (n=102)					
One month after vaccination	1	2	14	34	49
Five years after vaccination	8	17	48	29	0

RESULTS

A total of 160 subjects (85 males and 75 females) in group A and 154 (71 males and 83 females) in group B initially completed the vaccination program. Of these, 108 (52 males and 56 females) from group A and 102 (43 males and 59 females) from group B were available for retesting after five years. For group A subjects, mean anti-HBs antibody titre and mean GMT of the 108 vaccinees (68% of the total of 160 initial vaccinees in group A) a month after the third dose were 13,458.7 IU/L and 3651.5 IU/L, respectively. Five years later, the anti-HBs titre and GMT of these vaccinees were only 9% (1193.6 IU/L) and 5% (192.6 IU/L), respectively, of the original values. Similarly, for group B subjects, the anti-HBs antibody titre level and the GMT of 102 (66% of the total 154 initial vaccinees in group B) decreased from 16,843.7 IU/L and 4569.8 IU/L, to 843.6 IU/L (5% of the original titre) and 261.2 IU/L (6% of the original GMT), respectively (Table 1). There was no difference in the mean anti-HBs titres and GMTs between males and females, and no difference in results between those younger and older than eight years of age.

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The distribution of the anti-HBs titres in groups A and B a month after the complete vaccination and five years later are shown in Table 2. Thus, five years after the initial vaccination, there was a significant reduction in the anti-HBs titres in both groups, with 24 subjects below the accepted protective level (10 IU/L) and only three of the original 89 subjects with anti-HBs titre levels greater than 10,000 IU/L (all recipients of 10 µg dose).

Three subjects showed an increase in the anti-HBs levels five years after vaccination. All were females older than eight years of age; one was from group A (level increased from 121 to 294 IU/L) and two were from group B (4 to 83 IU/L, 9 to 1486 IU/L), and the latter two had an anti-HBs titre level of less than 10 IU/L a month after the initial complete vaccination.

Three vaccinees, two males and one female, all younger than eight years old and with high levels of antibodies initially and five years postvaccination, presented with acute viral hepatitis due to hepatitis A virus (HAV) infection (immunoglobulin [Ig] M anti-HAV-positive). All were icteric and clinically mild. No cases of clinical hepatitis B were identified.

DISCUSSION

Five years after vaccination, 89% of the vaccinees (186 of 210) still had protective levels of anti-HBs antibodies, but levels were markedly reduced to about 5% of the original mean titre and 6% of the original GMT. Twenty-four vaccinees (11%) had anti-HBs levels below the protective level of 10 IU/L; none of these reported icteric illness. Hadler and associates (6) have shown that five years after vaccination, 10% to 15% of vaccinees had 'undetectable' levels of antibody, comparable with our observation above. As shown in Table 2, the distribution of anti-HBs titre levels soon after vaccination and five years later were similar in groups A and B. Thus, the 10 µg hepatitis B vaccine dose is as effective and immunogenic as the 20 µg dose for mass vaccination of children.

The use of 5 µg of hepatitis B rDNA yeast vaccine administered to children between three months and 11 years of age at initial dose and one month later has been shown by Lai et al (7) to be as effective as 10 or 20 µg of the same vaccine given in three doses, despite lower anti-HBs antibody titres, presumably due to anamnestic responses. If this observation is confirmed by similar studies, the cost of the vaccination could be further and significantly reduced, hence making it more readily available to third world countries. Also, reducing the number of doses from three to two, given at a short interval, could improve compliance.

Antibody levels higher than the initial levels five years after vaccination – as seen in three of our patients – may be due to anamnestic responses. Two of these patients were poor responders because their anti-HBs levels remained under 10 IU/L a month after three doses of the vaccine, and they were found to have antibody levels of 93 and 1486 IU/L, respectively, at five years. While the rise of anti-HBs level from 9 to 1486 IU/L is striking, the increase from 121 IU/L and 41 IU/L, to 249 IU/L and 83 IU/L, respectively, is not impressive; the latter levels could be due to nonspecific variability or the declining phases of higher levels of anti-HBs following exposure to HBV infection. If this were truly an anamnestic response, it suggests that vaccinees are likely to be protected from HBV infection irrespective of the initial antibody level following vaccination. However, because antibody levels were not determined periodically, as in the study by Lai and associates (7) in whom 11 of 106 children (10%) were noted to have anamnestic response, it is difficult to exclude late responses to vaccination or subclinical HBV infection. Alanine aminotransferase, anti-HBc or HBsAg were not determined periodically or at the time of the last serum collection for anti-HBs. Thus, latent or asymptomatic HBV infection could not be identified. However, the three vaccinees who presented with jaundice had acute HAV infection as determined by positive sera for IgM anti-HAV, while their anti-HBs antibody levels were still high (342, 1070 and 1577 IU/L, respectively).

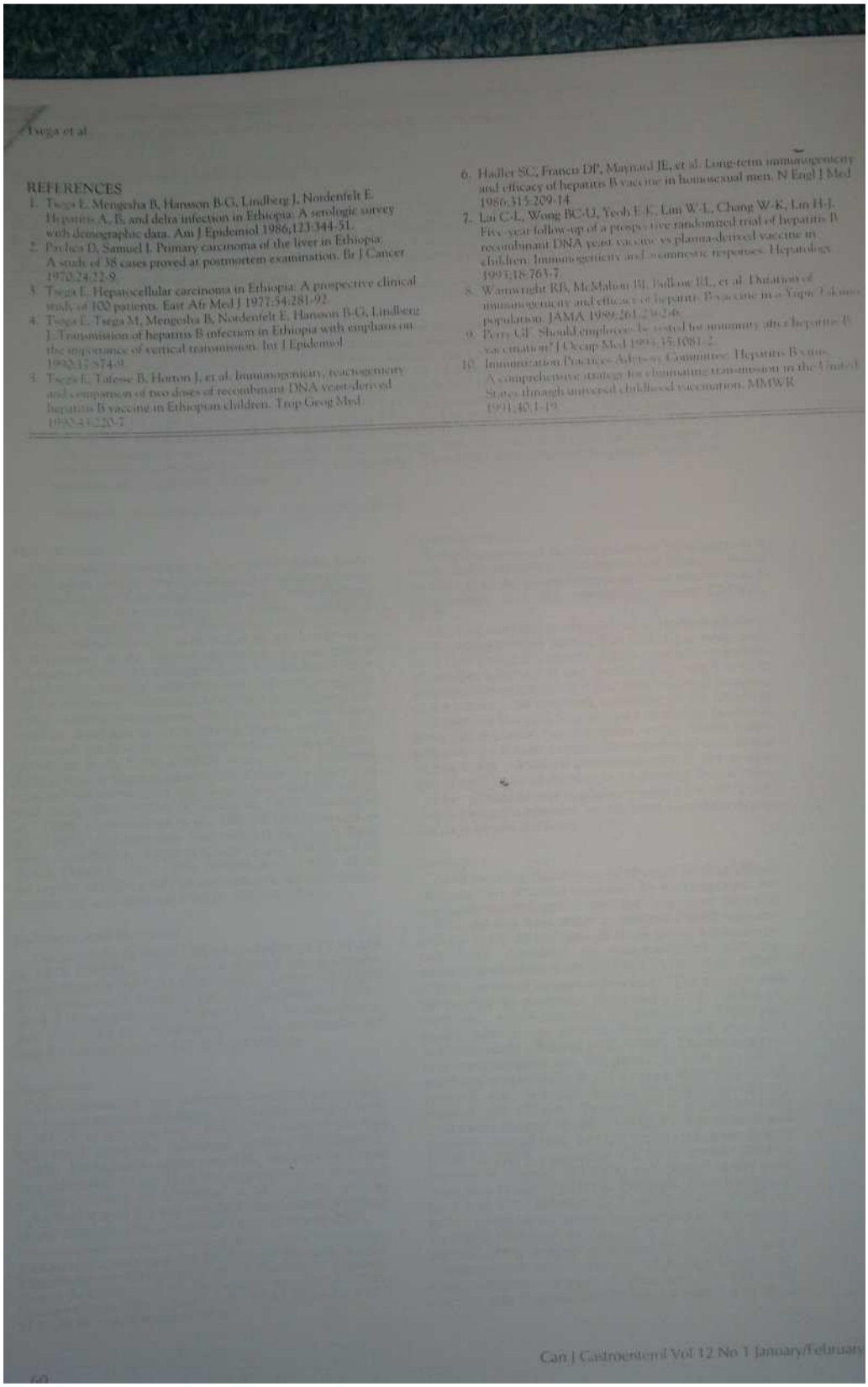
Although vaccine-induced antibody levels decline steadily over time, and up to 50% of adult and child vaccinees who respond adequately to vaccine may have low or undetectable antibody levels, immunological memory remains intact for at least seven to nine years (6,8,9). Based on this observation, recommendations from the United States Immunization Practices Advisory Committee state that there is no need for booster dose, and that serological testing to assess antibody level is not necessary in children and adults with normal immune status. Exceptions include hemodialysis and immunocompromised patients (10). Further recommendations will depend on a longer follow-up period of vaccinees.

CONCLUSIONS

This study confirms that 10 and 20 µg doses of hepatitis B rDNA yeast vaccine are equally immunogenic and protective against HBV infection for at least five years despite significant reduction of antibody level and GMT, with 11% of the vaccinees showing antibodies below the protective level. This is in keeping with our hypothesis that, in hyperendemic areas, anamnestic responses following vaccination would protect against HBV infection for a long time. While a longer period of follow-up is necessary, it is also advantageous for third world countries to conduct further studies using a 5 µg dose of the same vaccine administered twice instead of the currently recommended three doses, in order to improve compliance and reduce the cost of the vaccine.

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REFERENCES

1. Tsega E, Mengesha B, Hansson B-G, Lindberg J, Nordenfelt E. Hepatitis A, B, and delta infection in Ethiopia: A serologic survey with demographic data. Am J Epidemiol 1986;123:344-51.
2. Pavlica D, Samuel I. Primary carcinoma of the liver in Ethiopia: A study of 38 cases proved at postmortem examination. Br J Cancer 1970;24:22-9.
3. Tsega E. Hepatocellular carcinoma in Ethiopia: A prospective clinical study of 100 patients. East Afr Med J 1977;54:281-92.
4. Tsega E, Tsega M, Mengesha B, Nordenfelt E, Hansson B-G, Lindberg J. Transmission of hepatitis B infection in Ethiopia with emphasis on the importance of vertical transmission. Int J Epidemiol 1992;12:874-9.
5. Tsega E, Tafesse B, Hanson J, et al. Immunogenicity, reactogenicity and comparison of two doses of recombinant DNA yeast-derived hepatitis B vaccine in Ethiopian children. Trop Geog Med 1993;33:220-7.
6. Hadler SC, Francis DP, Maynard JE, et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986;315:209-14.
7. Lau C-L, Wong BC-U, Yeoh E-K, Lim W-L, Chang W-K, Lin H-J. Five-year follow-up of a prospective randomized trial of hepatitis B recombinant DNA yeast vaccine vs plasma-derived vaccine in children: Immunogenicity and anamnestic responses. Hepatology 1993;18:763-7.
8. Wainwright RB, McMahon BJ, Bulkow LL, et al. Duration of immunogenicity and efficacy of hepatitis B vaccine in a Yupik Eskimo population. JAMA 1989;261:2736-246.
9. Perry CM. Should employees be tested for immunity after hepatitis B vaccination? J Occup Med 1993;35:1081-2.
10. Immunization Practices Advisory Committee. Hepatitis B virus: A comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. MMWR 1991;40:1-19.

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Seroprevalence of cysticercosis in Bénin

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Abstract

We report the results of a seroepidemiological study on the prevalence of cysticercosis in Benin. Cluster sampling at 3 levels was performed in the 6 *départements* (Atacora, Borgou, Zou, Mono, Atlantique and Oueme) and 2625 serum samples, from 1329 adult females and 1296 adult males, were collected. Antibodies against *Taenia solium* cysticerci were first searched for by enzyme-linked immunosorbent assay and the 41 seropositive samples were then examined by enzyme-linked electroimmunotransfer blot assay (EITB). Thirty-five samples gave positive results in the EITB. The overall seroprevalence of cysticercosis was therefore 1·3% (95% confidence interval [95% CI] 0·9–1·9). The seroprevalence was 1·9% in males (95% CI 1·2–2·7) and 0·8% (95% CI 0·4–1·5) in females ($P<0·05$). A progressive increase in seroprevalence with increasing age was found. The highest seroprevalences were observed in Atacora and Atlantique, 2 non-Muslim *départements* (3·3% and 3·0%, respectively). This study demonstrated the public health importance of cysticercosis in Benin.

Keywords: cysticercosis, *Taenia solium*, seroprevalence, Bénin

Introduction

Cysticercosis constitutes a major public health problem in most tropical and sub-tropical developing countries and is one of the most important parasitic diseases of the central nervous system of humans worldwide (HEINZ & MACNAB, 1965; MAHAJAN, 1982). Cysticercosis in humans is due to infection by the larval stage (cysticercus) of the human tapeworm, *Taenia solium*. Humans are the only definitive host, but the larval stage can develop in faecal excretions and disseminate eggs in the environment, which infect pigs and (sometimes) humans, causing cysticercosis. The absence of well developed medical and sanitary infrastructures, and the lack of trained medical workers, explain the relative scarcity of reports from Africa (PREUX *et al.*, 1996). In South Africa, and in some other countries where sanitary and medical infrastructures are developed, this disease is well recognized (HEINZ & MACNAB, 1965; SHASHA *et al.*, 1986; PAMMENTER *et al.*, 1987; CHAMOUILLET *et al.*, 1997). Cysticercosis and neurocysticercosis have been described in Togo (DUMAS *et al.*, 1989) and in Bénin (ADJIDÉ *et al.*, 1996; AVODÉ *et al.*, 1996). Here we report the first study of cysticercosis seroprevalence carried out at a national level in tropical Africa.

Subjects and Methods

The republic of Bénin had a population of 4 606 244 in 1979 (INSAE, 1979) and is divided into 6 *départements*: Atacora (613 322 inhabitants), Atlantique (1 000 631 inhabitants), Borgou (766 571 inhabitants), Mono (634 679 inhabitants), Ouémé (822 534 inhabitants) and Zou (768 507 inhabitants), which are subdivided into 86 subprefectures and 258 communes.

Sample size

This serosurvey was primarily destined to evaluate the seroprevalence of human T cell lymphotropic virus I (HTLV-I) (DUMAS *et al.*, 1991). The sample size was calculated using the following formula (SCHWARTZ, 1993): $N=2\varepsilon_\alpha pq/e^2$; where $p=0·016$, the expected ratio corresponding to the HTLV-I seroprevalence reported in Ivory Coast by VERDIER *et al.* (1989), $q=1-p$, $\varepsilon_\alpha=1·96$ for $\alpha=0·05$, and $e=0·005$ (the desired precision of our results). The theoretical value of N was 2520.

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Sampling procedure

Sampling involved random selection by clusters at 3 levels (subprefectures, communes and houses), based on a list of all the subprefectures and communes in all 6 *départements*, and the population census (INSAE, 1979).

The first sampling level consisted of random selection of one subprefecture among 4 in each *département* each with their political divisions. The second level consisted of random selection of one commune among 4 in each subprefecture selected, and the third involved selection of the house to be surveyed, according to the following method: one house in a commune was chosen randomly from the available list; thereafter, every fourth house was selected for the survey. The number of individuals selected was calculated proportionately to the sample size of each political division and following the sex ratio.

The refusal rate was negligible—only 2·8% (77/2702)—due to encouragement by the local surveyors during the days before the survey.

Serology

After receiving the consent of all study subjects, blood samples were obtained from each by venepuncture, immediately centrifugated, and the sera were stored at –20°C. All sera were tested by enzyme-linked immunosorbent assay (ELISA) and all those giving a positive result were examined by enzyme-linked immunoelectrotransfer blot assay (EITB) (TSANG *et al.*, 1989).

Enzyme-linked immunosorbent assay. The antigen used for coating the microtitre plates was a crude soluble extract of *T. solium* cysticerci (GUERRA *et al.*, 1982) prepared from larvae isolated from infected pigs in a previous study (DUMAS *et al.*, 1989). The antigen protein concentration was 2·5 µg/mL in 0·05 M carbonate–bicarbonate buffer at pH 9·6, in a volume of 100 µL per well. After overnight incubation at 4°C, plates were washed with 1% (v/v) Tween-20™ in phosphate buffered saline (Sigma) at pH 7·2 (PBST). The positive and negative control sera, and the study sera, were diluted 1:200 in PBST containing 5% (w/v) bovine albumin fraction V (Sigma) (PBSTA) and placed in the plate wells in a volume of 100 µL per well. After incubation for 1 h at 37°C followed by 3 washings with PBST, alkaline phosphatase-labelled antihuman immunoglobulin (Ig)G (Behring) was added in a volume of 100 µL per well. After another incubation for 1 h at 37°C and 3 washings with PBST, 100 µL of *p*-nitrophenyl phosphate substrate was added to each well. The enzymatic reaction was stopped after 45 min by addition of 50 µL

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Figure. Electroimmunotransfer blots using anti-*Taenia solium* cysticercus antigen of 37 human sera from Bénin giving positive results by enzyme-linked immunosorbent assay. A. Lanes 1–13, positive sera from Atacora; lane 14, positive serum from Borgou; lanes 15 to 18, positive sera from Oueme; lane 19, positive serum from Zou; lane P, positive serum control; lane N, negative serum control. B. Lanes 20–35, positive sera from Atlantique; lane 36, negative serum from Atacora; lane 37, negative serum from Mono; lane P, positive serum control; lane N, negative serum control. Numbers indicate relative molecular masses.

there are very few population-based epidemiological studies. Two other studies have been conducted in Bénin, using ELISA. In Savalou subprefecture (*département* of Zou), a prevalence of 3·9% was found (AVODE *et al.*, 1996) and 3·5% was reported in the lacustrian village of Vekky, *département* of Atlantique (ADJIDE *et al.*, 1996). The lower seroprevalence rate found in our study was not due to the different immunological methods, as 95% (35/37) of our sera which were positive by ELISA were confirmed by EITB.

Our survey showed a predominance of positive males and a progressive increase of the prevalence with advancing age. Similar findings have been reported from Togo (DUMAS *et al.*, 1989).

In our survey, some regional variations have been observed. The prevalence rate was higher than the national level in 2 of the 6 *départements*: Atacora and Atlantique. The highest prevalence was observed in Atacora, a rural *département* where meat inspection was not properly carried out. Illegal meat marketing existed, leading to total lack of sanitary control. In many villages people butcher their own animals, offering the meat for sale. A low seroprevalence rate was observed in Borgou, where the majority of the inhabitants are Muslims and pork husbandry is therefore less common. Similar findings have been reported elsewhere. In North Africa, where the majority of the population are Muslims, cysticercosis is infrequent (MAHAJAN, 1982). On the other hand, cysticercosis is often described at a much higher prevalence in many sub-saharan African countries where animism and Christianity predominate (MAHAJAN, 1982; SHASHA *et al.*, 1991; NZISABIRA *et al.*, 1992; MICHEL *et al.*, 1993; ADJIDÉ *et al.*, 1996; PREUX *et al.*, 1996).

Only a few cases of neurocysticercosis have been reported from Bénin (GALLAIS, 1938; AVODÉ *et al.*, 1994), but its importance at the community level remains unknown.

This study demonstrated that cysticercosis is an important public health problem in Bénin and appropriate control measures should be taken. In endemic foci, effective and practical intervention is available through the chemotherapy of human taeniasis, and the cost of such intervention is acceptable, as it uses existing veterinary and medical services and inexpensive medication. For more rapid progress towards eradication and substantial reduction of the consequences of neurocysticercosis, short-term control programmes directed at identifying foci and treating all diagnosed or suspected human cases of taeniasis have been proposed (PAWLOWSKY, 1990). The immediate goal is to interrupt transmission from humans to pigs and to other humans. In Africa, there is a lack of both national surveillance and control programmes that are capable of encouraging, promoting, supervising and evaluating taeniasis/cysticercosis, and there is also a lack of funds to support such activities. Effective taeniacidal drugs are therefore often not available in rural endemic communities. The chemotherapy control measures must be supported by educational campaigns and significant improvements in personal hygiene and general sanitation within the endemic areas. Sanitary education should be given, and it is important to emphasize the danger of consuming raw or inadequately cooked pork and the need for safe disposal of human waste. In Bénin, 94% of those interviewed preferred partially cooked pork and 0·7% ate it raw (ADJIDÉ *et al.*, 1996; AVODÉ *et al.*, 1996). Similar results have been reported from Togo (DUMAS *et al.*, 1989). Development of animal husbandry practices should prevent pigs from having access to human faeces. In western Cameroon, MARTY *et al.* (1986) observed that pigs were often raised in cages but the inhabitants frequently defaecated inside the cages. In Bénin, the pigs are rarely raised by indoor husbandry (ADJIDÉ *et al.*, 1996; AVODÉ *et al.*, 1996). An effort should be made to increase veterinary inspection in most parts of Africa. In Bénin, previous studies have succeeded in increasing awareness of the local political and administrative authorities concerning cysticercosis and neurocysticercosis (ADJIDÉ *et al.*, 1996; AVODÉ *et al.*, 1996; PREUX *et al.*, 1996). Control measures must be adapted to local conditions and human behavioural patterns. The implementation of control programmes in Africa therefore depends on sociological studies to make the prophylactic measures understandable and acceptable to the population.

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References

Adjidé, C., Bouteille, B., Josse, R., Adjidé-Szmidt, V., Avodé, D. G. & Dumas, M. (1996). Prevalence de la cysticercose dans la commune lacustre de Vekky, Département de l'Atlantique (Bénin). *Bulletin de la Société de Pathologie Exotique*, **89**, 24–29.

Avodé, D. G., Bouteille, B., Avimadje, M. & Adjien, C. (1994). Epilepsie, hypertension intracrânienne, syndrome confusionnel et cysticercose cutanée, à propos d'un cas observé en milieu hospitalier au Bénin. *Bulletin de la Société de Pathologie Exotique*, **87**, 186–188.

Avodé, D. G., Capo-Chichi, O. B., Gandaho, P., Bouteille, B. & Dumas, M. (1996). Epilepsie provoquée par la cysticercose; à propos d'une enquête sociologique et culturelle réalisée à Savalou au Bénin. *Bulletin de la Société de Pathologie Exotique*, **89**, 45–47.

Chamouillet, H., Bouteille, B., Isautier, H., Bègue, A. & Lecadieu, M. (1997). Séroprévalence de la cysticercose, téniasis et ladrerie porcine, à la Réunion en 1992. *Médecine Tropicale*, **57**, 41–46.

Collomb, H., Larivière, M. & Philippe, Y. (1961). Cysticercose cérébrale (premier cas au Sénégal). *Bulletin de la Société Médicale de l'Afrique Noire de Langue Française*, **6**, 695–700.

Collomb, H., Larivière, M., Philippe, Y. & Ayats, H. (1964). Foyer de cysticercose de la Basse Casamance (Sénégal). *Bulletin et Mémoire de la Faculté Mixte de Médecine et de Pharmacie de Dakar*, **12**, 148–155.

Dumas, M., N'Diaye, I. P., Daumens, J. P. & Gueye, M. (1976). Cysticercose cérébrale (2 nouveaux cas sénégalais). *Bulletin de la Société Médicale de l'Afrique Noire de Langue Française*, **21**, 203–211.

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Dumas, M., Grunitzky, E. K., Deniau, M., Dabis, F., Bouteille, B., Belo, M., Pestre Alexandre, M., Catanzano, G., Dardé, M. L. & d'Almeida, M. (1989). Epidemiological study of neurocysticercosis in northern Togo (West Africa). *Acta Leidensia*, **57**, 191–196.

Dumas, M., Houinato, D., Verdier, M., Zohoun, T., Josse, R., Bonis, J., Zohoun, I., Massougbodji, A. & Denis, F. (1991). Seroepidemiology of human T-cell lymphotropic virus type I in Benin (West Africa). *AIDS Research and Human Retroviruses*, **7**, 447–451.

Gallais, P. (1938). Deux cas de cysticercose cérébrale avec manifestations épileptiques. *Bulletin de la Société de Pathologie Exotique*, **31**, 945–949.

Giordano, C., Vedrenne, H., Dago-Ekrib, A., Doucet, J., Nozais, J. P., Piquemal, M. & Vidal, H. (1976). Cysticercose cérébrale à forme confusionnelle avec aspects périodiques à l'EEG, étude anatomo-clinique. *Médecine d'Afrique Noire*, **23**, 43–51.

Grunitzky, E. K., Balegnu, A. K., M'Bella, M., Belo, M., Sadzo, A., Bouteille, B. & Dumas, M. (1995). La cysticercose chez des malades neurologiques en milieu hospitalier à Lomé, Togo. *Annales de Médecine Interne*, **146**, 419–422.

Guerra, G., Flisser, A., Canedo, L. & Laclette, J.-P. (1982). Biochemical and immunological characterization of antigen B purified from cysticerci of *Taenia solium*. In: *Cysticercosis: Present State of Knowledge and Perspectives*, Flisser, A., Willms, K., Laclette, J. P., Larralde, C., Ridaura, C., Beltran, F. & Vogt, M. W. (editors). New York: Academic Press, pp. 437–451.

Heinz, H. J. & Macnab, G. M. (1965). Cysticercosis in the Bantu of Southern Africa. *South African Journal of Medical Sciences*, **30**, 19–31.

INSAE (1979). *Recensement Général de la Population et de l'Habitat*. Cotonou, Benin: Institut National de la Statistique et de l'Analyse Economique du Benin.

Mahajan, R. C. (1982). Geographical distribution of human cysticercosis. In: *Cysticercosis: Present State of Knowledge and Perspectives*, Flisser, A., Willms, K., Laclette, J. P., Larralde, C., Ridaura, C., Beltran, F. & Vogt, M. W. (editors). New York: Academic Press, pp. 39–46.

Marty, P., Mary, C. & Pagliardini, G. (1986). Courte enquête sur la cysticercose et le taeniasis à *Taenia solium* dans un village de l'Ouest Cameroun. *Médecine Tropicale*, **46**, 181–183.

Michault, A., Rivière, B., Fressy, P., Laporte, J. P., Bertil, G. & Mignard, C. (1990). Apport de l'EITB au diagnostic de la neurocysticercose humaine. *Pathologie et Biologie*, **38**, 119–125.

Michel, P., Callies, P., Raharison, H., Guyon, P., Helmet, L. & Genin, C. (1993). Epidemiologie de la cysticercose à Madagascar. *Bulletin de la Société de Pathologie Exotique*, **86**, 62–67.

Nzisabira, L., Nzengiyumva, G., Bouteille, B., Ndwiragiye, A., Niyongabo, Th., Bigirumana, V., Dumas, M. & Aubry, P. (1992). La cysticercose dans la province de Kayanza (Burundi). *Bulletin de la Société de Pathologie Exotique*, **85**, 374–377.

Pammenter, M. D., Rassouw, E. J. & Dingle, C. E. (1987). Serological detection of cysticercosis in two rural areas of South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 242–244.

Pawlowsky, Z. S. (1990). Perspectives on the control of *Taenia solium*. *Parasitology Today*, **6**, 371–373.

Preux, P. M., Melaku, Z., Druet-Cabanac, M., Avode, G., Grunitzky, E. K., Bouteille, B., Cruz, M. & Dumas, M. (1996). Cysticercosis and neurocysticercosis in Africa: current status. *Neurological Infections and Epidemiology*, **1**, 63–68.

Schwartz, D. (1993). *Methodes Statistiques à l'Usage des Médecins et des Biologistes*, 4th edition. Paris: Flammarion Médecine Sciences.

Shasha, W. & Pammenter, M. D. (1991). Sero-epidemiological studies of cysticercosis in schoolchildren from two rural areas of Transkei, South Africa. *Annals of Tropical Medicine and Parasitology*, **85**, 349–355.

Shasha, W., Van Dellen, J. & Cakata, F. (1986). Cysticercosis: an analysis of 141 cases in South Africa. *South African Journal of Epidemiology and Infection*, **1**, 94–97.

Simac, C., Michel, P., Andriantsimahavandy, A., Esterre, P. & Michault, A. (1995). Use of enzyme-linked immunosorbent assay and enzyme-linked immunoelectrotransfer blot for the diagnosis and monitoring of neurocysticercosis. *Parasitology Research*, **81**, 132–136.

Tsang, V. C. W., Brand, J. A. & Boyer, A. E. (1989). An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases*, **159**, 50–59.

Verdier, M., Denis, F., Sangaré, A., Barin, F., Gershi-Damet, G. M., Rey, J. L., Léonard, G., Moumer, M. & Hugon, J. (1989). Prevalence of antibody to human T-cell leukemia virus type I (HTLV-I) in populations of Ivory Coast, West Africa. *Journal of Infectious Diseases*, **160**, 363–370.

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Announcement

1999 Award in honour of Fred L. Soper (1893–1976) for publications in the field of Inter-American Health

This is an announcement and call for submission of nominations for the 1999 award in honour of Fred L. Soper, former Director of the Pan American Health Organization (the World Health Organization Regional Office for the Americas) from 1947 to 1958.

The Award is presented annually to the author or authors of an original scientific contribution comprising new information on, or new insights into, the broad field of public health, with special relevance to Latin America or the Caribbean or both. This may consist of a report, an analysis of new data, experimental or observational, or a new approach to analysing available data. Preference is given to studies involving more than one discipline and to papers related to infectious diseases, a life-long concern of Dr Soper.

Only papers already published in scientific journals listed in the Index Medicus or in the official journals of the Pan American Health Organization are eligible for consideration. Furthermore, the award is limited to contributions by authors whose principal affiliation is with teaching, research or service institutions located in the countries of Latin America and the Caribbean (including the Centers of the Pan American Health Organization).

The Award consists of a certificate and a prize of US$ 1000.

Papers submitted by or on behalf of their authors may be considered for the Fred L. Soper Award. For the 1999 Award, only papers published during the calendar year 1998 will be considered; all submissions must be received by 31 March 1999 at the following address: Executive Secretary, PAHEP, 525 23rd Street NW, Washington, DC 20037, USA.

Amir sultan (MD), 2014

Table 1. Seroprevalence of *Taenia solium* cysticercosis according to age and sex in Bénin, 1989

Age (years)	Population in 1979[a] Male	Female	No. of subjects Total Male	Female	Positive by ELISA[b] Male	Female	EITB[c] Male	Female	Prevalence (%) by EITB[c,d] Male	Female	Total
<20	1322 762	1269 136	302	285	4	1	4	0	1·3 (0·4–3·4)	0 (0–1·3)	0·7 (0·2–1·7)
20–29	267 672	428 771	438	411	8	3	6	2	1·4 (0·5–3·0)	0·5 (0·1–1·7)	0·9 (0·4–1·8)
30–39	203 677	263 419	228	291	5	2	5	2	2·2 (0·7–5·0)	0·7 (0·1–2·5)	1·4 (0·5–2·8)
40–49	142 794	166 262	144	170	3	2	3	2	2·1 (0·4–6·0)	1·2 (0·1–4·2)	1·6 (0·5–3·7)
50–59	100 125	105 295	82	88	3	3	3	3	3·7 (0·8–10·3)	3·4 (0·7–9·6)	3·5 (1·3–7·6)
≥60	171 144	165 187	102	84	4	3	3	2	2·9 (0·6–8·4)	2·4 (0·3–8·3)	2·7 (0·9–6·2)
Total	2208 174	2398 070	1296	1329	27	14	24	11	1·9 (1·2–2·7)	0·8 (0·4–1·5)	1·3 (0·9–1·9)

[a]INSAE (1979).
[b]Enzyme-linked immunosorbent assay.
[c]Enzyme-linked immunoelectrotransfer blot assay.
[d]95% Confidence interval in parentheses.

Table 2. Seroprevalence of *Taenia solium* cysticercosis according to region and sex in Bénin, 1989

Département	Population in 1979[a] Male	Female	No. of subjects Total Male	Female	Positive by ELISA[b] Male	Female	EITB[c] Male	Female	Prevalence (%) by EITB[c,d] Male	Female	Total
Atacora	297 434	315 888	182	209	10	5	8	5	4·4 (1·9–8·5)	2·4 (0·8–5·5)	3·3 (1·8–5·6)
Atlantique	484 763	515 868	258	278	11	6	10	6	3·9 (1·9–7·0)	2·2 (0·8–4·6)	3·0 (1·7–4·8)
Borgou	377 739	388 832	217	162	1	0	1	0	0·5 (0–2·5)	0 (0–2·3)	0·3 (0–1·5)
Mono	297 830	336 849	176	200	0	1	0	0	0 (0–2·0)	0 (0–1·8)	0 (0–1·0)
Oueme	390 914	431 620	250	248	4	1	4	0	1·6 (0·4–4·0)	0 (0–1·5)	0·8 (0·2–2·0)
Zou	359 494	409 013	213	232	1	1	1	0	0·5 (0–2·6)	0 (0–1·6)	0·2 (0–1·2)
Total	2208 174	2398 070	1296	1329	27	14	24	11	1·9 (1·2–2·7)	0·8 (0·4–1·5)	1·3 (0·9–1·9)

[a]INSAE (1979).
[b]Enzyme-linked immunosorbent assay.
[c]Enzyme-linked immunoelectrotransfer blot assay.
[d]95% Confidence interval in parentheses.

of 2N NaOH to each well. Each sample was tested in duplicate.

Absorption (OD) was measured at 405 nm with an LP 400 spectrophotometer (Diagnostic Pasteur). To enhance the measurement specificity, OD values at 690 nm were subtracted from those at 405 nm (ΔOD) and the difference between the OD values for the test sera and a pool of sera negative for cysticercosis, ($\Delta OD - \Delta OD_{neg}$), was calculated. The reaction threshold was the mean ($\Delta OD - \Delta OD_{neg}$) value of 100 sera from persons with other parasitic and bacterial infections (including syphilis, malaria, toxoplasmosis, distomatosis, hydatidosis, schistosomiasis, ascariasis and filariasis) plus 3 SD, which was 0·400. Values ≥0·400 were considered to be positive. The sensitivity and specificity of the ELISA, determined with sera from 14 human cases of cysticercosis proven by histological examinations and the 100 sera from persons with diverse bacterial and parasitic diseases, were 85·7% (12/14 positive) and 92% (8/100 positive), respectively.

Enzyme-linked immunoelectrotransfer blot assay. The immunoblotting technique was carried out as described by TSANG *et al.* (1989) and modified by MICHAULT *et al.* (1990) and SIMAC *et al.* (1995). Sera were diluted 1:20. The gel was 12·5% acrylamide (0·3% bis-acrylamide). Electrophoresis was done for 1 h at 500 V, 10 W and 35 mA at +10°C in a discontinuous buffer system. Each well contained 10 µL solution containing 15 µg of sodium dodecyl sulphate-treated crude cysticercus antigen (protein concentration 2·22 g/L). Electrotransfer to nitrocellulose was carried out for 40 min at +6°C, 500 V, 10 W, 250 mA. The nitrocellulose strips were incubated with sera diluted 1:20 for 1·5 h at room temperature before washing and incubation with 1:1000 antihuman IgG conjugated with peroxidase (Sanofi Pasteur) for 1 h at room temperature. Following further washing, the strips were developed in diaminobenzidine–hydrogen peroxide substrate solution.

The EITB has been reported to be 100% specific for cysticercosis (TSANG *et al.*, 1989). More than 7 parasitic antigens have been described in *T. solium* cysticerci (TSANG *et al.*, 1989). The presence of one or more of the 6 antigens with M_r=14, 18, 21, 24, 32 and 43 was considered as a positive result.

Results

Blood samples were collected from 2625 subjects (Table 1). The mean age of females was 31·3 years (SD=14·1), and that of the males was 32·1 years (SD=15·4). The difference between the mean ages was not statistically significant (Student's *t* test).

Forty-one sera gave positive results by ELISA; 37 were available for EITB, and 35 gave positive results, an overall seroprevalence of 1·3% (Tables 1 & 2; Figure). The difference between the rates in males (1·9%) and females (0·8%) was significant (P<0·05).

The seroprevalence rate varied widely in the different *départements* of Bénin (Table 2).

Discussion

This population-based seroepidemiological survey was the first to report the extent of human cysticercosis in Bénin. The overall prevalence of seropositivity found (1·3%) was lower than that of 2·4% reported in the northern part of Togo, a neighbouring country (DUMAS *et al.*, 1989), and the rates in South Africa and Madagascar, 8·5% and 18% respectively (HEINZ & MACNAB, 1965; MICHEL *et al.*, 1993).

Cases of cysticercosis among hospital patients in West Africa have often been reported (COLLOMB *et al.*, 1961, 1964; DUMAS *et al.*, 1976; GIORDANO *et al.*, 1976; AVODE *et al.*, 1994; GRUNITZKY *et al.*, 1995), but

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East African Medical Journal Vol. 75 No. 7 July 1998
DEEP VENOUS THROMBOSIS IN A UNIVERSITY TEACHING HOSPITAL, ADDIS ABABA, ETHIOPIA
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DEEP VENOUS THROMBOSIS IN A UNIVERSITY TEACHING HOSPITAL, ADDIS ABABA, ETHIOPIA

A. GEBREMEDHIN and M. SHAMEBO

SUMMARY

This paper reports the results of a five-year prospective study designed to obtain information on the demographic characteristics, risk factors and complications of deep venous thrombosis (DVT) in sixty-six Ethiopians. There were 44 females and 22 males with a female to male ratio of 2:1. Their age ranged between 13 and 80 (mean = 34±12.8) years. Fifty-one subjects (77%) were below the age 41 years. Sixty-three cases (95%) presented with lower and three with upper limb DVT. In 26 females (40%) pregnancy and pregnancy related conditions such as childbirth, abortions and Caesarean Section were the risk factors for their DVT. Immobilization was considered a risk factor in 12 cases (18%) and 12 other patients did not have an apparent risk factor for their DVT. Post-thrombotic syndrome was observed in 26% of the study population during the follow-up period which ranged from two months to seven years (median = 1 year). Twelve percent of the patients developed recurrent DVT and 9% had non-fatal pulmonary embolism. Venous thrombosis is common in Ethiopians and it usually affects pregnant and young adults. The risk factors and complications observed were similar to those documented in the literature.

INTRODUCTION

The epidemiological studies of DVT have been confined mainly to the inpatient population. As a result, its true incidence in the general population has not been definitely determined; although recent studies done in urban populations in the Western World indicate incidence rates of 1.6 per 1000 persons per year(1,2). Current data on the incidence, risk factor distribution and complications of DVT from developing countries are lacking or not available here.

Some population-based studies done in Africa in the seventies have drawn attention to the rarity of thromboembolic disorders in indigenous Africans in contrast to the high incidence in populations of the affluent world(3). However, studies done both in South Africa and Nigeria among post operative patients with objective end-points (^{125}I-Fibrinogen leg scans, Ultrasonographic studies and Venography) have shown a similar incidence of DVT in native Africans and Europeans(4,5).

Little or no information is available on the frequency risk factors and complications of venous thromboembolic disorders in Ethiopians. The objectives of this study were to determine the demographic characteristics and to find out the risk factors and complications of DVT in patients regularly attending a clinic in a university teaching hospital, Addis Ababa, Ethiopia.

MATERIALS AND METHODS

Patients with DVT seen in the wards and at the bi-weekly haematology follow-up and referral clinic of Tikur Anbessa Hospital (TAH), in Addis Ababa, Ethiopia, were prospectively studied during the period between January 1992 to December 1996.

The study group consisted of 48 new consecutive cases of DVT seen in the 5-year study period and 18 follow-up cases (diagnosed before 1992). Forty-six subjects were referred to the clinic for follow-up upon discharge from the Medical, Surgical and Obstetrics and Gynaecology wards of TAH. The remaining 20 cases came from different health institutions in the country.

Clinical data were recorded on a preset protocol that contained information on the demographic characteristics, duration of symptoms, limbs affected, initiating event (precipitant), history of oral contraceptive use, family history and the date of diagnosis of DVT.

Results of the physical examination such as the circumference of the affected limb in contrast to the normal limb, calf, arm or thigh discomfort, oedema and venous distention and pain on dorsiflexion of the foot (positive Homan's sign) were recorded. Laboratory studies included complete blood count including baseline platelet count, prothrombin time (PT), partial thromboplastin time (PTT),

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blood group and venographic studies when available. The type, duration and complications of treatment such as bleeding and/or thrombocytopenia and Warfarin induced skin necrosis were also noted. Included also was duration of the follow-up and complications observed, such as pulmonary embolism, recurrent thrombosis and the post-thrombotic syndrome.

After the study subjects achieved a therapeutic range of PT, follow-up visits to the clinic were scheduled at two weeks from discharge, monthly for 6 months, every 3 months until one year and then every 6 months thereafter. Data was updated during each visit and at the end of the study period.

RESULTS

During the five-year study period, 44 females and 22 males with DVT were seen in the clinic, making a female to male ratio of 2:1. Table 1 shows the age and sex distribution of the study subjects. The age of the study subjects ranged between 13 to 80 (mean = 34±12.8) years. The age range for females was 15 to 64 (mean = 31±9.5) and that of the males 13 to 80 (mean = 40±16.9) years. Fifty-one subjects (77%) were below the age of 41 years. The duration of symptoms before presentation ranged between one day to two months (median=7 days).

Table 1

Age and Sex distribution of patients with DVT

Age groups (yrs)	Sex		Total	
	Female	Male	No.	%
11-20	5	2	7	10.6
21-30	18	5	23	35.0
31-40	15	6	21	31.8
41-50	4	5	9	13.6
51-60	1	2	3	4.5
61+	1	2	3	4.5
Total	44	22	66	100 0

The frequency of the various risk factors observed is depicted in Table 2. Pregnancy and related conditions such as childbirth, abortions and Caesarean Section were observed in 26 (59%) of the 44 females. Fifteen of these females (34%) had DVT during the puerperal period, and in 12 cases it developed within the first ten days after delivery. Four females developed DVT during pregnancy, one in the first and three during the third trimester. The remaining five and two females had Caesarean Section and abortions respectively. Seven females (16%) gave history of oral contraceptive use with durations ranging from three months to six (median=1.5 years). Prolonged bed-rest was a risk factor in 12 cases (18%) and the causes of immobilization were severe and complicated pneumonia, congestive heart failure, stroke, pancreatitis and tuberculous peritonitis. Seven cases developed DVT after major surgery, including prostatectomies, abdominal hysterectomies and general abdominal surgeries. Five patients had malignancy before they developed DVT and a 56-year old additional male patient developed oesophageal cancer one year after the diagnosis of left lower limb DVT.

Table 2

Risk factor distribution in patients with DVT

Risk factors	Sex		Total	
	Female	Male	No.	%
Pregnancy	4	-	4	6.0
Puerperium	15	-	15	22 7
Caesarean section	5	-	5	7 6
Abortion	2	-	2	3.0
Surgery	4	3	7	10.6
Trauma	1	1	2	3.0
Immobilization	4	8	12	18.2
Malignancy	3	3	6	9.1
Oral contraceptive use	7	-	7	10.6
Varicosity	2	1	3	4 5
Obesity	1	-	1	1 5
Systemic lupus Erythematosus	1	-	1	1.5
No apparent risk identified	4	8	12	18.2

In 12 patients (18%), there was no apparent risk factor observed at presentation. These were eight males and four females with a mean age of 3.11 ±11.6 (range 13 to 45) years. There was no family history of venous thrombosis in the patients studied. The clinical features, relevant laboratory studies and complications of DVT are shown in Table 3. The diagnosis of DVT was based on clinical evaluation alone in 47 patients. In the remaining 19 cases venography was done and was positive in all. Sixty-three cases (95%) had lower-limb DVT at presentation.

Table 3

Clinical features, complications and laboratory studies in patients with DVT.

Characteristics	Sex		Total	
	Female	Male	No.	%
Affected limb:				
left upper limb	3	-	3	4.5
left lower limb*	28	12	40	60.6
right lower limb	15	10	25	37.8
Arm, calf and/or thigh swelling	44	22	66	100.0
Arm, calf and/or thigh pain	44	22	66	100.0
Positive Homan's sign	35	9	44	66.6
Complications:				
Postthrombotic synd.	11	6	17	25.7
Recurrent DVT	5	3	8	12.1
Pulmonary embolism	4	2	6	9.1
Diagnosis: clinical	35	12	47	71.2
venography	9	10	19	28.8
Death	4	2	6	9.1

* 2 females who presented with left upper limb DVT developed left lower limb DVT, later.

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CASE REPORT

PULMONARY HYDATID CYST AS A CAUSE OF RECURRENT HAEMOPTYSIS AND RESPONDING TO TREATMENT WITH ALBENDAZOLE

Getachew Aderaye, MD[1]

ABSTRACT: *A 28 year old Ethiopian male who presented with recurrent haemoptysis, fever, and multiple well defined masses on a chest x-ray and subsequently responded to treatment with albendazole is reported. Complete remission of the symptoms and improvement of chest x-ray findings was attained within 2 months of initiation of treatment. The unusual presentation of pulmonary hydatid cyst and the response to pharmacotherapy is briefly reviewed.*

INTRODUCTION

Pulmonary hydatid cysts infrequently present as multiple and bilateral diseases. They are usually asymptomatic but when a cyst ruptures, a massive leak of hydatid fluid could result in anaphylactic shock and a minor leak can communicate with a bronchus and lead to superinfection and abscess formation. Haemoptysis is rare in hydatid disease. The treatment of pulmonary hydatid is careful surgical removal of the cyst. Surgery however can be technically difficult in patients with multiple and bilateral disease and between 10 to 30% of patients will anyway have recurrence of the cyst within 5 years of surgery (1,2). Furthermore, the perioperative mortality increases from 1% to 20% in successive surgeries. Since mid 1980s, chemotherapy using the benzimidazole compounds, notably albendazole have proved to be either totally or partially effective in the treatment of some of these cases (3,4). We report here a case of pulmonary hydatid cysts with uncommon clinical presentation and who responded to treatment with albendazole.

CASE REPORT

T.M was a 26 year old male from Sidamo when he first presented to Yirgalem Hospital 2 years ago with complaints of cough and intermittent haemoptysis. He was empirically started on anti-TB medication (streptomycin, thiazina) which he took for one and a half years but showed no improvement. Instead, his symptoms got progressively worse with frequent and frank haemoptysis, low grade fever, left sided chest pain and weight loss. He was brought and admitted to Ras Desta Hospital in Addis Ababa where he had a chest x-ray taken for the first time. The chest x-ray showed

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bilateral, well circumscribed oval and circular lesions and obliteration of the left costophrenic angle. (Figure 1). Sputum smear examination for acid fast bacilli (AFB) was repeatedly negative and sputum culture grew aspergillus species. A diagnosis of multiple fungus balls (aspergillomas) was entertained and subsequently referred to the Chest Unit of the Tikur Anbessa Hospital (TAH) for further evaluation and management.

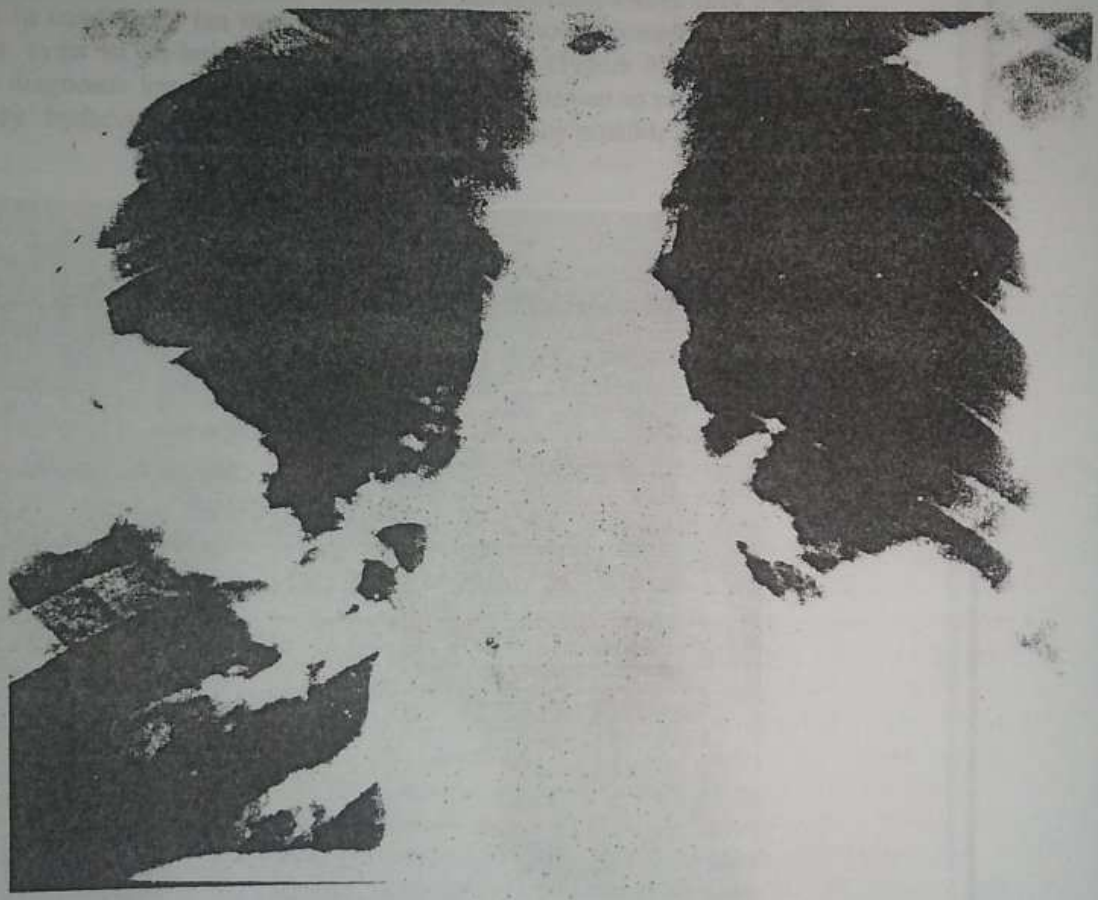


Figure 1. A postero-anterior and lateral films of bilateral pulmonary hydatid cysts, Tikur Anbessa Hospital, January 1996.

Investigations at TAH revealed, Hgb = 13.9gm%, WBC=7000/mm³, ESR 12 mm in the 1st hr. Urine analysis, liver and renal function tests were all normal. Sputum smear examination was negative for AFB. Bronchoscopic aspirate grew aspergillus species. The cytological examination was negative for malignant cells.

Ultrasound of the pulmonary masses were reported as cystic in consistency but there were no daughter cysts or hydatid sand observed. With a diagnostic impression of multiple pulmonary hydatid cysts with superinfection, patient was started on antibiotics (ampicillin, gentamycin) and albendazole 400 mg PO twice a day. Ten days after initiation of treatment, the patient started to show signs of improvement and by the end of one month he was completely free of symptoms. He was given another course of albendazole for 4 weeks as an outpatient and his chest x-rays after 2 months of treatment showed complete resolution of the cyst fluid (Figure 2). At present patient is being followed up regularly at our Chest Clinic for any possible recurrence.

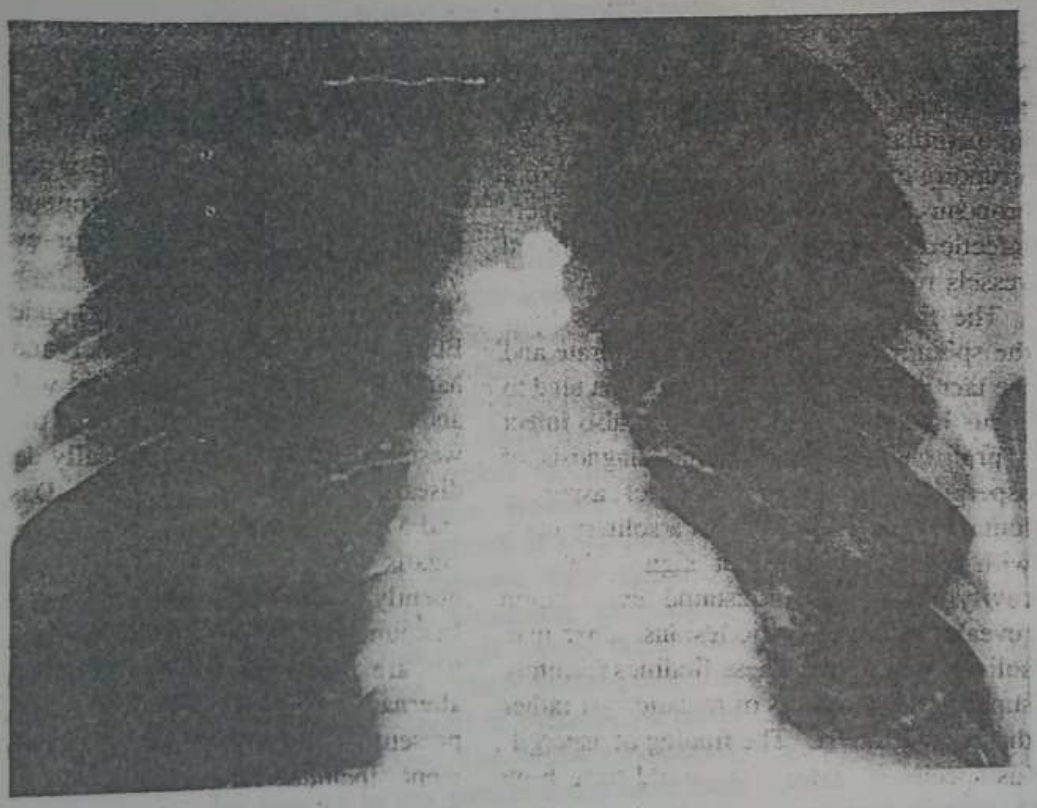


Figure 2. Complete resolution of cyst fluid with a residual surrounding halo, Tikur Anbessa Hospital, 2 months after treatment with albendazole.

DISCUSSION

Our patient remained a diagnostic problem for 2 years due to the uncommon clinical presentation and lack of diagnostic facilities. The definitive diagnosis of pulmonary hydated cyst requires an ELISA test which is the most sensitive and specific serologic test. Indirect Hemagglutination Test (IHA) is positive in about 60% of cases. Unfortunately these tests are usually kept in reference laboratories and are not easily available for routine use. Most patients with cystic hydatid disease are asymptomatic until such-time the cyst ruptures spontaneously or as a result of infection and the patient develops cough productive of purulent sputum that contains fragments of hydatid membrane. Our patient most likely had a rupture of a cyst with communication to a bronchus leading to a continuous superinfection and erosion of surrounding blood vessels resulting in gross haemoptysis.

The finding of aspergillus species from the sputum and bronchoscopic aspirate and, the fact that pulmonary aspergilloma tend to occur in a preformed cavity and also infect a preformed cyst makes the diagnosis of aspergilloma tempting. However, aspergillomas commonly present as a solitary mass with typical air crescent sign within the cavity. Besides, ultrasound examination revealed multiple cystic lesions rather than solid mass lesions. These findings therefore supported a diagnosis of hydatid cyst rather than aspergilloma. The finding of aspergillus species in our patient could have been due to endobronchial colonization rather than infection by the fungus.

The treatment of cystic hydatid disease is principally surgical. Surgery however is associated with significant perioperative mortality and recurrence. Besides, multiple bilateral cysts and disseminated hydatid disease are technically difficult to operate. The benzimidazole compounds, initially flubendazole and mebendazole, had been tried for the treatment of cystic hydatid disease but the results were discouraging owing to the poor concentration of the drugs both in serum and the cysts (5,6). Later on however, encouraging results were obtained using albendazole and this was accounted by the presence of high concentration of the active metabolite, albendazole sulphoxide both in the serum and the cysts (7). Analysis of pooled data of all drug trials using albendazole between 1983 and 1989 on 500 patients demonstrated an overall success rate of 80% (30% cure and 50% reduction in size) (8,9). The drug safety profile included transient hepatotoxicity which rarely required discontinuation of the medication. Our patient did extremely well with the treatment and is presently being followed up for evidences of recurrence.

Cystic hydatid disease is endemic in Ethiopia. A study by Fuller and Fuller have shown that the disease is widespread and the highest prevalence is in the Southwestern Ethiopia, with clinically detectable disease occurring in 5% of the Dassanetch and Myangtom tribes (10,11). The disease entails significant morbidity and is frequently encountered in our clinical practice. In countries like ours where surgical facilities are not widely available, the need for alternative medical treatment is obvious. At present, the indications for medical treatment include inoperable hydatid cysts, recurrent disease, multiple cysts and those who are not fit to undergo surgery. As we learn more on drug pharmaco-kinetics including the optimal dose and duration of treatment that will attain an even better cure rate, medical treatment is likely to replace surgical treatment.

REFERENCES

1. Schiller, CF. Complication of Echinococcus cyst rupture. *JAMA* 1966;195:220-2.
2. Mattaghian H, Saidi F. Post operative recurrence of hydatid disease. *Br J Surg* 1978;65:237-42.
3. Morris DL, Dykes PW, Dickson B, Barrier SE, Bogan JA, Burrow FGD. Albendazole in hydatid disease. *Br Med J* 1983;296:103-4.
4. Saimogggt AG, Gremieux AC, Hay JM, *et al*. Albendazole as a potential treatment for human hydatidosis. *Lancet* 1983;(ii):652-6.
5. Okelo GBA, Chematai AK. The treatment of hepatic hydatid disease by using mebendazole: a report of 16 cases. *East Afr Med J* 1981;58:608.
6. Braitharaite PA. Long term high dose mebendazole for cystic hydatid disease of the liver: failure in two cases. *Aust NZ J Surg* 1981;51:23-7.
7. Morris DL, Dykes PW, Mariner S, *et al*. Albendazole - objective evidence of response in human hydatid disease. *JAMA* 1985;253:2053-7.
8. Horton RJ. Chemotherapy of Echinococcus infection in man with albendazole. *Trans Roy Soc Trop Med Hyg* 1989;83:97-102.
9. Nahimas J, Goldsmith R, Soibelman M, Elon J. Three to seven years follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994;88(3):295-304.
10. Fuller GK, Fuller DC. Hydatid disease in Ethiopia with some immunodiagnostic test results. *Am J Trop Med Hyg* 1981;30(3):645-52.
11. Fuller GK. Hydatid disease among the Dassanetch of South West Ethiopia. *Trans Ray Soc Trop Med Hyg* 1976;70:353-4.

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Tesfaye Mengesha, Teshale Seboxa, 1998. *Ethiop Med J*, 36.

LETTER TO THE EDITOR

AMODIAQUINE: THE EXEMPTED ANTIMALARIAL DRUG IN ETHIOPIA

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In the 1993 Ethiopian Medical Association Annual Conference, we reported our work on antimalarial drug resistance of *Plasmodium falciparum* isolates in Gondar and the surrounding areas. Out of thirty-two isolates tested 10 (31%) showed resistance to chloroquine, out of which one was both chloroquine and amodiaquine resistant; the amodiaquine resistance (3.1%) was reported to be possibly due to cross resistance to chloroquine (1). No resistance was observed to the sulfadoxine-pyrimethamine combination (Fansidar^{R}). Isolates for the study were collected from patients harbouring *P.falciparum* monoinfection in Kola Diba, and from those coming to the Gondar Teaching Hospital. Species identification was done by a standard Giemsa staining procedure (2), and drug resistance studies were performed using the WHO microtest method (3).

The recent report by the Malaria Control Unit of the Ministry of Health, however, shows the failure rate of chloroquine to have increased to over 75% (Alemirew, D. *et al.*, EMA Continuing Medical Education Programme, December 1996). This raises the possibility of formulating a policy to replace chloroquine by sulfadoxine-pyrimethamine as a first line drug. This policy was backed by the experience of other African countries, like Kenya and Malawi, that have already made such a policy a reality. This raises the question of having other cheap, and efficacious antimalarials into focus. Related to this, recent reviews by the Liverpool School of Tropical Medicine and Hygiene on the comparative cure rates of antimalarials have shown amodiaquine to be an efficacious antimalarial in Africa (partly reported in TDR news No. 52). The amodiaquine status in Africa also showed 93-100% cure rate and a fast fever clearance time of 1.1 days (4,5).

Due to the efficaciousness of the other 4-aminoquinoline, i.e. chloroquine, amodiaquine has never been used in Ethiopia. Therefore, we do not expect genuine resistance, though the possibility of cross resistance to chloroquine might exist. Considering the ratio of amodiaquine to chloroquine resistance (1:10), seen in our study, making an adjustment to the present chloroquine resistance status might result in up to 8% amodiaquine cross-resistance (4), giving a cure rate of $\geq$90% which is in line with reports from other African countries (5). Though as 4-aminoquinolines, chloroquine and amodiaquine belong to the same group, and cross-resistance may be expected, the

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difference in their pharmacokinetics makes cross-resistance less frequent. Chloroquine is partly metabolized into mono-desethyl derivatives and eliminated in urine, while amodiaquine is a pro-drug and excreted mainly in the bile (6). Earlier pharmacokinetic data for amodiaquine (3) show high potency with 0.1 ug/ml maximum therapeutic concentration, compared to 0.2 ug/ml in chloroquine. This shows a wider amodiaquine therapeutic index, which may be useful if the need arises for dosage increment against emerging tolerant strains. With these advantages, and given the need for 2nd line antimalarials in Ethiopia, we suggest an in-depth study of this "exempted" drug regarding its toxicity, cure and recrudescence rates, fever and parasite clearance time, etc.

REFERENCES

1. Mengesha T, Seboxa T. Drug resistant falciparum malaria in Gondar, North Western Ethiopia. Paper presented at the Ethiopian Medical Association, 29th Annual Conference, May, 1993.
2. Cheesbrough M. Medical laboratory manual for tropical countries, 1981. Cambridgeshire, England. pp. 519.
3. Black RH, Canfield CJ, Clyde DF, Peters W, Wernsdorfer WH. Chemotherapy of malaria (2nd revised edition), Bruce-Chwatt LJ. (Ed). *WHO*, 1986, Geneva pp. 261.
4. Sowunmi A, Salako LA. Evaluation of the relative efficacy of various antimalarial drugs in Nigerian children under five years of age suffering from acute uncomplicated falciparum malaria. *Annal Trop Med Parasitol* 1992;861:1-8.
5. Muller O, VanHensbrock MB, Jafar S, Drakeley C, Okorie C, Joof D, Pinder M, Greenwood B. A randomized trial of chloroquine, amodiaquine and pyrimethamine sulfadoxine in Gambian children with uncomplicated malaria. *Trop Med Intl Hlth* 1996;1:124-32.
6. Pussard E, Verdier F. Antimalarial 4-aminoquinolines: Mode of action and pharmacokinetics. 1194. *Fund Clinc Pharmacol* 8:1-17.

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RANDOMISED CLINICAL TRIAL OF INSULIN ALONE OR INSULIN IN COMBINATION WITH SULFONYLUREA IN THE TREATMENT OF NON-INSULIN DEPENDENT DIABETIC PATIENTS WITH SECONDARY REFRACTORINESS TO SULFONYLUREAS

Berhane Seyoum, MD[1], Jemal Abdulkadir, MD, CM, FRCP(E)[1].

ABSTRACT: *A total of 40 NIDDM patients with secondary failure to sulfonylureas were randomly put on insulin alone or insulin in combination with sulfonylurea regimen and they were crossed over after controlling the blood glucose. The objective of the study was to assess whether insulin alone or insulin in combination with sulfonylureas is better to achieve good metabolic control for NIDDM patients with secondary failure to sulfonylureas. They were 26 females and 14 males. The mean age was 50.7±9.4 years (range 33-76). The mean duration of diabetes was 9.0±1.2 years (range 1-20 years). Nine patients (22.5%) had first degree family history of diabetes mellitus. Eight patients (20%) had hypertension and 12 patients (30%) had nephropathy. Background retinopathy and neuropathy were found in 19 patients (47.5%) and 18 patients (45%), respectively. In 32 patients (80%) the combination of sulfonylureas with insulin did not bring any significant change in blood glucose level, however, in eight patients (20%) there was significant difference in the blood glucose control. In conclusion, the results obtained show no significant difference in glucose control whether insulin was given alone or in combination with sulfonylureas in NIDDM patients with secondary failure. Therefore, we recommend insulin therapy alone for NIDDM patients with secondary failure to sulfonylureas.*

INTRODUCTION

First line treatment of Non-insulin dependent diabetes mellitus (NIDDM) consists of diet, exercise and oral agent (usually a sulphonylurea). Many patients develop secondary refractoriness to oral agents after 10 to 14 years of treatment (1,2). At this stage, the alternatives that are usually considered are transferring patients to insulin alone or to a combination of insulin with sulphonylurea. The idea of using an insulin-sulphonylurea combination in NIDDM patients with secondary refractoriness to oral agents is not new (2-7). Some authors had reported that the addition of

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glibenclamide to insulin resulted in a significant improvement of metabolic control in NIDDM patients with secondary refractoriness to sulfonylurea while others have argued that the benefit from the combination was negligible (8-10) and that the increased costs and added side effects far outweigh the marginal benefit (11). This study was undertaken to assess whether insulin alone or insulin in combination with sulfonylureas is better for NIDDM patients with secondary refractoriness to sulfonuylureas.

PATIENTS AND METHODS

A total of 41 NIDDM patients were recruited from the follow-up clinic of the Tikur Anbessa Diabetes Centre. All patients were taking 20 mg or more of glibeneclamide and their blood glucose was steadily above 200 mg% for at least 2-3 months. The sample size was determined according to a cross over design (12). The power of the study was 90%. During entry, each patient had undergone a general physical and laboratory examinations. Kidney function tests, urine analysis, total cholesterol and its fractions and triglycerides were determined at the National Research Institute of Health using standard methods (13).

The exclusion criteria were: 1) Patients with known heart, kidney and liver dysfunctions. 2) Patients with acute infections such as acute febrile illness, pneumonia, etc. 3) Patients taking drugs which interact with sulphonylurea like steroids, hydrochlorothizides, coumarins, etc. 4) Patients with known HIV infection. 5) Patients with vomiting and diarrhoea.

There was a run in period of 3-8 weeks. During this time all patients were advised to observe strict dietary control and perform regular exercise according to their ability. All patients were also given glipizide 10 mg 12 hourly. During this run in period, only one patient responded. This patient achieved fasting blood glucose values of less than 140 mg/dl and was excluded from the study. Subsequently all patients were randomly assigned to either of the two regimes based on the radom table. The regimens were insulin alone or insulin in combination with sulfonylureas. Half of the patients were put on the insulin alone regimen and the other half were put on insulin in combination with sulfonylureas. Once the desired target was achieved, patients were crossed over after a wash out period of two weeks. Those who were taking insulin alone were instructed to add sulfonylureas and those who were taking the combination treatment were told to stop sulfonylureas. The insulin used was intermediate acting NPH Human insulin (ELI LILLY and Company, Indianapolis, USA). It was given at 8-9 pm. The sulphonylurea was glipizide 10 mg administered at least half an hour before breakfast. In order to assure compliance the insulin vial was checked and the number of tablets were counted on every visit. All patients were taught how to inject insulin and they were as well told to maintain their usual diet and exercise.

The target was to achieve fasting blood glucose < 140 mg%. In order to achieve this target the insulin dose was adjusted according to the blood glucose values whereas the glibizide dose was fixed at 10 mg/day. Fasting blood glucose was done twice per week. Once the desired target was achieved random blood glucose was determined at 4-5 pm to assess the effect of the sulphonylureas during the day time. Subsequently, the patients were maintained on the same dose of insulin without

manipulation for 2 weeks, then they were crossed over. Before cross over there was 1-2 weeks of wash out period during which time, no intervention was done. It was not possible to conceal the cross over point because of the nature of the illness. Similarly, patients were followed on the other arm of the regimen until the desired target is achieved. The measuring variable used was the amount of insulin required to achieve the desired target. The minimum amount of insulin expected to be reduced with the addition of sulfonylurea was four units.

Informed consent was obtained from each participating patient after the purpose of the study had been explained to them, following the guidelines of the Helsinki convention for human experimentation. The paired T-test was used for statistical analysis.

RESULT

According to the selection criteria, 41 NIDDM patients were included in the study. One patient was excluded during the run in period because he achieved normal blood glucose with glipizide. Therefore, the study was done on 40 patients and all have completed the study. Out of the 40 patients 26 (65%) were females and 14 (35%) were males. The mean age was 50.7±9.4 years, the range being (35-76 years). The mean duration of diabetes was 9.0±1.2 years (range 1-20 years). Nine (22.5%) patients had first degree family history of diabetes mellitus. Hypertension and nephropathy was found in 20% and 30% of the patients respectively; however, no one had hypertensive heart disease or renal failure. Half of the patients had background retinopathy and neuropathy, 47.5% and 45% respectively. Patients were followed for a mean total of 10.9±2.3 weeks (range 8-16 weeks) excluding the run in and wash out periods. During the study one patient developed severe painful neuropathy which did not respond to analgesics. This patient improved after 6 months spontaneously. There was no new development of complications in all patients. The combination of sulphonylurea with insulin lowered the blood glucose level by 4.4 mg% (mean fasting blood sugar before combination was 122.6±2.6 mg% and after combination 118.2±17.1 mg% T-paired test $p<0.05$) and the insulin level by 1.1u (mean insulin before combination was 33.4±13.8 units and after combination 32.3±12.9 units T-paired test $p<0.05$). The mean blood glucose before any intervention was 309.1 mg%. Almost all patients (87.5%) gained weight at the end of the study. The mean weight gain was 2.3±1.5 kg. Total cholesterol and triglycerides dropped significantly (Table 2).

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py alone (10,14-16). However, our study concurs with the conclusion made by Peters et al (11), who found that, combination therapy with insulin plus sulphonylureas only marginally improves overall diabetic control. Achieving this slight response increases costs and the risk of side effects, as a result their conclusion was that this form of therapy should not be used for NIDDM patients with secondary failure to sulfonylureas.

All patients were given glipizide during the run in period. Although there is no major advantage for glipizide over glibenclamide in terms of blood glucose control (17), glipizede was given to our patients in order to improve compliance. We found only one patient who responded to glipizide and this patient was excluded from the study. Besides, after patients achieved the target, we determined blood glucose levels at 4-5 pm in order to see the effect of sulfonylureas during the day time as the sulfonylureas were given in the morning. Our findings show that only 3 patients achieved blood glucose levels less than 160 mg/dl at this time of the day. Therefore, changing glbenclamide to glipizide does not have any advantage, and moreover, sulfonylureas were not helpful in controlling the blood glucose at 4-5 pm unlike the findings by other authors (14).

Weight gain is generally regarded as a side effect of insulin therapy. In this study, improvement in glycemic control was significantly correlated with weight gain. This weight gain during insulin therapy is a sign of the effectiveness of insulin rather than a side effect. It is known that after 10 years of their diagnoses only 50% of the NIDDM patients continue to respond adequately to sulfonylureas (14). Our finding is in agreement with this report, the mean duration of diabetes in our patients is 9.0 ± 1.2 years.

In keeping with previous reports (18,19), serum lipid levels dropped in 26 (65%) of the patients during insulin therapy. In previous studies, patients in whom glycemic control was markedly improved (with a decrease in glycosylated haemoglobin of 3 percent) by multiple insulin injection, serum triglyceride concentrations decreased by 50% (13). However, in another study serum triglycerides did not change after six months of treatment with a two insulin injection regimen during which glycosylated haemoglobin decreased by 2% and body weight increased by 5 kg (20). Thus, although short-term intensive insulin therapy under carefully controlled conditions reduces antiatherogenic changes in serum lipoproteins, these effects may be diminished by factors such as weight gain and suboptimal glycemic control.

A hitherto neglected but important factor in advocating a particular treatment regimen for patients with NIDDM is whether the treatment improves subjective well-being. With respect to insulin therapy, we found that the majority (more than 90%) of our patients reported a dramatic improvement in their subjective well being. Although, initially many of the patients had a negative attitude about insulin injection and willingness to continue insulin therapy, after they saw a marked improvement in their subjective feeling of well-being and metabolic control, they were convinced to continue the insulin injection for life.

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REFERENCES

1. Lebovitz HE, Feinglos MN. The oral hypoglycemic agents. in Ellenberg M, Rifkin H (eds.): Diabetes mellitus. Theory and practice. ed. 3. New Hyde Park, NY, Medical Examination publishing Co Inc, 1983,pp.591-610.
2. Dunchan GG, Joiner CL, Lee CT. Sulfonylurea symposium: methods of selection of diabetic patients sensitive to sulfonylurea compounds. *Metabolism* 1956;964-71.
3. Friedlander OE. Use of tholbuthamide in insulin resistant diabetes report of a case. *New Engl J Med* 1957;257:11-4.
4. Feldman JM, Lebovitz HE. Endocrine and metabolic effects of glibenclamide. Evidence for an extra pancreatic mechanism of action. *Diabetes* 1971;20:745-55.
5. Hamelbeck H, Klein W, Zoltobrocki M, Schoffling K. Glibenclamide insulin-combinations be handling beim Sekundar-versagen der sulfonylharnstoff-therapie. *Dtsch Med Wochenschr* 1982;107:1581-3.
6. Bieger WP, Dlugosch R, Rettenmeier et al. Trial of sulfonylurea in combination with insulin in the therapy of diabetes type 1 and 2. *Klin Wochenschr* 1984;62:631-9.
7. Longnecker MP, Elsenhans VD, Leiman SM, Owen OE, Boden G. Insulin and sulfonylurea agent in NIDDM. *Arch Intern Med* 1986;146:673-6.
8. Groop L, Harno K, Nikkila EA, Palkonen R, Tolppanen EM. Transient effect of the combination of insulin and sulfonylurea (glibenclamide) on glycemic control in non-insulin dependent diabetics poorly-controlled with insulin alone. *Acta Med Scand* 1985;217:33-9.
9. Sachse G, Mases E, Federlin K. Kombinationtherapie mit insulin und sulfonyl-harnstoffen bei Sekundarversagen der ulfonylharnstofftherapie. *Dtsch Med Wochenser* 984;109:419-21.
10. Lins PE, Lundbald S, Trotzig P, Adamson U. Glibenclamide improves the response to insulin treatment in NIDDM with secondary failure to sulfonylurea therapy. *Acta Med Scand* 1988;223:171-9.
11. Peter LA, Davidson BM. Insulin plus a sulfonylurea agent for treating type 2 diabetes. *Ann Int Med* 1991;1150:45-53.
12. Colton T. Statistics in medicine. First edition. Inferences on proportion. Little, Brown and company. 1974;pp141-6.
13. Kattermann R, Jaworek D, Moller G, Assmann G, Bjorkhen I, Sevensson L et al. Multicenter study of a new enzymatic method of cholesterol determination. *J Clin Chem Clin Biochem* 1984;22:245-51.
14. Yki-Jarvinen H, Kauppila M. Kujansuu E et al. Comparison of insulin regiments in patients with non-insulin dependent diabetes mellitus. *New Engl J Med* 1992;327:14-26-33.

15. Schade DS, Mitchell WJ, Griego G. Addition of sulfonylurea to insulin treatment in poorly controlled type II diabetes. A double-blind, randomised clinical trial. *JAMA* 1987;257:2441-5.
16. Osci K, O'Dorisio TM, Falko JM. Concomitant insulin and sulfonylurea therapy in patients with type II diabetes effects of glucoregulation and lipid metabolism. *Am J Med* 1984;77:1002-9.
17. Groop L, Groop PH. Stenma S, Salaranta C, Totterman K-J, Fyhrquist F, Melander A. Comparison of pharmacokinetics, metabolic effects and mechanisms of action of glyburide and glipizide during long term treatment. *Diabetes Care* 1987;10:671-8.
18. Agardh C-D, Nilsson-Ehle P, Schersten B. Improvement of the plasma lipo-protein pattern after insitution of insulin treatment in DM. *Diabetes Care* 1982;5:322-5.
19. Pfeifer MA, Grunzell JD, Best JD, Judzewitrats RG, Halter JB, Parte D Jr. The response of plasma triglyceride, cholesterol and lipoprotein lipase to treatment in non-insulin dependent diabetic subjects without familial hyper-triglyceridemia. *Diabetes* 1983;32:525-31.
20. Groop L, Widen E, Fraussila-Kallunki A, *et al*. Different and effects of insulin and oral antidiabetic agents on glucose and energy metabolism in type II (Non-insulin dependent) diabetes mellitus. *Diabetologia* 1987;32:599-605.

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Original article

Atopic eczema in school children

Kibrebeal Melaku

Abstract: Information of Atopic eczema is sparse in Ethiopia. This survey was conducted to determine the prevalence of eczema among school children in Addis Ababa, Ethiopia, in 1995. A standardized self-administered questionnaire developed by the International Study of Asthma and Allergies in Children (ISAAC) was used. A total of 2951(52.8%) were females. A 29.2% of life time experience of recurrent itchy rash and 22.1% of 12 months prevalence of rash was reported by the children. However, only 11.2% of children reported having eczema. Sleep disturbing rash, a sign of severe eczema, was reported by 2.6% of children. By using a simple standardized questionnaire, an internationally comparable local data was generated by this study. However, further studies with a more objectives diagnostic criteria and skin prick test are recommended. [*Ethiop. J. Health Dev.* 2000;14(0):0-00]

Introduction

Atopic eczema (synonymous with Atopic dermatitis) is an inflammatory skin disorder characterized by itching and a predilection for the flexure sites)1). Although Atopic eczema is not a life threatening disease, the constant scratching associated with it may result in skin damage, secondary infection and sleep loss to both children and parents (1). Despite this burden of morbidity, little is known about the causes of Atopic eczema (1). Moreover, several studies have suggested that the prevalence of eczema have increased two-to three-fold over the past 30 years (3). Some of this increase could probably be related to variation in the diagnostic criteria. However a genuine increase in the magnitude of eczema have been noted in several studies that have used objective criteria and methods (3). Although several population-based studies of Atopic eczema have been conduced in Europe (4), comparison of disease prevalence between countries have been virtually impossible because of lack of standardized diagnostic criteria suitable for use in epidemiological studies (4,5).

In order to make international comparisons feasible, the International Study of Asthma and Allergies in Childhood (ISAAC) group prepared a simple and standardized questionnaire. This survey instrument can be applied worldwide to describe the magnitude and geographic distribution of Atopic eczema and other allergic diseases (6). In Ethiopia, few hospital based studies have been done to determine the pattern and prevalence of skin diseases in general (7-13). No community based survey has been done to specifically study the prevalence of Atopic eczema except the Jimma study which was conducted at about the same time with this study.

The aim of this study was to determine the prevalence of Atopic eczema in school children. Moreover, by using a simple standardized questionnaire, we have made international comparison feasible with other studies which have used similar module.

Methods

This study was a descriptive, cross sectional study. Subjects were recruited from schools in Addis Ababa, the capital city of Ethiopia. At the time of this survey, Addis Ababa had 153 junior secondary schools. Study schools were selected using a multi-stage sampling strategy. First, out of the 153 schools, 28 were selected by simple random sampling. The list of schools was obtained from the Addis Ababa Education Bureau. From each school, two sections of the 7th grade students were selected

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by simple random sampling. In schools wee there were only two or less sections, all sections were included. Then, all students who were aged 13 and 14 were enrolled in the study. Children of these age were selected because of their ability of fill the self-administered questionnaire.

The sample size calculation was based with the intention of detecting a 2% difference of severe Atopic eczema between two centers (for instance one having 3% and the other having 5% prevalence of severe eczema), with the study power of 99% and at 99% significant level. These assumptions required a sample size of 3000. However, a far less sample size was adequate to detect the prevalence of eczema symptoms as they are more common than symptoms of severe Atopic eczema (6).

The data was collected using a standardized and self-administered questionnaire developed by ISAAC (6). The study was part of a multi-center international study. The questionnaire was adapted and translated into Amharic, the national language. Standard guidelines for translation of the questionnaire from English into the local language was used (14). The questionnaire was pre-tested on children who didn't participate in the actual study to evaluate its validity locally. Trained physicians were available to give explanation on how to fill the questionnaire and to give clarification for any inquiry made by the students.

The questions to determine the prevalence of Atopic eczema and related symptoms were as follows.

1. Have you ever had a itchy rash that was coming and going for at least 6 months?
2. Have you had this itchy rash at any time in the last twelve months?
3. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?

If yes to question 3:

4. In the last 12 months, how often, on average, have you been kept awake at night by this itchy rash? (Never in the last 12 months, less than 1 night per week, 1 or more nights per week).

All respondents were asked:

5. Have you ever had eczema?

N.B. For a detail information, please refer to the ISAAC manual (6).

Operational Definitions

1. The presence of an itchy, relapsing skin rash in the preceding 12 months that had affected the skin creases (flexural parts) was considered as Atopic eczema.
2. Atopic eczema symptoms resulting in sleep disturbance for one or more nights per week was considered as severe Atopic eczema.

The questions were based on a diagnostic criteria that were being developed by UK national case control study, which set out to develop a minimum list of reliable discriminators for other Atopic Dermatitis (15).

Data was then processed using EPI-Info-Version 6 statistical software. Prevalence rates were calculated for the major symptoms. Ethical approval was obtained from Faulty of Medicine, Addis Ababa University.

Results

A total of 2951 children aged 13 and 14 years completed the questionnaire. There were 1558(52.8%) females and 1393(47.2%) males with female to male ration of 1.1:1. Of these, 1619(54.9%) were 13 years old while the remaining 1332(45.1%) were 14 years old.

Table 1: prevalence of Atopic eczema and related symptoms in the last 12 months among school children in Addis Ababa, Ethiopia, 1995(N = 2951).

	Frequency	Percentage
Recurrent Itchy rash ever	863	29.2%
Reported having eczema	331	19.9%
Atopic eczema*	587	11.2%
Severe eczema*	77	2.6%

*Full-fill the operational definition (see methods and materials section).

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The Life-time prevalence of a recurrent, itchy rash was found to be 29.2% while only 22.1% reported similar rash in the past 12 month period. However, only 331(11.2%) of children reported having eczema the prevalence of symptoms of severe eczema in the last 12 months was 2.6%. There was a male preponderance for both symptoms of eczema ever and severe eczema, but only the former reached a significant level at p-value <0.05 (OR=1.34 95% CI (1.06,1.70)). On the other hand, the prevalence of rash ever was found to be slightly higher among girls than boys but didn't reach significant level (OR (95%CI)=0.92(0.78,1.08)).

Discussion

This survey has provided local data concerning the prevalence of symptoms of eczema among school children of Addis Ababa, by using a simple standardized questionnaire.

Comparisons of the findings of this study is made with the results of the various ISAAC collaborative centres which have used similar module (1). Accordingly, the 12 month prevalence of symptoms of self reported eczema in our study was found to be 19.9% which was similar to studies done in Ibadan, Nigeria (17.7%) and Helsinki, Finland (17.3%) but different from results found from Jemma, Ethiopia (3.2%) and Beijing, China (1.6%) (1). A similar variation was also noted in the prevalence of reported eczema ever (life-time occurrence). Accordingly, the prevalence of reported eczema in our study was found to be 11.2% which is similar to that observed in Eldoret, Kenya (13.2%) and Recife, Brazil (11.4%) but different from studies done in Jemma, Ethiopia (5.6%) and in Kutaisi, Georgia (4.1%) (1). These findings clearly demonstrate the geographic variation in the occurrence of eczema among children. This could serve as a starting point for looking at etiologic factors of eczema. The severity of eczema was assessed by asking the frequency of sleep disturbing rash rather than by determining the extent of skin involvement due to the difficulty of using such criteria in a questionnaire method (6).

Accordingly, the prevalence of symptoms of severe Atopic eczema was found to be 2.6% in our study which is similar to other studies done in Eldoret, Kenya (2.4%) and from Cape town, South Africa (2.3%) but different from the results found from Bombay, India (0.1%), Beijing, China (0.2%) and Jemma, Ethiopia (0.8%) (1).

A significant variation in the prevalence of reported symptoms of Atopic eczema and severe eczema has been noted between and within countries inhabited by similar ethnic groups (1). For instances, both the prevalence of reported symptoms of eczema and severe eczema was noted to be higher in this study as compared to the Jemma, Ethiopia study, which is the 2nd ISAAC study center in Ethiopia (1). No obvious reason was found which would explain the apparent difference seen between the two centres. However, some unidentified genetic, environmental and socio-economic factors might have played a role.

It is interesting to note that an itchy rash, which is one of the most important symptoms of Atopic eczema, can occur as the result of various infestations and skin infections. Since these skin diseases are very common in many of the developing countries like ours, it might be worth to do studies with more objective criteria (15) to verify how much they strongly correlate with findings obtained by the questionnaire method.

In conclusion, this study has shown that the prevalence of Atopic Eczema among school children of Addis Ababa is significantly high. However, further studies with a more objective criteria (15) and skin prick test are recommended.

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Reference

1. Hywel W, Colin R, Alistair S, Nadia AK, Gabriel A, Ross A et al. World-wide variations in the prevalence of symptoms of Atopic Eczema in the International study of Asthma. J Allergy Clin Immunol. 1999;103:125-28.

2. Schultz Larsen F. The epidemiology of Atopic dermatitis. Mongr Allergy 1993;31:9-28.

3. William's HC. Is the prevalence of Atopic dermatitis increasing? Clin Exp Dermatol. 1992;17:385-91.

4. Burkhoter D, Schiffer P. The Epidemiology of Atopic diseases in Europ. A review. Allergy Clin Immunol News. 1995;7:113-24.

5. William's HC, Burnery PGJ, Pembroke AC, Hay RJ. Validation of the UK diagnostic criteria for Atopic dermatitis in a population setting. BR J Dermatol. 1996;135:12-7.

6. International Study of Asthma and Allergies in Childhood (ISAAC) Manual. Munster: University of Munster; 1992.

7. Jose I. Figueroa, L-Claire F, Aynalem A, Rod J Hay, Pediatric Dermatology. 1996;13(5):378-381.

8. Dagnew MB, Erwin G. Epidemiology of common transmissible skin diseases among primary school children in North West Ethiopia. Trop Geogr Med 1991;43:152-155.

9. Mumcuoglu KY, Miller J, Miller O, Ben Yshaif F, Klavs S. The prevalence of ecto-parasites in Ethiopian migrants. Isr 5 J Med Sci. 1993;26:371-373.

10. Manzor J. Skin diseases in Addis Ababa. A preliminary review. Ethiop Med J 1981;19:123-134.

11. Schaller KF. 1963. Skin diseases in Ethiopia. Ethop Med J 1981;19:123-134.

12. Warndorff JA. 1978. Topical treatment. Ethiop Med J 1979;16:159-162.

13. Shibeshi D. Pattern of skin diseases at the Ethop-Swedish pediatric Hospital. Dermatology Unit, Department of Internal Medicine, Addis Ababa University, Ethiopia (unpublished data).

14. Weiland SK, Beasley R, Strachan D. Guidelines for the translation of questionnaire. Munister, Germany: ISAAC phase one coordinating committee;1993.

15. William's HC, burney POGJ, Hay RJ, et al. The UK working party's diagnostic criteria for Atopic dermatitis I: Derivation of a minimum set of discriminators of Atopic Dermatitis. Br J Dermatol 1994;131:383-96.

16. William's HC. So how do we define Atopic eczema? A practical Manual for researchers wishing to define Atopic eczema. Nottingham; university of Nottihgham. Department of dermatology; 1997.

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Kibrebeal Melaku, Yemane Berhane, 1999. *Ethiop Med J*, 37.

PREVALENCE OF WHEEZE AND ASTHMA RELATED SYMPTOMS AMONG SCHOOL CHILDREN IN ADDIS ABABA, ETHIOPIA

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ABSTRACT: *This survey was conducted as part of the international multi-centre study to determine the prevalence of wheeze and asthma-related symptoms among school children in Addis Ababa, Ethiopia. It was conducted in June 1995. A standardized self-administered questionnaire developed by the International Study of Asthma and Alergies in Children (ISAAC) was used. The questionnaire was designed to offer a standardized epidemiological instrument to ensure comparison of results from wide range of countries. A total of 2951 children aged 13 and 14 years were enrolled into the study, of which 1393 (47.2%) were males and 1558 (52.8%) were females. Five hundred fifty seven children (18.2%) reported that they have had wheezing at least once in the last 12 months while only 84 children (2.8%) reported to have "Bronchial Asthma". One hundred eighty eight (6.4%) and 214 (7.3%) children reported sleep disturbance and limitation of speech associated with attacks, respectively. Nocturnal cough was reported by 805 (27.3%) children. Eight hundred two (27.2%) children reported exercise-induced wheeze. This study revealed that the prevalence of wheeze and other asthma related symptoms to be fairly high among school children of Addis Ababa, Ethiopia. Moreover, the majority of these children do not seem to know their problems and therefore, remain untreated. Further studies are recommended to precisely determine the prevalence of wheeze and asthma using more objective methods such as bronchial hyper-responsiveness test.*

INTRODUCTION

There is a serious concern about the recent rise in the prevalence and severity of asthma in both developing and developed countries (1-3). Moreover, there is poorly explained variations in its prevalence and severity worldwide (1,2,4,5).

Cultural and language differences in expressing symptoms in different parts of the world may partly explain the variation in the prevalence and severity of asthma.

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(2,6). Although the presence of Bronchial Hyper-responsiveness (BHP) has been used in the working definition of asthma, symptoms remain the corner stone of large-scale epidemiological surveys of asthma in children (4,6-10). Accordingly an international research group known as International Study of Asthma and Allergic Disease of Children ("ISAAC") has developed a simple standardized questionnaire using the common asthma related symptoms to reduce variations in survey instruments (2,5-9).

In Ethiopia, few studies have been conducted to determine the prevalence of bronchial asthma (11-14). Nearly all of those studies were based on cases drawn from hospital records (11-13). Therefore, the purpose of this study was to determine the prevalence of wheeze and asthma-related symptoms among school children using ISAAC questionnaire. School children were selected mainly because they were readily accessible. This study will provide a unique opportunity to compare results with other studies done elsewhere using a similar instrument. In addition, it will provide a baseline information for future assessment of trends in the prevalence of bronchial asthma and clues for etiological studies as well.

SUBJECTS AND METHODS

The study utilized a descriptive cross-sectional design. It was conducted in June 1995. Subjects were recruited from schools in Addis Ababa, the capital city of Ethiopia. The list of schools was obtained from the Addis Ababa Education Bureau. At the time of the survey, Addis Ababa had 153 junior secondary schools. The study schools were selected using a multi-stage sampling strategy. First, out of the 153 schools 28 were selected by simple random sampling. In each school, two sections of the seventh grade students were selected by simple random sampling. In schools where there were only two or less sections, all sections were included. Then, all students who were aged 13 and 14 years were asked to participate in the study. Children of this age were selected because of their ability to fill the self-administered questionnaire at the time.

The sample size calculation was based on the intention of detecting a 2% difference of severe asthma between two centers (one having 3% and the other having 5% prevalence of severe asthma), with the study power of 99% and at 99% significant level. These assumptions required a sample size of 3000. However, a far less sample size is adequate to detect an over all prevalence of asthma symptoms as they are more common than symptoms of severe asthma (1).

The data were collected using a standardized self-administered questionnaire developed by ISAAC. The study is part of a multi-centre international study. It was adapted and translated into Amharic, the national working language. Translation was made by two individuals into Amharic and back translated into English by another two persons to check on the consistency of the translation. The questionnaire was pretested on children who did not participate in the actual study to evaluate its validity locally. Trained physicians were made available to give explanation on how to fill the questionnaire and to give clarification for any inquiry made by the students.

Data were collected concerning experience of wheeze ever and self reported asthma. For those who claimed to have wheezes during the previous 12 months, additional inquiries were made as to whether wheezing has been sleep disturbing and speech-limiting. Further inquires were

also made with regard to exercise-induced wheeze and nocturnal cough. Ethical approval was obtained from the Addis Ababa University.

Data were processed using Epi Info version 6.03 statistical software. Prevalence figures were calculated for the total study population and separately by sex.

RESULTS

Among the total of 2951 students aged 13 and 14 years who completed the questionnaire, 1393 (47.2%) were males and 1558 (52.8%) were females with male to female ratio of 1:1.1. Of the respondents, 1619 (54.9%) were 13 years old while the remaining 1332 (45.1%) were 14 years old (Table 1).

Of all the respondents, 537 (18.2%) had ever experienced wheezing while only 308 (10.4%) reported wheezing in the past 12 months (Table 2). There was no difference in the prevalence of ever wheeze and asthma between boys and girls with Odds Ratio (95% CI) of 1.11 (0.92,1.35) and 0.97 (0.61, 1.53), respectively.

Table 1. Age and sex composition of the study children, Addis Ababa, Ethiopia, 1995. (n = 2951).

Variable		Frequency	Percent
Age			
	13 year	1619	54.9
	14 year	1332	45.1
	Total	2951	100.0
Sex			
	Male	1393	47.2
	Female	1558	52.8
	Total	2951	100.0

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Table 2. Prevalence of reported ever wheeze and diagnosed asthma among school children in Addis Ababa, Ethiopia, 1995. (n=2951).

	Male	Female	OR (95 CI)
Wheeze ever*1			
Yes	265	1123	
No	272	1284	1.11 90.92,1.35)
Total	537	2407	
Reported having Ashthma*2			
Yes	39	1344	
No	45	1502	0.97 (0.61,1.53)
Total	84	2846	

*1 7 Missing responses (5 male and 2 female)
*2 21 Missing responses (10 male and 11 female)

Though the reported rate of suffering from asthma related symptoms was found to be fairly high, only 84 (2.8%) reported that they had been diagnosed as having asthma. Within the previous 12 months, 161 (5.4%) of all the respondents had experienced four or more attacks of wheeze, while 177 (6.0%) had sleep disturbing wheeze. Speech disturbance due to wheeze was experienced by 206 (7.9%) of the school children (Table 3). In addition, exercise-induced wheeze was experienced by 802 (27.2%) and nocturnal cough by 805 (27.3%) of the school children (Table 4).

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which is similar to that observed in Singapore, 2.4%, and Iran, 2.7%; but different from studies done in Brazil, 16.1%, Morocco, 12.1%, and Scotland, 21.4% (3). These findings clearly demonstrate the geographic variations in the occurrence of asthma among children. This could serve as a starting point for looking at etiologic factors of asthma.

It is interesting to note that the "asthma-ever" prevalence rate of 2.8% is much lower than the prevalence of wheeze ever (18.2%). One possible reason for such remarkable discrepancy could be under-diagnosis and treatment of asthma in children. Experiences elsewhere have also shown that such direct questions are found to be of less reliable measures for epidemiological purpose (16).

Severity of asthma was measured in ISAAC questionnaire by asking for sleep disturbance and limitation of speech due to severe wheeze in the past 12 months. Accordingly, the prevalence was 2.5% and 7.3% for sleep disturbance and limitation of speech, respectively. The prevalence rates from other countries are on the lower side (3). A possible explanation for such differences could be difference in the availability and accessibility of the health care system that are needed for the management of asthma in the different countries.

The prevalence of exercise-wheeze was found to be 27.3% in our study. This is similar to studies done in Capetown, South Africa (21.4%), Scotland, UK (31,4%). On the other hand, very low results are reported from Georgia (6.6%) and Jimma, Ethiopia (1.0%). It is also interesting to note that the 12-month prevalence of exercise-related wheeze was found to be higher than the 12-month wheeze. This may be a real finding or could possibly be a result of over-reporting by adolescents (3).

The very high prevalence of nocturnal cough found in our study (27.3%) was similar to reports from Nigeria (31.7%), and Hong Kong (27.7%) but different from reports from Jimma, Ethiopia (2.0%) and Greece (6.7%). This variable showed a higher prevalence than any other question suggesting that in addition to estimating asthma, it may be measuring other respiratory disease conditions (3).

From this survey the following conclusions may be drawn. First the prevalence of wheeze among school age children of Addis Ababa is fairly high. Second, the majority of the affected children do not seem to be aware of the problem and hence are bound to remain untreated.

In view of the fairly high presence of asthma-related symptoms, we recommend further studies to be carried out using more objective methods such as measuring bronchial hyper-responsiveness to verify whether they support results obtained by the questionnaire methods.

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REFERENCES

1. ISAAC Coordinating Committee-Manual for International Study of Asthma and

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Allergies in childhood (ISAAC). Bochum and Aukland, ISAAC Coordinating Committee, 1992.
2. Pearce NE, Weiland SK, Keil U, *et al*. Self reported prevalence of asthma symptom in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. *Eur Respir J* 1993;6:1455-61.
3. Writing group. World-wide variation in prevalence of symptoms of asthma, allergic rhino-conjunctivitis, and atopic eczema. *Lancet* 1998;351(9111):1225-32.
4. Asher MI, Keil U, Anderson HR, *et al*. International study of asthma and allergic in childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-91.
5. Robertson CF, Bishop J, Sennhauser FH, Mallo J. International comparisons of asthma prevalence in children. Australia, Switzerland, Chile. *Paediat Pulomonol* 1993;16:219-26.
6. Burney PGJ, Laitnen LA, Perdizet S, *et al*. Validity and repeatability of the IVTALD (1984) Bronchial symptoms questionnaire: an international comparison. *Eur Respir J* 1989;2:940-5.
7. Show RA, Woodman K, Ayson M, *et al*. Measuring the prevalence of bronchial hyper responsiveness in children. *Int J Epidemiol* 1995;24:597-602.
8. Asher MI, Patternore PK, Harrison AC, *et al*. International comparison of the prevalence of asthma symptoms and bronchial hyper-responsiveness. *Am Rev Res Dis* 1998;138:524-9.
9. Jenkins MA, Carlin Jr. Validation of questionnaire and bronchial hyper-responsiveness and hyper-responses against assessment in the diagnosis of asthma. *Int J Epidemiol* 1996;25:609-16.
10. Ehrlich RI, Du Toit D, Jordaan E, Volmink JA, Weinberg EG, Zwarenstein M. Prevalence and reliability of asthma symptoms in primary school children in Cape Town. *Int J Epidemiol* 1995;24(6):1138-45.
11. Erksson M. Management of bronchial asthma in Ethiopia children. *Ethiop Med J* 1982;20:39-45.
12. Lester FT. Bronchial asthma in Addis Ababa. *Ethiop Med J* 1992;30:225-32.
13. Siyoum B. Bronchial asthma in Jimma. *Ethiop Med J* 1992;30(4):225-32.
14. Yemanberhan H, Bekele Z, Venn A, Lewis S, Parrey E, Britton J. Prevalence of wheeze and relation to atopy in urban and rural Ethiopia. *Lancet* 1997;350:85-90.
15. Stewart AW, Asher MI, Clayton TO, *et al*. The effect of season-of-response to ISAAC questions about asthma, rhinitis, and eczema in children. *Int J Epidemiol* 1997;26:126-36.
16. Strachan DP. Epidemiology. In:Silverman M, ed. Childhood asthma and other wheezing disorders. London, Chapman and Hall, 1995;pp.7-31.

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CASE REPORT

PERIPHERAL NEUROPATHY FOLLOWING ADMINISTRATION OF NERVE TISSUE ANTIRABIES VACCINE

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ABSTRACT: *In 1997, two patients were admitted to Tikur Anbessa Hospital with complaints of ascending paralysis in all extremities following administration of sheep brain tissue anti-rabies vaccine following a rabies exposure. The paralysis had started after 14 daily subcutaneous injections of the Fermi type nerve tissue vaccine. After an eight week stay in the hospital with supportive care and physiotherapy, the patients showed remarkable improvement. They received a booster dose of vaccine while in the hospital, with no deterioration in their neurological status and were discharged.*

INTRODUCTION

Rabies is widespread in Ethiopia and the incidence of human rabies is one of the highest in the world (1). In Addis Ababa, 462 human rabies cases were reported in 1992-93 (1). Between 1964-1975 a total of 62,751 people received post-exposure Fermi type nerve tissue vaccine most of the exposures (98.9%) are due to dog bite (2).

Anti-rabies vaccine was introduced by Pasteur in 1894. This vaccine sometimes produces a variety of adverse effects including anaphylaxis, local skin reactions, localized and generalized lymphadenopathy, fever, malaise in addition to various form of neurological complications. Of the complications, neuroparalysis is said to occur once in every 600 persons vaccinated with Semple rabies vaccine (3,4,5). A five fold increase in the incidence rate of neuroparalytic complications is reported in those who received 14 injections when compared to those who received 7 or fewer injections (6). The risk of neurologic reactions following vaccination with duck embryo vaccine (DEV) is approximately one per 25,000 vaccinated individuals; a risk that is clearly lower than that of nerve tissue vaccine (7). In fact these complications are much less frequent in those vaccinated with human diploid cell vaccine (HDCV) (8). These safer vaccines (HDCV and DEV) are not readily available or are too expensive for use in third world countries (9). This report addresses the problem of neuroparalytic complications related to post-exposure nerve tissue vaccine administration in two cases.

CASE 1.

A 16 year old girl from Butajira (in the central part of Ethiopia) was a victim of an

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unprovoked bite by a rabid dog 34 days prior to hospital admission. During the attack she sustained several scratches over her body and a bite on her right leg. She received 15 doses of post exposure antirabies vaccine and one booster. The vaccine is given as 5ml subcutaneously doses for 14 days and a boosters on day 24, 34 and 44. She tolerated the vaccine very well except for minor local reactions.

Four days after she received her first booster dose she started to experience dysesthesia and mild pain in her right leg. Within a day she developed weakness in the same leg and then in the opposite. The weakness progressed rapidly and within two days she was unable to walk. Later she noted weakness of her upper extremities and voice changes. She had no diplopia, nasal regurgitation or difficulty of swallowing or sphincter dysfunction. She denied history of fever, chills, headache, joint pain or diarrhoea. She had history of upper respiratory tract infection one month before the onset of her recent complaints. Although she had been fully vaccinated as a child the only recently administered vaccine was that for rabies post exposure treatment.

Although physical examination of this girl revealed stable vital signs, she was unable to move herself in bed. She had multiple healed scratch marks on the legs and a fresh scar on her right below the knee. She was able to count up to 12 with a single breath. The vital capacity was not measured. The mental status and cranial nerve examination were normal. The muscle power both in the upper and lower extremities was 0/5 with hypotonia and areflexia. The plantar response was flexor bilaterally. The sensory examination was normal. She was admitted to the hospital as a case of Guillaine Barre's syndrome to rule out secondary to neuroparalytic complication of antirabies vaccine.

The laboratory results were: WBC 4500/mm^3, Hgb 12.5gm%, ESR 5mm/hr and a cerebrospinal fluid analysis showed a cell count of 10/mm^3 (all were lymphocytes), protein 15gm% and glucose 66mg%. The nerve conduction study showed moderate to severe, motor axonal degeneration types of polyneuropathy.

She received the last booster dose of vaccine while she was in the hospital, and did not show any deterioration in her motor function. During 4 weeks in the hospital she was treated with physiotherapy and showed an improvement in her muscle power, but the muscle stretch reflex remained absent. On discharge from the hospital muscle power became 2/5 and she was advised to continue the physiotherapy at home.

CASE 2.

A 41 year old man from Addis Ababa was bitten on his right leg by unprovoked dog one month prior to hospital presentation. He began post exposure vaccination for rabies two days after the brain of the dog was diagnosed as positive for rabies. He received 14 daily doses of Fermi vaccine 5ml each subcutaneously, which he tolerated very well. Three days after he received the 14th vaccine injection he developed numbness in his right leg followed by weakness of both legs. Within one day the weakness involved the upper extremities and, within few days he was unable to stand. He was admitted to Ras Desta Hospital in Addis Ababa where he stayed for two weeks and was subsequently referred to Tikur Anbessa Hospital for respiratory support. There was no history of fever, headache, sore throat, joint pain, diarrhoea, vomiting or symptoms of upper respiratory

tract infection. He did not have sphincter dysfunction. There is no history of other vaccination. He is married and a father of 4 children.

On physical examination the patient showed stable vital signs. He was able to count upto 18 with a single breath and his vital capacity was 3500 ml, or 50ml/kg. He was unable to raise himself from a supine position. He had a healed scar on the external aspect of the right knee joint. His higher function and cranial nerve examinations were normal. The muscle power in all extremities was 0/5. The muscle tone was hypotonic, the muscle stretch reflexes were absent and the plantar response was equivocal bilaterally. Sensory examination for pain, temperature, light touch, vibration and position sense were normal.

The investigation on admission revealed WBC of 4500/mm^3, Hgb 12.5gm%, ESR 100mm/hr. Cerebrospinal fluid was a clear and Pandy test was strongly positive. Nerve conduction study showed a moderate to severe motor, axonal degeneration type of polyneuropathy.

During his stay in the hospital for 2 weeks he was managed with physiotherapy. He received his last booster dose of the anti-rabies vaccine following which there was no further deterioration in his muscle strength. On discharge his muscle power of all extremities improved from grade 0 to 2 out of five and was able to sit up supported. He was advised to continue his rehabilitation at home.

DISCUSSION

Both of these patients presented with an absence of muscle stretch reflexes and a gradual onset of an ascending paralysis of all extremities, without affection to the sensation, sphincter or mental functions. These clinical features are suggestive of a disorder affecting the peripheral nerves, which was confirmed by the nerve conduction study, showing moderate to severe motor axonal degeneration type of neuropathy.

The neurological symptoms started after the patients received 14 doses of post-exposure sheep brain tissue anti-rabies vaccine. It was possible to hypothesize that the vaccine to be the cause of the advanced peripheral neuropathy, although it is difficult to establish a cause and effect relationship beyond the temporal association.

In many countries anti-rabies vaccine prepared from the nervous tissue of a variety of animals are used in human post exposure rabies prevention(2). Neurologic reactions to the neural antigen in these vaccines are not uncommon and make their use hazardous. Neuroparalytic complications occur in up to 1 in 600 vaccinees, with a fatality rate of 10%-25%(4,5).

Because myelin in adult neural tissue vaccine, contributes to the risk of neuroparalytic complications, a highly immunogenic antirabies vaccine has been produced in the relatively myelin free neural tissue of suckling mice. However, even this suckling mouse brain vaccine is not free of complications. It produces neuroparalytic complications in approximately 1:8000 persons vaccinated. Most of the neurological complications after administration of suckling mouse brain vaccine are of the Guillain-Barre syndrome type and there is a case fatality rate of 20% to 50% (10,11).

Attempts have been made to reduce the frequency of these serious neuroparalytic complications related to antirabies vaccines by producing vaccines in brain tissue or cell cultures. For example the DEV developed in the United States reduced the incidence of the neurological reactions to 1:32,000

persons. However, the immunological potency was low and requires multiple injections to induce protective immunity (1,7).

The anti-rabies vaccines prepared from adult animal neural tissue which continues to be used in many countries are associated with different neurological complications. The neurological complications have an incidence of approximately up to 1:1600 (4). To reduce the serious reactions associated with these vaccines and to increase vaccine immunogenecity, HDCV was developed in 1963 (12). The HDCV has resulted in a higher level of immunogenic potency and safety as compared with the previously prepared anti-rabies vaccines (13).

Both of the patients described in the report received the booster dose of the nervous tissue antirabies vaccine while in the hospital. There was no neurological deterioration after the booster. In fact, improvement continued and finally they were discharged from the hospital in a good state. Even though it is difficult to make a recommendation, it may be unsafe to avoid the booster dose with a fear of further deterioration of the neurological condition which can be handled promptly with respiratory support if in case they develop respiratory failure. Alternatively omitting the booster dose will put patients at a higher risk of developing rabies, because nerve tissue vaccine (NTV) has a weaker immunogenecity than the HDCV (4). The booster in NTV is important in development of immunogenic potency.

It is alarming to find two cases of serious complications to rabies post exposure vaccination within a short period of time. Rabies is fatal and commonly encountered viral disease in Ethiopia. Better vaccines with higher immunogeneicity and lower neurologic reactions are to be made available.

REFERENCES

1. Fekadu M, Taye E, Wondimagegnehu A, *et al.* Possible human to human transmission of rabies in Ethiopia. *Ethiop Med J* 1996;34:123-7.
2. Fekadu M. Rabies in Ethiopia. *Am J Epidemiol* 1982;115:266-72.
3. Emanuel A, Imorkis G, Jack N. Neurological complications following anti-rabies vaccination. *JAMA* 1953;151:188-91.
4. Gibbs FA, Gibbs EL, Carpenier PR, *et al.* Comparison of rabies vaccines grown on Duck embryo and nervous tissue. *N Engl J Med* 1961;265:1002-3.
5. Rubin RH, Gregg MB, Sikes RK. Rabies in citizins of the United States, 1963-1968:Epidemiology, treatement, adn compoications of treatment. *J Infect Dis* 1969;120:268-73.
6. Hemachudha T, Phanuphank P, Johnson RT, *et al.* Neurologic complication of semple type rabies vaccine: Clinical and Immunological Study. *Neurology* 1987;37:550-6.
7. Rubin RH, Hattwick MAW, Jones S, Gregg MD, Schwartz VD. Adverse reactions to Duck Embryo Rabies Vaccine: Range and incidence. *Ann Intern Med* 1973;78: 643-9.
8. Plotkin A. Rabies vaccine prepared in human cell cultures: Progress and Prospectives: Reviews of infectious diseases 1980;2:433-8.

9. Fuenzalida E, Palacios R, Borgono M. The use of rabies vaccine prepared in suckling mouse brain. In: proceedings of the 12th International symposium on rabies, Talloires, 1965, Badel and new York Karges, Vol 1, pp.339-345, 1966.
10. Held JR, Adaros HL. Neurological disease in man following adminstration of suckling mouse brain antirabies vaccine. *Bull WHO* 1972;46:321-7.
11. Toro E, Vergaro L, Roman G. Neuroparalytic accidents of antirabies vaccination with suckling mouse brain vaccine. *Arch Neurol* 1977;34:694-700.
12. Kenneta W Bernard, Philip W Smith, Fred J Kader, *et al*. Neuroparalytic illness and human Diploid Cell Rabies Vaccine. *JAMA* 1982;248:3136-8.
13. WHO/rabies research 1997;47:10.

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The objective of this study was therefore to make a preliminary assessment on outpatient [illegible]unselling in four referral hospitals in Addis Ababa and suggest pertinent [illegible]ommendations in order to upgrade this service in the hospitals. Outcomes of such survey would also serve as a baseline data to monitor any progress made towards the improvement of patient counselling in the hospitals.

METHODS

[illegible] cross-sectional institution based assessment of patient counselling in outpatient pharmacies was conducted from the beginning of April to mid May 1998 in four referral [illegible]spitals in Addis Ababa, namely, Tikur Anbessa Hospital (H1), Yekatit 12 Hospital [illegible]2), Menilik II Hospital (H3), and Zewditu Hospital (H4).

Structured questionnaires of two types, containing both close and open-ended questions [illegible]re designed for conducting the interview with patients. The survey mainly concentrated on patients who had prescriptions containing psycotropic and cardiovascular agents, broad-spectrum antibiotics and ENT preparations.

[illegible] know the approximate number of patients who got their prescriptions filled with and without any form of counselling, observation were also made for 5 hrs every day for six [illegible]eks. The total number of patients who got no written or verbal information (including [illegible]g labels) was recorded. In the conduct of the survey, communication techniques reported elsewhere were adopted and employed (8).

SUBJECTS

[illegible]total of 200 adult patients, 81 females and 119 males were included in the study. 50 [illegible]tpatients who definitely had to be given counselling services were randomly chosen from each of the hospitals.

RESULTS AND DISCUSSION

[illegible]ble 1 shows the socio-demographic characteristics of patients surveyed. The [illegible]ucational background of the patients is an important factor in comprehending [illegible]onversation with the pharmacist and counselling instructions. Illiteracy has been [illegible]ntified as one of the barriers in effective communication (6). As shown in the table, a [illegible]nificant percentage of patients (22.5%) are illiterate. Thus, it is vital that patients in his group get verbal instructions to make sure that they do not forget basic information. [illegible]e elderly is the other group that requires special counselling. In this study, although [illegible]out 6.5% of the patients were aged between 65 and 80 years, none of them received sufficient counselling time.

A case study has shown that many elderly hospital outpatients do not get the best from their medications because they do not understand them and as a result, about 70% of non-compliance rate has been recorded (6). It has been suggested that elderly patients require long consultation period of up to 15 minutes to comprehend instructions on their medications (9).

Table 1: Socio-demographic characteristics of patients interviewed, May 1998 (n = 50 in each of the hospitals).

	Percent encountered				Cumulative
Socio-demographic characteristics	H1	H2	H3	H4	(%)
Sex					
Male	62	60	52	36	52.5
Female	38	40	48	64	47.5
Age group					
18-25	24	22	26	28	25.0
26-44	52	52	50	48	50.5
45-64	16	18	16	22	18.0
65-80	8	8	8	2	6.5
Level of education					
Illiterate	26	24	20	20	22.5
Elementary school (grade 1-6)	6	6	4	12	7.0
Junior high school (grade 7-8)	6	6	8	12	8.0
High school (grade 9-12)	10	46	48	44	37.0
University diploma and +	52	18	20	12	25.5

Table 2 shows dispensing time, average number of prescriptions filled per hr by a pharmacist and percentage of patients who received medications without any counselling in the four hospitals. As shown in the table, a pharmacist fills prescriptions ranging from 50 (H4) to 83 (H1) per hr. The large volume of work that each pharmacist performs in the hospitals is manifested in the time that the pharmacist spends with each patient. It is apparent from the table that the time each patient spends with the pharmacist is too short (for example, less than a minute in 90% of the cases in H1) to comprehend the information, if any, provided by the pharmacist or solicited by the patient. Thus, it would not be surprising, if some of the patients forget the verbal information they were given regarding their medication.

Due to this large volume of work, providing appropriate counselling service is difficult and hence, a significant percentage of the patients (ranging from 50% in H4 to 78.3% in H1) did not receive counselling. It was observed that, in many cases, the patients do not ask for information, they simply listen to the person standing behind the counter.

Studies have shown that patients who took an active part in discussion with pharmacists were likely to receive more information than passive patients. Information given to passive and active patients also vary. In one study conducted in UK, it was found out that on average, passive patients received significantly less information than active patients, 8.5 and 15.9 items respectively, and recalled less, 2.7 and 4.4 items, respectively (10).

The results of this survey revealed that either the patients do not know whom to ask about their medication or that they had a problem in identifying the pharmacist. Of the 200 patients interviewed, 83.5% of them did not try to seek counselling from the pharmacist. The majority (60%) thought that the only duty of the pharmacist was to dispense drugs. Moreover, 61.5% of the patients indicated that the advice they would like to solicit from the pharmacist/dispenser regarding their medications was only on the frequency of administration.

Table 2: Dispensing time, average number of prescriptions filled per hr by the pharmacist and percentage of patients who received medications without counselling in the four hospitals, May 1998 (n = 50 in each of the hospitals).

Description	Percent encounters				
Dispensing time (min)	H1	H2	H3	H4	% Cumulative
< 1	90	68	72	54	71.0
1-2	8	20	22	34	21.0
3-4	2	12	6	12	8.0
Average number of prescriptions filled per hr	83	67	63	50	65.75
Patients who received no counselling	78.3	55.2	60.3	50	60.95

The fundamental requirement in effective communication is that the information received is the same as that given, and is understood by the receiver the way the sender intended it to be. Thus, communication consists of a sender, a message and a receiver. When applied to communication between pharmacist and patient, the sender and receiver are individuals while the message may be conveyed orally, or in writing as a label, or leaflet. Table 3 records the awareness of the patients regarding some vital information about their medication. The most frequent types of drug information desired by the patients as well as those offered by the pharmacist were frequency of administration (ranging from 74% in H3 to 94% in H4) and route of administration (ranging from 32% in H1 to 80% in H2 and H4). However, this does not necessarily mean that the patients got sufficient or clear instructions from the pharmacist. For example, while patients were instructed to take their medications three times a day, their most common schedule was to take them in the morning, at noon and in the evening. Thus, a dose that is meant to be taken over a period of 24 hrs is used up in about 12 hrs or less. Moreover, about 8% of the prescriptions were filled with frequency of administrations that were vague. For instance "2 x 3" may mean two tablets per day in 8 hrs interval or 3 tablets in 12 hrs interval. Hence, dosage regimen is also assumed to be not practical for the patients. Percentages of the prescriptions that provided information on contraindications (average of 9.5%) and adverse drug reactions (average of 7%) were low. Previous allergic history of the patients, if any, was not asked. Drug-drug interactions are assumed to be the concern of the physician even if the pharmacist has the obligation to ask the patient prior to dispensing.

In order to assess the awareness of the patients regarding the importance of counselling, they were asked "From whom do you seek advice regarding your medication?" The results (Table 4) indicate that the majority of the patients do not know that the pharmacist

is the most qualified health care professional who is capable of giving appropriate advice on drugs. As shown in the table, the majority (67.5%) of the patients indicated that they prefer physicians for drug consultations. Asked why they prefer the physicians, the majority (60%) stated that the physicians were the ones who diagnosed their diseases and prescribed the medications. Surprisingly, pharmacists were preferred by only 9.5% of the patients.

Table 3: Percent response by the patients to the question: "Have you been counselled/informed on your medication?", May 1998 (n = 50 in each of the hospitals).

	Percent "Yes"				Cumulative
Type of consultation	H1	H2	H3	H4	(%)
Name of the drug	2	2	20	18	10.5
Dose of the drug	12	34	46	44	34.0
Type of dosage form	26	34	28	38	31.5
Route of administration	32	80	46	80	59.5
Frequency of administration	86	82	74	94	84.0
Storage conditions	4	24	12	18	14.5
Drug-drug interactions	2	2	6	12	5.5
Adverse effect of the drug	2	4	8	14	7.0
Contraindications	2	4	18	14	9.5
Written information	14	4	4	10	8.0

Table 4: Preference of patients for health personnel with matters relating to drug advice, May 1998 (n = 50 in each of the hospitals).

	Percent response				Cumulative	
Preference	H1	H2	H3	H4	Total No	%
Physicians only	74	72	58	66	135	67.5
Pharmacists only	4	4	16	14	19	9.5
Both	22	24	26	20	46	23.0

In the absence of a formal counselling system and a mutual understanding between pharmacists and other professionals, a lot of patients get their prescription filled with little or no guidance from any of the health professionals. As a result, significant percentage (29%) of patients may use their medications improperly. 16% of the patients indicated they discontinue their medications once the symptoms of their diseases subside, while the remaining (13%) reported that they keep their medications for future use (Table 5). This improper use of drugs has its own negative consequences in drugs such as antibiotics. In such situations, the expected therapeutic results will not be obtained and the risk of developing resistance would be high. The majority of the patients (average of 71%, i.e., ranging from 58% in H3 to 82% in H2) confirmed that they would take the full course of their medications, because most of them had chronic diseases.

Elsewhere, it has been shown that a) patient satisfaction and compliance are related; b) there is often failure among patients to remember or understand instructions; c) patient understanding and recall can be improved through better instructional techniques (in that patients remember best the first instruction given to them); d) emphasized instructions are

better recalled; e) repetition of instructions enhances recall; f) the fewer the instructions given, the greater proportion of information remembered, and g) simplification of information enhances recall (11).

Table 5: Comparison of percentage of patients who indicated to take full course of medication and those that discontinue when symptoms subside, May 1998 (n = 50 in each of the hospitals).

	Percent response				Cumulative
Pattern of drug utilisation by patients	H1	H2	H3	H4	%
Complete full course of medication	76	82	58	68	71
Discontinue medication when symptoms subside and keep it for future use	14	8	24	18	16
Discontinue medication when symptoms subside and discard the remaining	10	10	18	14	13

CONCLUSIONS

In the hospitals surveyed, pharmacists mainly perform routine dispensing duties with inadequate patient counselling and this may be partly attributed to the large number of prescriptions they fill. Under such circumstances, it appears that pharmacists consider patient counselling optional and they only provide it when asked. As a result, the majority of the patients did not receive appropriate counselling on their medications and a large number of them may use their medications improperly.

RECOMMENDATIONS

The association of communication, counselling, and compliance represents factors which may significantly contribute to enhancing patient care and to the fulfilment of the pharmacist's professional role. It is through effective communication and counselling by the pharmacist that they can make an invaluable contribution to the correct and safe use of dispensed medicines.

Therefore, in order to reverse the existing situation, appropriate measures have to be taken which include among other things: a) the pharmacist should always wear indicative badge for identification purposes; b) awareness should be created in the society to recognize the pharmacist as the expert on drugs; c) drug labels should be written clearly to be easily understood by the ordinary patient; d) the communication skills of the pharmacist should be upgraded, and e) patient counselling should be given the most serious and urgent attention.

Only such a co-ordinated effort will bring about better results, both in terms of exploiting the full potential of the pharmacist and improving the quality of service rendered to the general public. The most important attributes are the willingness to talk as well as to listen.

REFERENCES

1. Harman, R. J., (Ed.), Communication, Counselling and Compliance in Handbook of Pharmacy Health-Care, The Pharmaceutical Press, London, 1990, pp 411-424.
2. Nicholas, G. P. Ambulatory Patient Care, in Remington's pharmaceutical sciences, 18th ed. MACK Publ., 1990, pp 1715-1730.
3. Balon, A. J. D. (1986), Pharm. J., 237: 452.
4. Karig, A. W. and Hartshorn, E. A., Counselling Patients on their Medications, Drug Intelligence Publications, INC., Hamilton, Illinois, USA, 1991, p xiii.
5. Gerald, G. B. and William, E. S. (1974), Amer. J. Hosp. Pharm, 31: 247-253.
6. Stone, P. and Curtis, S. J. (1995), Dispensing Prescriptions, in Pharmacy Practice, 2nd ed., Farrand Press, London, pp 61-88.
7. Ketema, H. and Yirdaw, M. (1992), Eth. Pharm. J., 10:61-71.
8. Mariame, I., Yvonne, T. and Keith, S. (1975), Amer J. Hosp. Pharm., 32: 828-831.
9. Harman, R. J., (Ed), Care for the Elderly, in Hand Book of Pharmacy Health-Care, The Pharmaceutical Press, London, 1990, pp 405-410.
10. Blenkinsoop, A., Robinson, E. and Panton, R. Do pharmacy customers remember the information given to them by the community pharmacist? in Social Pharmacy, Innovation and Development, Harding, G., Nettleton, S. and Taylor, K. (Eds), The Pharmaceutical Press, London, 1994, pp 84-94.
11. Hargie, O. and Morrow, N., Pharmacist-Patient Communication: The Interpersonal Dimensions of Practice in Social Pharmacy, Innovation and Development, Harding, G., Nettleton, S. and Taylor, K. (Eds), The Pharmaceutical Press, London, 1994, pp 95-111.

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RHEUMATIC HEART DISEASE AMONG SCHOOL CHILDREN IN ADDIS ABABA CITY: AWARENESS AND ADEQUACY OF ITS PROPHYLAXIS

Kebede Oli, MD[1], Jenny Porteous, PhD[2].

ABSTRACT: *One of the objectives of this large scale cross-sectional study of school children of the Addis Ababa city was to assess the status of rheumatic heart disease (RHD) prophylaxis among rheumatic heart disease patients identified during the survey. Awareness about the presence of the illness in those affected and reasons for poor coverage, when detected, were also assessed. Sixty of the 9388 school children surveyed were found to have rheumatic heart disease. On interviewing parents of the children with rheumatic heart disease, ten acknowledged being informed of their children's cardiac illness. Of these parents, 15% (or 9/60) had some idea that their children had heart disease related to some form of infection. However, only two of the nine (22%) children whose parents had some idea about their disease were on regular monthly benzathine penicillin prophylaxis in the previous 12 months preceding the interview. Three (33%) of the nine children had six or fewer injections in the 12 months preceding the interview. The remaining 4 parents (44%) reported that their children took treatment that included injections only at the time of initial diagnosis several years earlier and had not had any follow up since then. Their reasons for not seeking medical care for their children included lack of information on prophylaxis, inability to pay for the treatment and distance of the health facilities. The lack of awareness and the extremely low rate of regular prophylaxis, therefore, highlight the need for an urgent control programme that takes active case detection, treatment access and health education into consideration.*

INTRODUCTION

In Ethiopia rheumatic heart disease (RHD) is a serious problem in clinical practice and accounts for nearly 50% of cardiac admissions in teaching hospitals (1,2). RHD is known to have a more aggressive course in developing countries (3). The aggressive nature of the disease in developing countries has been ascribed to many factors. There

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is a strong evidence for RHD recurrences resulting from repeated untreated infections (lack of secondary prophylaxis) playing an important role in its rapid evolution (4,5).

A prior community-based Ethiopian survey found very low levels of prophylaxis for RHD (6). Moreover, in spite of the existence of evidence supporting (7,8,9) the efficacy of secondary prophylaxis, no National or Regional control programme for RHD has ever been initiated in Ethiopia. Lack of awareness about RHD and its prophylaxis among parents (or their children) may be some of the reasons for the low levels of prophylaxis.

Therefore the main objectives of this study were to: 1) determine the level of awareness of parents and/or their children of the presence of a cardiac lesion among those detected to have lesion during the survey, 2) determine the proportion of known cases on no or inadequate prophylaxis, and 3) identify reasons for inadequate prophylaxis in those who were aware of the presence of the disease and based upon this study's findings, recommendations will be made to improve rates of RHD prophylaxis.

POPULATION AND METHODS

A school-based screening of grade 7 and 8 children for the presence or absence of RHD using the auscultatory method was carried out in a randomly selected government and private schools in Addis Ababa. Grades 7 and 8 were selected because these children were expected to have the highest prevalence of RHD (3). The study protocol was approved by the Research and Ethical Committee of the Faculty of Medicine (Addis Ababa University).

The lists of all government and private schools within Addis Ababa were obtained from the [illegible]cation Bureau for the Addis Ababa Region. The register contained information on sex, grades, class size and status of the school (i.e. government or private). A list of all government and private schools in Addis Ababa City teaching grades 7 and 8 classes comprised the study's sampling frame. These included 47 government and 20 private schools. Children in government schools accounted for about 86% of the study population. After stratifying the schools into government and private, probability sampling proportional to population size was done to select 10 schools from the government listing and 5 schools from the list of private schools to yield a sample of about 9,000 children from government and 1,000 children from private schools. All students in grades 7 and 8 in the selected schools were included in the study. The sample size was determined by the funds made available to the study.

A day or two before the physical examinations a research assistant went into each classroom in the selected schools to interview children and collect information on their age, sex and address. The children were also asked if they had any knowledge of having any cardiac illness in themselves. The information collected was re-checked for its completeness on the same day by the research assistant himself. A second more detailed questionnaire for parents of affected children was also completed to assess their awareness of the presence of heart disease in those children subsequently confirmed to have RHD. The questionnaire items also assessed adequacy of secondary prophylaxis and reasons for adequate compliance, when present. The parent interview questionnaire is appended. The second interview was carried out by a trained nurse (M.B.) working in the Cardiology Unit of the Department of Internal Medicine (Addis Ababa University). A diagnosis of RHD

was based on an initial clinical evaluation followed by echocardiographic and Doppler studies. The initial clinical evaluation consisted of two stage evaluations. The first stage, auscultation for the presence of significant heart murmurs (10), was carried out by a trained nurse. The second stage of the evaluation involved the re-examination of children identified as having significant heart murmurs by the nurses and/or those who gave history of heart disease, for the presence of murmurs suggestive of RHD and other serious cardiac lesions. This was done by an experienced cardiologist. The cardiologist referred those children with murmurs suggestive of RHD and other serious cardiac lesions to Tikur Anbassa Teaching Hospital for further confirmatory tests.

RESULTS

Out of a total of 32,398 eligible school children in grades 7 and 8 attending all 47 government schools, 8,896 (27.5%) were included in the study. Similarly 21.4% (1157 out of 5396) of all eligible grade 7 and 8 school children from all 20 private schools were elected. The response rates for the government and the private schools were 93.4% (8365/8896) and 88.4% (1023/1157) respectively. The mean age of the children was 14.0 $\pm$ 1.3 years and 52% of them were girls.

Awareness about the presence of rheumatic heart disease: Three hundred nineteen children (3.4%) of the total 9388 reported that they had heart disease during the first interview. A significantly greater number of children in government schools reported the presence of a heart disease (3.8% versus 0.3%; Fisher's exact $p<0.001$). These children were examined by both a nurse and a cardiologist. Only six of these children were subsequently confirmed to have rheumatic heart disease with two of them in congestive heart failure and receiving treatment. These were traced to one of the cardiology clinics in the city. Each initiated prophylaxis after developing symptoms of heart failure. An additional fifty four confirmed cases were detected among those who did not report the presence of heart disease. Based upon the parent interviews of the 60 children who were confirmed to have valvular heart disease ten acknowledged they had been informed of their children's cardiac illness. Nine of the 60 parents had some idea that their child had heart disease due to some form of infection. Two of these nine (22%) children were on regular monthly benzathine penicillin prophylaxis in the previous 12 months preceding the interview. Three (33%) of the nine children had six or fewer injections in the 12 months preceding the interview. The remaining 4 parents (44%) reported that their children took treatment that included injections only at the time of initial diagnosis several years earlier and had not had any follow up since then.

Adequacy of prophylaxis and reasons for poor coverage: Seven out of nine (78%) parents who were aware that their children had a heart problem did not take their children for regular prophylaxis. The reasons given by these parents for not taking their children for regular prophylaxis included: 1) lack of awareness about the significance of prophylaxis (4 parents), 3)difficulty with paying for treatment (2 parents), and 3) difficulty with attending the follow up clinics due to distance (1).

Table 1. A list of government and private schools in Addis Ababa randomly selected for rheumatic heart disease screening programme, 1995.

Name of the school	No. of grade 7 & 8 students
Government	
1. Kelemework	1,583
2. Berhane Zare	662
3. Medhanialem	1,233
4. Mekanisa	169
5. Umer semeter	1,054
6. Kebena	333
7. Entoto Amba	1,150
8. Tsehay chora	668
9. Tinsae Berhan	457
10. Arebegnoch	1,587
Government total	8,896
Private	
1. Indian Community	156
2. Indian National	192
3. Lideta Boy's	496
4. Yemen Community	167
5. Lutheran	146
Private Total	1,157
Grand Total	10,053

DISCUSSION

Not surprisingly only 15% (9/60) of the parents of children with RHD had some awareness about the disease status of their children, and only two of these nine (22%) sick children were on regular prophylaxis. Not many studies have looked into this

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aspect of the management of rheumatic heart disease. One of the WHO collaborative reports comments that the proportion of cases giving a history of prophylaxis before initiation of their intervention programme was about 25% (7).

There could be several factors responsible for such a state of affairs. Possible explanations include lack of access to medical care, unavailability of the services, inability to pay for treatment and inadequate knowledge about the significance of rheumatic heart disease prophylaxis. The majority of the parents available for interview in this survey clearly stated that they had no knowledge about the significance of the prophylaxis. Other reasons given were inability to pay for treatment and/or unavailability of health facilities in the nearby areas. This is also compatible with the complete absence of any rheumatic heart disease control programme in the country.

Studies suggest that rheumatic heart disease may take a more aggressive course in developing countries with cardiac disability, indications for surgery and death occurring at a younger age (3,11,12). The aggressive nature of the disease in developing countries has been ascribed to many factors. The virulence of streptococcus group A (13,14), the frequency and severity of carditis during the initial attack of rheumatic fever, and rheumatic recurrences have all been studied in some detail (15-19). It has been suggested that the more virulent 'rheumatogenic strains' of streptococci may be more common in the developing world (14,15), but further confirmation is required. Moreover, prospective studies of acute rheumatic fever from developing countries have not shown a higher frequency of carditis at the onset of rheumatic fever (15-17).

There is also strong evidence that rheumatic recurrences resulting from untreated repeated streptococcal infections play an important role in the rapid evolution of rheumatic heart disease (4,5). A higher cure rate for acute rheumatic carditis has been shown to occur when recurrences were prevented (4,5,19), whereas there was more death and progression to rheumatic heart disease and a greater requirement for value replacement among patients who suffered frequent recurrences (4,20).

In Ethiopia rheumatic fever and rheumatic heart disease are serious problems in clinical practice with early morbidity (1,2). In spite of prophylaxis being a feasible and cost-effective method of intervention that can both improve the cure rate of rheumatic carditis and prolong survival (7,8), no community control program currently exists in the country. Although figures are not available to us there are children from the low socioeconomic group that could not be reached through this screening since school attendance is not compulsory in Ethiopia. It is possible that rates for RHD and its prophylaxis in this less privileged category of children are worse. This study looked at school children only because it was felt that this is a group more accessible for initial intervention programmes.

As has been shown most of the RHD children in this study were unaware of their illness. The very few who had some knowledge about their illnesses were not on adequate prophylaxis. Other came to the medical attention and started prophylaxis after the disease and symptoms had advanced to the stage when treatment is least effective. We, therefore, feel that there is a need for an urgent control programme that takes active case identification, health education, and cost of treatment into account.

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REFERENCES

1. Abraham G. Pattern of cardiovascular disease among adult hospitalised Ethiopians. *Ethiop Med J* 1982;20:63-8.
2. Muhe L, Daniel E, Abegaz B. Pattern of cardiac admissions to the Ethio-Swedish Children's Hospital (1981-1985;abstract). *Ethiop Med J* 1986;24:198.
3. McLaren MJ, Hawkins DM, Koornof HJ, *et al.* Epidemiology of rheumatic heart disease in Black Children of Sweto, Johnannesburg. *Br Med J* 1975;3:474-7.
4. Majeed HA, Yousof AM, Khuffash F, *et al.* The natural history of acute rheumatic fever in Kuwait: a prospective six year follow up report. *J Chronic Dis* 1986;39:361-9.
5. Rheumatic fever working party. Joint report. *Circulation* 1965;32:457-76.
6. Oli K, T.Haimanto R, Forsgren L, Ekstedt J. Rheumatic heart disease prevalence among schoolchildren of an Ethiopian rural town. *Cardiology* 1992;80:152-5.
7. Strasser T, Dondog N, Kholy A. El, *et al.* The community control of rheumatic fever and rheumatic heart: report of a WHO international cooperative project. *Bull WHO* 1981;59:285-94.
8. WHO technical report series. no.764, 1988 (Rheumatic fever and rheumatic heart disease: report of a WHO study group).
9. The community control of rheumatic fever and rheumatic heart disease: report of a WHO international cooperative project. *Bull WHO* 1992;70:213-8.
10. Levine S, Harvey SP. Clinical Auscultation of the heart. Philadelphia, Saunders, 1959, P41.
11. Roy SB, Bhatia ML, Lazaro EJ *et al.* Juvenile mitral stenosis in India. *Lancet* 1963;2:1193-5.
12. Markowitz M. Observations on epidemiology and preventability of rheumatic fever in developing countries. *Clin Ther* 1981;4:240-51.
13. Bisno AL. Group A streptococcal infections and acute rheumatic fever. *N Enlg J Med* 1991;325:783-93.
14. Zabriskie JB. Rheumatic fever: the interplay between host genetics, microbe. *Circulation* 1985;71:1077-86.
15. Majeed HA, Khan N, Dabbag M, *et al.* Acute rheumatic fever during childhood in Kuwait. The nature of initial attacks. *Ann Trop Paediatr* 1981;1:13-20.
16. Veasy LG, Weidmeir SE, Orsmond GS, *et al.* Resurgence of actue rheumatic fever in the intermountain area of the United States. *N Engl J Med* 1987;316:421-7.
17. Sanyal SK, Thapar MK, Ahmed SH, *et al.* The initial attack of acute rheumatic fever in North India: a prospective study of the clinical profile. Circulation [illegible] 9:7-12.

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18. Tompkins DG, Boxerbaum B, Linman J. Long-term prognosis of rheumatic fever patients receiving regular I.M. benzathine penicillin. *Circulation* 1974;45:543-51.
19. Sanyal SK, Berry AM, Duggal S, *et al*. Sequelae of the initial attack of acute rheumatic fever in children from North India: a prospective 5-year follow-up study. *Circulation* 1982;65(2):375-9.
20. Markowitz M. The decline of rheumatic fever: role of medical intervention. *J Paediatr* 1985;106:545-50.

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COMMUNICATIONS

A PRELIMINARY ASSESSMENT OF OUTPATIENT COUNSELLING IN FOUR REFERRAL HOSPITALS OF ADDIS ABABA

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ABSTRACT: *A cross-sectional institution based preliminary assessment of patient counselling in outpatient pharmacies was conducted from April to mid May 1998 in four referral hospitals in Addis Ababa. A total of 200 patients were interviewed using a pre-prepared questionnaire. The results of the survey indicate that only 39% of the patients received counselling on their medications. According to the responses of 71% of the patients and observations made, the dispensing time was less than a minute and this was too short to adequately interact with the pharmacist. In these hospitals, it was found out that the pharmacist fills up to 83 prescriptions per hr and due to this large volume of work, the majority of the patients (61%) did not receive appropriate counselling. 84% and 59.5% of the patients got information, verbally and/or in written form, on frequency and route of administration respectively. 8% of the prescriptions were filled with vague labels on frequency of administrations. Low percentage of the prescriptions provided information on contraindications (9.5%) and adverse drug reactions (7%). 83.5% of the patients did not try to seek advice on their medications and the majority of them (60%) thought that the only duty of the pharmacist was to dispense drugs. As a result, patients prefer physicians (61.5%) to pharmacists (9.5%) on matters relating to drug advice. Due to inadequate counselling, 29% of the patients may use their medications improperly. 16% of the patients indicated that they discontinue their medications once the symptoms of their diseases subsided, while the remaining 13% keep their medications for future use.*

Key words: Counselling, outpatient, prescription, dispensing time, drug labelling

INTRODUCTION

Whether prescription or non-prescription drugs are dispensed, counselling patients on their medications is one of the principal responsibilities of the pharmacist. The term counselling has been increasingly used to describe the sympathetic interaction between the pharmacist and the patient, which may go beyond conveyance of straightforward information about the medicine and how, when and for how long to use it (1).

Counselling may be defined as a service consisting of giving advice on problems and broadly as part of an interaction which can usefully occur between the pharmacist and the patient (2). It includes the potential for contributing to the most suitable choice of

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medication and resolving problems of administration or use of medication. As such, counselling is a form of exchanging ideas and opinions between dispenser and patient rather than a unilateral provision of information by the dispenser. Whether asked by the patient or not, the pharmacist must make sure that the patient obtains sufficient information on the medication dispensed including which foods, drinks and other medicines should be avoided; side effects if any, and what to do if they occur, etc. (2,3). General counselling phrases (information) that should be provided by the pharmacist include: *Use the drug as directed; Do not exceed or reduce the prescribed dosage; Do not use the drug for other conditions except as directed by your physician; Read the label and examine each dose of medication each time you use a dose; Never take a dose of medication in the dark or in poor lighting; Do not take other medications (prescription or non-prescription) unless you have first discussed them with your physician or pharmacist; Do not share your medication with other people; Keep your medication in a cool place/refrigerator; Keep all medication out of the reach of children, and Discard/do not keep unused/outdated medication that you no longer need* (4).

Proper patient counselling therefore ensures that the patient will use his/her medication safely and appropriately. As a health-care provider, the pharmacist is obliged morally, ethically and legally to provide patients with adequate and clear information on the drugs to maximize the therapeutic outcome and prevent conceivable problems during therapy (5). However, in many cases, the information that the pharmacist conceivably could share with the patient may be awesome (2).

In the absence of counselling or compliance drug preparations may be misused in various ways such as administering overdosage (dose more than prescribed at any one of administration, or taking the same medication from two or more different bottles simultaneously); underdosage (dose less than prescribed at any one administration, omitting one or more doses, or discontinuing the drug before the prescribed time); a dose at a different time than specified in the directions or a dose in a form other than that specified in the directions; medication using the wrong route of administration; someone else's medications, and two or more medications which are contraindicated therapeutically.

The effect of pharmacist counselling on patients attending a clinic with hypertension, previously considered resistant to treatment was reported. They found out that factors contributing to poor control were non-compliance and drug side effects. Counselling and suggesting modifications to treatment regimens achieved better control of blood pressure in a significant number of cases (6).

Even though patient counselling is one of the basic services expected to be rendered by the pharmacist, it seems that it has received little attention in Ethiopia. It is presumed that shortage of qualified pharmaceutical personnel, lack of preparedness of the practising pharmacist, lack of professional management and community perception have significantly contributed to the existence of several problems in the clinical practice of pharmacy in general and patient counselling in particular (7).

Review

Acute Bacterial Meningitis

Daniel Fekade, MD*

Synopsis

Baterial meningitis is a serious infection of the membranes covering the brain and spinal cord which may occur in epedemics. The three most common causative agents of bacterial meningitis are N. meningitidis, S. pneumonia and H. influenzae type b. The bacteria are spread by direct close contact with discharges from the nose or throat of an infected person. Meningitis has to be excluded by examination of the cerebrospinal fluid (CSF) in a febrile patient with altered mental state. Prompt empirical treatment should be started as soon as the diagnosis is made since any delay in the initiation of antimicrobial therapy could be fatal. The most frequent long term complications are hearing loss, learning difficulties, and seizures. Prevention depends on use of vaccines, rapid diagnosis, and prompt treatment of personal contacts. The current knowledge on the epidemiology, investigation, management and prevention of bacterial meningitis is reviewed. [J Ethiop Med

Introduction

The causes of bacterial meningitis are different in different age groups, Table 1. However, the three commonest causes of community-acquired bacterial meningitis in both adults and children are: Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae type b.

Other causes of bacterial meningitis occur infrequently (< 5% of cases) in both children and adults and include E. coli, Listeria monocytogenes and group B streptococci in the new born period, and S. aureus and Gram negative bacilli, following head injury and neurosurgical procedures.

Meningitis caused by all three common meningeal pathogens occurs endemically throughout the world, but the majority of cases occur in the tropics. Since the introduction of H. influenzae conjugate vaccines the incidence of heamophilus meningitis has decreased dramatically in countries where the vaccine had been used extensively.

Meningococcal meningitis causes widespread epidemics in countries that lie within the "African Meningitis Belt". Ethiopia forms the eastern boundary of the Meningitis Belt, (Figure 1). Epidemics occur during the hot dry season, between January to the end of June. They occur in cycles of five to ten years, and each epidemic may last one to three years. Serogroups A & C are responsible for the majority of the epidemics in Africa. [1]

Several large epidemics had occurred in Ethiopia, the latest one during 1989/90, affecting over 40,000 people [2,3] (Figure 2).

Pathogenesis and Clinical presentation

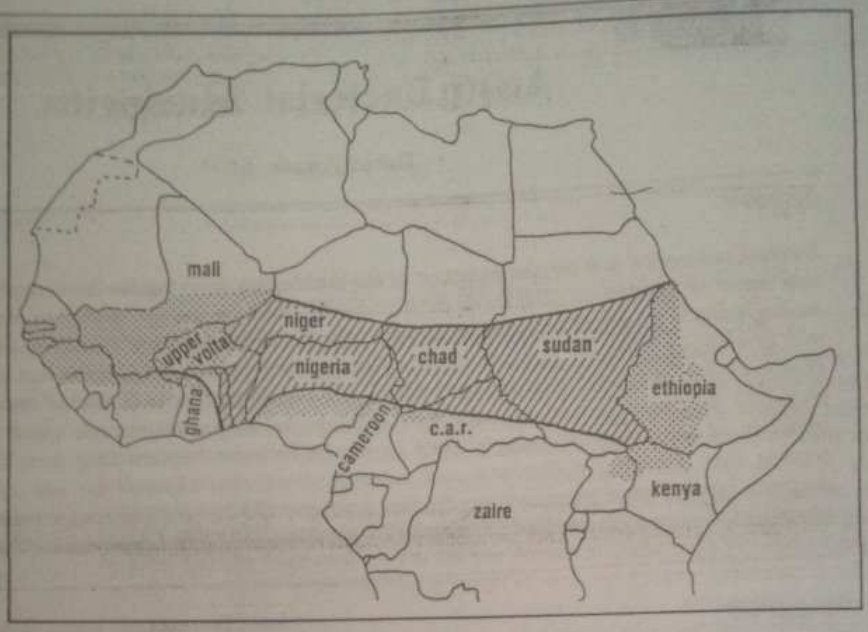
The bacteria are spread by direct close contact with discharges from the nose or throat of an infected person. Bacteria that cause meningitis first "colonize" the nasopharynx. They have pili/fimbriae to attach to the muscosal epithelial cells. Some organisms have IGA protease that cleaves secretory IGA in the

Table 1: Causes of bacterial meningitis by age groups

Bacteria	% of cases
N. meningitidis	10-30% in adults 30-40% in children up to 15 years Very rare in infants
S. Pneumoniae	#0-50% in adults 10-20% in children Up to 5% in infants
H. influenczae	1-3% in adults 35-45% in children Rare in infants

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Figure 1.
The African
Meningitis Belt



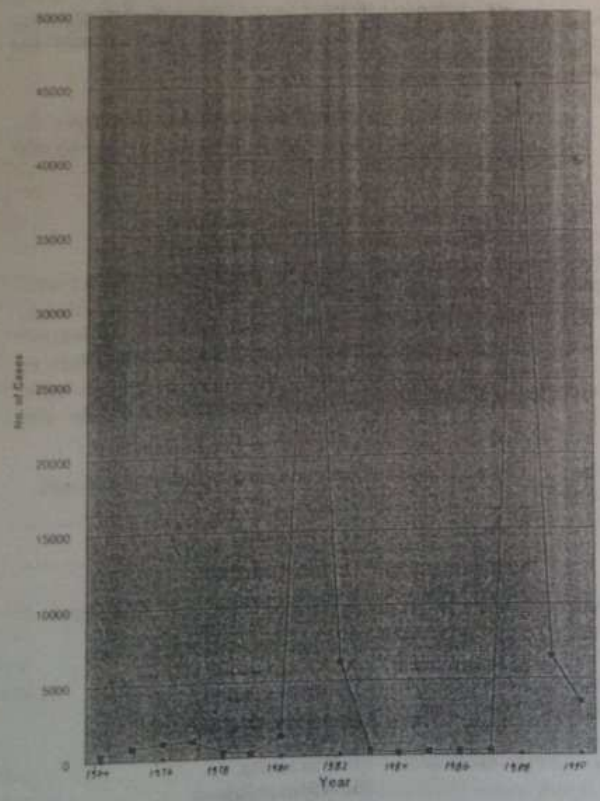


Figure 2: Meningococcal Meningitis in Ethiopia (1974-1990)

nasopharynx. The common meningeal pathogens also posses a thick capsular polysaccharide that resists complement mediated killing. [4]

Bacteria then invade the blood stream, traverse the blood brain barrier and seed the choroid plexus, where they multiply. There is increased permeability of the blood brain barrier and loss of cerebral autoregulation and an exudative inflammatory response in the cerebrospinal fluid, CSF.

Cytokines, in particular, tumor necrosis factor (TNF), interleukins-1 and 6 (IL-1 and IL-6) are responsible for some of the pathophysiologic changes associated with acute bacterial meningitis. In one study circulating levels of TNF correleated with outcome of patients with meningococcal meningitis. [5]

Most of the clinically observed symptoms and signs are the result of infection in an anatomically confined space, i.e. the brain and the spinal cord.

Fever, headache, menigisums and signs of cerebral dysfunction (confusion, declining consciousness) are found in 85% or so of

patients. The menigismus may be subtle, or obvious and accompained by Kernig's and Brudzinski' signs.

Other clinical findings include cranial nerve palsies (affecting III, IV, and VI), focal cerebral signs, seizures and vomiting. Papilloedema is an unusual manifestation in patients with bacterial meningitis.

Some patients with bacterial meningitis may not present with the "classic" signs and symptoms. Neonates as well as infants often do not have menigismus, but may present with change in state of alertness, listlessness, refusal to feed or respiratory distress. In the elderly the onset may be insidious with lethargy, obtundation, no fever and variable signs of meningeal inflammation.

In the febrile patient with an altered mental state, meningitis has to be excluded by examination of the CSF.

Specific etiologic diagnosis may be suggested by certain symptoms/signs. For example, about 50% of patients with meningococcemia have a prominent rash. Systemic complications like shock may also occur.

Both local suppurative and immunologic complications may occur in patients with meningococcal meningitis. Immunologic complications include immune complex arthritis, or cutaneous vasculitis with blistering skin lesions. Survivors can suffer long term complications, including hearing loss, mental retardation, paralysis, and seizures.

Diagnosis

The diagnosis of bacterial meningitis is based on examination of the CSF. The CSF white cell count is usually in the rage of 1000-5000/UL, with a neutrophilic predominance. The CSF protein is raised (100-500mg/dL) in virtually all patients with bacterial meningitis. A decreased CSF glucose (< 40mg/dL) is found in about 60% of patients and the CSF: serum glucose ration below 0.3 in about the same proportion of patients.

The CSF Gram stain is positive in 60-80% of cases of bacterial meningitis, and has a specificity of nearly 100%. However, the probability of detecting the organism by Gram stain may decrease to 40-60% in patients who had received antibiotic, and the yield of CSF culture similarly falls to below 50% from 70-85%.

Tabel 2: Recommendation for the treatment of bacterial meningitis based on CSF Gram stain

Organism	Preferred antibiotic
Gram stain unavailable, or no stainable organism	Ceftriaxone or cefotaxime
Gram positive cooci	Ceftriaxone or cefotaxime *
Gram negative cocci	Penicillin G
Gram negative bacilli	Ceftraixone or cefotaxime

* vancomycin, in areas where cephalosporin resistance had been reported.

In patients whose CSF pattern is consistent with bacterial meningitis but whose CSF Gram stain is negative, a latex agglutination test for bacterial antigen should be done on the CSF. The sensitivity of the latex agglutination test ranges from 50-80% and specificity is high. However, a negative test never rules meningitis caused by a specific meningeal pathogen.

Management

The care of patients with bacterial meningitis includes bogh supportive and definitive measures.

Patients with suspected bacterial meningitis should have their CSF examined within 30 minutes, and if the findings are consistent with bacterial meningitis, antimicrobial therapy should be instituted based on the results of the Gram stain or bacterial antigen tests (Table 2). However, if no organism can be identified, empirical antibiotics should be started based on the patient's age.

Recommendations on which drug to use empirically in the emergency setting for presumed bacterial meningitis have changed, in light of altered susceptibility pattern of the common meningeal pathogens.

Although strains of N. meningitidis have emerged that are relatively resistant to penicillin, the prevalence of penicillin-resistant meningococci is not at a worrying level at present.

Reprots from developed countries show that about a quarter of the strains of H. influenzae type b, produce B-lactamase and are resistant to amplicillin. Resistance to chloroampbenicol has also been described in some areas.

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The frequency of penicillin resistance in pneumococci has also been increasing in recent years in some regions. The prevalence of penicillin resistant pneumococci in Europe and the United States is between 20-30%. In South Africa strains resistant to both penicillin and chloramphenicol have been isolated. Penicillin resistant Streptococcus pneumoniae have also been reported from Zambia and Kenya. This has important implications on the treatment of pneumococcal meningitis in resource poor countries where the alternative third generation cephalosporins are either unavailable or unaffordable. In a report from South Africa chlorapmphenicol had been reported to be an unsatisfactory replacement for the treatment of penicillin resistant pneumococcal meningitis.

Penicillin remains the drug of choice for the treatment of meningococcal meningitis. Crystalline penicillin G 12-16 million units intravenoulsy in 4-6 divided doses for seven to ten days is the recommended treatment.

Penicillin is the drug of choice for the treatment of pneumococcal meningitis, when the organism is susceptible to penicillin.
Antimicrobial regimen for the therapy of meningitis caused by penicillin-resistant S. pneumoniae is third generation cephalosporins alone or in combination with vancomycin; in areas where pneumococci resistant to cephalosporins have been isolated.

Third generation cephalosporins, (ceftriaxone, cefotaxime) are recommended for empirical treatment of meningitis caused by Haemophilus influenzae.

The available evidence on adjunct dexamethasone treatment confirms reduced incidence of hearing loss in patients with H. influenzae meningitis; however, clinical data are insufficient for a recommendation that this drug be used routinely for meningitis caused by other organisms. [6]

Prevention and control

Large outbreaks of meningococcal meningitis occur in the "Meningitis Belt" of Africa. Mass vaccination of high-risk groups with meningococcal polysaccharide divalent vaccine (Serogroups A & C) had been shown to be effective for the control of epidemics. The vaccine gives about 80% protective immunity, and lasts 3 to 5 years.

The vaccines against H. influenzae type b are safe and highly effective and have now become part of routine childhood immunization programs in the developed countries.

Chemoprohylaxis of "close" contacts of patients with meningitis caused by N. meningitidis or H. influenzae is useful to reduce secondary cases; rifampicin or doxycycline is recommended for chemoprohylaxis.

References

1. Moore PS. Meningococcal meningitis in Sub-Saharan Africa: A model for the epidemic process. Clin Infect Dis 1991. 14: 515-525.
2. Habtegaber E, Mamo E. 1984. Meningococcal meningitis in Ethiopia, 1974 – 1983 and strategies for control. Eth J Hlth Dev, 1: 47-63.
3. Fekade D, Zawde D. 1992. Epidemic meningococcal meningitis among adult Ethiopians in Addis Ababa, Ethiopia. Eth Med J 30: 135-142.
4. Tundkel AR, Scheld WM. Acute meningitis. In: Mandell GL Bennet JE and Dolin R, editors. Principles and practice of infectious diseases 4th ed. Churchill Livingstone, 1995: 831-865.
5. Waage A, Halstensen A, Espevik T, Brandtzaeg P. Cytokines in meningococcal disease, In: Griffin GE, editor. Clinical infectious diseases. Bailliere 1994: 1: 97-108.
6. Mcintyre PB, Berkey CS, King SM et al. 1997. Dexamethasone as adjunctive therapy in bacterial meningitis. A meta-analysis of randomized clinical trails sicnce 1988. JAMA 11: 925-931.

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PREVALENCE AND CLINICAL FEATURES OF TUBERCULOSIS IN ETHIOPIAN DIABETIC PATIENTS
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PREVALENCE AND CLINICAL FEATURES OF TUBERCULOSIS IN ETHIOPIAN DIABETIC PATIENTS

Y. FELEKE, J. ABDULKADIR and G. ADERAYE

ABSTRACT

Objective: To determine the prevalence and clinical characteristics of tuberculosis (TB) in diabetic patients.
Design: This study was a cross-sectional survey based on the retrospective analysis of data on tuberculosis in diabetic patients.
Settings: The study was conducted at Endocrinology and Metabolism Unit of the Department of Internal Medicine, Faculty of Medicine, Addis Ababa, Ethiopia.
Subjects: Seventy eight cases of tuberculosis among 1352 diabetic patients were included from September 1989 to 1996.
Main outcome measures: Clinical evaluation, chest x-ray, acid fast bacilli (AFB) in sputum or measures tissue and histopathologic characteristic of biopsy specimens.
Results: Seventy eight cases of TB were identified among 1352 diabetic patients giving a prevalence of 5.8%. Among 1352 diabetic patients, 713 (52.7%) were males, 639 (43.3%) were females, 619(45.8%) were IDDM and 733 (54.2%) were NIDDM. The mean age of the 71 TB patients whose records could be retrieved was 34.0±11.9 years, 42(59%) were males, 29 (41%) were females. Fifty four (76.1%) were IDDM and 17(23.9%) were NIDDM, 17 of the IDDM had clinical characteristics of malnutrition-related diabetes mellitus (MRDM), 36 (56%) of 71 patients were admitted for management. The three most common symptoms of tuberculosis were fever (80.5%), sweating (80.4%) and cough (70.5%). Twenty six (36.6%) of 71 cases were positive for AFB and six (8.5%) were seropositive for HIV. Fifty six (78.9%) had pulmonary, eight (11.2%) had extrapulmonary and seven (9.8%) had disseminated TB. Forty eight of 53 abnormal chest x-ray showed unilateral involvement. Thirty eight of 41 (53.6%) had completed TB treatment, relapse occurred in seven (9.8%), eight (11.2%) are currently on treatment, 13 (18.3%) were lost to follow up, four (5.6%) defaulted and three (4.2%) died. The relative risk of developing TB in IDDM and NIDDM patients was being 26 times and seven times than the general population respectively.
Conclusion: The prevalence of tuberculosis in the diabetic population is high and this warrants a prospective study to determine association between pulmonary tuberculosis and diabetes.

INTRODUCTION

Tuberculosis has long been a major cause of morbidity and mortality in Ethiopia. According to the Ministry of Health report of 1995(1), tuberculosis accounted for 3.7% of outpatient morbidity, 9.4% of hospital admissions and 27% of hospital deaths(1,2). The group mainly affected is that between 15 and 45 years. In such an environment with underdeveloped health care facilities and a high prevalence of tuberculosis, diabetics could be expected to be at an even greater risk of contracting the disease than non diabetics because of their generally poorer state of health engendered by inadequate diabetic control.

Available information on the prevalence of tuberculosis in diabetics in sub-Saharan Africa shows that it is common and its prevalence is significantly higher in diabetics than in the general population(3-6). A report from this country by Lester *et al*(7) in 1976 showed a prevalence of 13% in 404 diabetics. Another report by Lester in 1984(8) showed a higher prevalence level of 3.9% in 847 patients. These recent prevalence levels in African diabetics are comparable to those reported in American diabetics in the pre-insulin era(5) suggesting that the current high prevalence of tuberculosis in African diabetics is related to the high prevalence of tuberculosis in the population and the shortcomings of diabetes care in Africa than to an inherently higher susceptibility of African diabetics to tuberculosis.

Amir sultan (MD), 2014

The purpose of this study on tuberculosis in Ethiopian diabetics was to determine its current prevalence and clinical characteristics, as seen in patients attending the diabetic clinic of the Endocrinology and Metabolism unit of the Department of Internal Medicine, because the earlier report on this topic from Ethiopia in 1984(8) antedated the HIV epidemic.

MATERIALS AND METHODS

A review of the records of 1352 patients seen at the endocrinology and metabolism unit of the Department of Internal Medicine, Medical Faculty, Addis Ababa University, from September 1989 to 1996 revealed 78 cases had developed tuberculosis. The records of 71 of them could be retrieved for analysis. Diagnosis of pulmonary tuberculosis was based on clinical evaluation plus chest x-ray and/or detection of acid fast bacilli (AFB)) in the sputum. Extrapulmonary tuberculosis was diagnosed on the basis of clinical evaluation and demonstration of acid fast bacilli in tissue or body fluid or the characteristic histopathology in a biopsy specimen. The patients were seen at the diabetes clinics where follow up occurs at intervals of eight to 20 weeks, after the initial period of stabilisation, and every seven to 15 days during pregnancy.

RESULTS

Among the 1352 diabetic patients, seventy eight cases of TB were identified, giving a prevalence of 5.8%. Seven hundred and thirteen (52.7%) were males, 639 (47.3%) females; 619 (45.8%) IDDM and 733 (54.25%) were NIDDM. Among the 71 patients, 54 (76.0%) had IDDM and 17(23.9%) NIDDM. Seventeen of the IDDM patients, had clinical characteristics commensurate with those of malnutrition-related diabetes mellitus (MRDM)(9). The age, sex and type of diabetes in patients diagnosed with tuberculosis is shown in Table 1. The mean age of the 71 patients with tuberculosis was 34±19 years (range 14-85 years)

Table 1

Age and sex distribution of diabetic patients with tuberculosis

	IDDM (N = 54)						NIDDM (N = 17)							
	M	%	F	%	Total		M	%	F	%	Total		Grand Total	
Age in years					No	%					No	%	No	%
14-19	2	3.7	0	-	2	3.7	0	-	0	-	0	-	2	2.8
20-29	18	33.3	8	14.8	26	48.1	0	-	0	-	0	-	26	36.6
30-39	11	20.3	8	14.8	19	35.2	1	5.8	1	5.8	2	11.8	21	29.6
40-49	3	5.6	1	1.9	4	7.4	2	11.8	3	17.6	5	29.4	9	12.8
50-59	1	1.9	1	1.9	2	3.7	2	11.8	6	35.3	8	47.0	10	14.1
60+	1	1.9	0	-	1	1.9	1	5.8	1	5.8	2	11.8	3	4.2
Total	36	66.7	18	33.3	54	100.0	6	35.3	11	64.5	17	100	71	100

Table 2

Clinical characteristics of tuberculosis in diabetic patients

Presenting complaint	Yes		No		No information	
	No.	(%)	No.	(%)	No.	(%)
General	57	80.5	5	7.0	9	12.7
Fever	57	80.5	7	9.9	7	9.9
Sweating	57	80.5	5	7.0	9	12.7
Weight loss	5	7.0	40	56.3	26	36.6
Contact with chronic cougher						
Pulmonary	54	76.1	9	12.7	8	11.3
Cough	12	16.9	0	-	59	83.1
Chest pain	4	5.6	67	94.4	-	-
Haemoptysis						
Extra pulmonary	1	1.4	-	-	-	-
Swelling of wrist joint	1	1.4	-	-	-	-
Abdominal pain and diarrhoea	1	1.4	-	-	-	-
Hematuria	1	1.4	-	-	-	-
Lymphadenopathy						

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Clinical characteristics of patients with tuberculosis are shown in Table 2. Fifty six (78.9%) patients had pulmonary TB, eight (11.2% had extrapulmonary TB and seven (9.9%) had disseminated tuberculosis. The duration of symptoms of tuberculosis varied from two weeks to one year. The most frequent symptoms of tuberculosis were fever, sweating and cough which occurred in 57 (80.5%), 57 (80.5%) and 54 (76%) patients respectively. The duration of diabetes at the time of diagnosis of tuberculosis in diabetic patients ranged from onset to 25 years.

Among those with extrapulmonary tuberculosis, one patient presented with swelling and pain in the wrist joint. Synovial biopsy showed histopathology consistent with tuberculous arthritis. The patient completed his antituberculosis therapy, but two years later developed smear positive pulmonary tuberculosis and was found to be HIV positive.

A patient who presented with persistent pyuria and later found to have an echodense mass in the right kidney was admitted to the surgical ward with the diagnosis of hypernehproma. He subsequently developed cough, sweating and pleural effusion whose analysis and cytology were consistent with tuberculosis. Urine culture for AFB was not done. Both pleural effusion and renal mass resolved following antituberculosis treatment.

Two other patients presented with productive cough, fever, and sweating. Their physical findings and chest x-rays were normal but sputum specimens were positive for AFB. At the time of this study, both of them had completed treatment and were doing well during last follow up visits. Twenty six (46.4%) of 56 pulmonary tuberculosis patients were smear positive for AFB. Erythrocyte sedimentation rate was normal in two (both of whom had extrapulmonary tuberculosis), elevated in 64 patients, unrecorded in five of 71 patients. The mean fasting blood glucose (FBG in 59 of 71 patients at the time of diagnosis of tuberculosis (those for whom the information was available was 301.3mg/dl. Only six of the 59 patients had FBG less than 140mg/dl.

Chest x-ray reports were available in 58 of the 71 patients, while findings were normal in five, and abnormal in 53 of the patients showing unilateral single or multiple lobe involvement in 48 and bilateral involvement in five. Among 53 chest x-rays, pulmonary parenchymal involvement consisting of interstial, alveolar infiltration or consolidation was found in 33 of 53 chest x-rays, pleural effusion in seven, interstitial infiltrate in three, pneumothorax in two, cavitary lesion with infiltration in five, atelectasis in three, lung absecess in one. Four of abnormal chest x-rays showed honey combing appearance with interstitial infiltrate which was suggestive of bronchiectasis in four patients. Six of the 71 patients were HIV positive. Patients were treated with three to five antituberculosis drugs(INH, rifampicin, ethambutol, pyrazinamide and streptomycin) for six to 12 months. Thirty six (50.7%) patients required admission to the hospital for control of diabetes and management of their tuberculosis treatment.

Outcome of treatment in patients with tuberculosis: Thirty eight (53.6%) out of the 71 diabetic patients with tuberculosis had completed antituberculosis treatment. Four (5.6%) patients defaulted while relapse occurred in seven (9.8%) patients out of which four had bronchiectasis. Six (8.5%) patients were still on treatment while three patients had died. In two of the deaths, the cause of death was not related to tuberculosis. One died of hepatic coma, the second of sepsis and the third patient developed evidence of intracranial space occupying lesion while she was on antituberculosis treatment though the cause remained undetermined since no autopsy was done.

The mean duration of follow up after completion of treatment in 58 patients was 20±15.2 months (range of one month to six and a half years), 13 (18.3%) patients were lost to follow up after initiation of antituberculosis medication.

DISCUSSION

The tuberculosis prevalence of 5.8% in our patients was higher than the 3.9% previously reported from this country by Lester in 1984(8). This may be due to an increasing incidence of tuberculosis in the community and poor control of diabetes which increases the risk for infections in general. Tuberculosis was more common in IDDM than in NIDDM and in the former group more common among MRDM diabetics which is a most socioeconomically underprivileged group)(9). The mean ±SD age of our diabetic patients with tuberculosis was 34±19 years as compared to our diabetic patients which was 41±14.4 years. A study done by Vucinie et al.(11) from Yugoslavia, showed that most tuberculosis diabetic patients were IDDM but more than 40 years old while the highest prevalence of tuberculosis was in those aged 60-70 years (40%). The relative risk for our IDDM and NIDDM respectively being 26 and seven times that in the general population(10).

The most frequent symptoms were fever, sweating and cough (80.4%, 76% and 80.4% compared to the report by Vucinie *et al*(11) which showed fever and weight loss in 82% and 72%, respectively. In this report, 8.4% of diabetic patients with tuberculosis were seropositive for HIV which could be one of the reasons why the prevalence of tuberculosis is increasing in diabetic patients. There was no strong association between development of tuberculosis and duration of diabetes in our study but there was a significant association between development of tuberculosis and degree of hyperglycaemia because 93% of tuberculosis patients were severely hyperglycaemic (the mean fasting blood glucose level of 301.3 (112.9 mg/dl) at the time of diagnosis of tuberculosis. The mean FBS of those without tuberculosis was 191.2±60mg dl (p value <0.05)

In our study, 81.6% of the patients developed

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uberculosis within ten years of the diagnosis of iabetes as compared to the study done by Turner(12) which showed 62% developing tuberculosis in similar ime period. In our patients, radiographic appearance at the start of treatment showed a unilateral involvement in 82.2% and bilateral involvement in 17.8% as compared to the study done by Luntz(13) which showed 43% and 57% respectively.

Ninety per cent of our patients had a high ESR and multiple lobe involvement which was similar to the report by Ekim *et al*(14). Relapse rate in our study was ten per cent as compared to the study done by Turner, in which relapse rate was 36%(12). This could be attributable to the improved combination antituberculosis treatment in our cases. The combination of three to five antituberculosis drugs (ethambutol, INH, pyrazinamide, streptomycin and rifampicin) were given to the patients based on the national policy of tuberculosis management(1,2). However, in situations of scarcity of drugs, the anti TB drug regimen was appropriately modified.

In general tuberculosis was diagnosed in diabetic patients at an advanced disease stage and 50% of the patients required inpatient management. The young IDDM patients were mainly affected. In order to justify these facts, it is advisable to do a prospective study and also determine the serology status of HIV in the diabetic patients.

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REFERENCES

1. National Tuberculosis and Leprosy Control Programme, Ministry of Health, Ethiopia. 1997; 1st Edit. 9-27
2. Guideline for the National Tuberculosis Control Programme In Ethiopia, Addis Ababa, 1992.
3. Mugusi, F. Diabetes and pulmonary tuberculosis in Tanzania (Dissertation). Dar es Salaam, Tanzania, University of Dar es Salaam, 1987.
4. Mugusi, F., Swai A.B.M., Alberti K.G.M.M and McLarty D.G. Increased prevalence of diabetes mellitus in patients with pulmonary tuberculosis in Tanzania. *Tubercle* 1990; 71:271-276
5. McLarty, D.G., Pollitt, C. and Swali, A.B.M. Diabetes in Africa. *Pract. Diabetes Digest.* 1992; 3:35-40.
6. Adetuyibi A. Diabetes in the Nigerian African Review of Longterm complications. *Trop. geogr. Med.* 1976; 155-168.
7. Lester, F.T., Abdulkadir, J., Lalson, D. and Qanta P. Diabetes Mellitus Clinical features in 404 Ethiopians. *Ethiop. Med. J.* 1976; 14:185-197.
8. Lester, F.T. Tuberculosis In Ethiopian Diabetics. *Ethiop. Med. J.* 1984; 22:129-133.
9. Abdulkadir, J., Mengesha, B., Weldegebriel, Z., Keen, H., Worku, Y., Gebre, P., Bekele, A., Urga, K. and Tadesse, A.S. The Clinical and hormonal (C-peptide and glucagon) profile and liability to ketoacidosis during nutritional rehabilitation in Ethiopian patients with malnutrition-related diabetes mellitus. *Diabetologia.* 1990; 33:222-227.
10. Demissie, M., Gebeyehu, M. and Berhane, Y. Primary resistance to antituberculosis drugs in Addis Ababa, Ethiopia. *Int. J. Tuberc. Lung. Dis.* 1997; 1:64-67.
11. Vucinic, V., Duric, O., Zugic., V. and Videnovic, J. Tuberculosis in patients with diabetes mellitus Tuberc and lung disease 1995; 76:39.
12. Warmick, M.T. Pulmonary tuberculosis and diabetes mellitus. *Quart. J. Med.* 1957; 26:31-41.
13. Luntz, G.R.W.N. Management of Tuberculos diabetic *Brit. Med. J.* 1957; 2:1082-1086.
14. Ekim, N.N., Cilli, A., Guz, G. and Gazi A.M. Pulmonary tuberculosis in diabetic patients. *Tuberc. Lung Dis.* 1995; 76:28.

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Distribution of fluoride and fluorosis in Ethiopia and prospects for control

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Summary

A review and mapping of fluoride test data for 270 water sources in 126 communities and examination of the literature of fluorosis distribution in Ethiopia show that this health problem extends beyond the Rift Valley into some highland communities. Fluoride concentrations above 5.0 mg/l in the Rift Valley were found mostly in hot springs (100% of all sources), lakes (78%), shallow wells (54%) and boreholes (35%) and the lowest concentrations (below 1.5 mg/l) in springs and rivers. Analysis of hydrochemical, economic and demographic factors in the spatial distribution of high-fluoride domestic water sources indicates that the fluorosis problem has become more serious in the Rift Valley in recent decades. Considerable spatial variation in the occurrence of fluoride, even within the same communities, and the presence of some low-fluoride water sources in the Rift Valley offer possibilities for geochemical exploration for acceptable domestic sources. The defluoridation programme in the Wonji irrigation scheme illustrates the problems faced by a large rural community in a developing country. Possibilities for control are examined and recommendations made for the development of alternative water sources and promising defluoridation methods using locally available materials and technologies.

keywords fluoride distribution, environment, fluorosis, prevention, defluoridation, alternative water sources, Ethiopia

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Introduction

Fluoride occurs in all rock types but the highest concentrations of fluoride in water sources, and the highest prevalence and intensity of dental and skeletal fluorosis, are found around intracontinental hot spots, aborted rift zones and Andean type magmatic belts. Fluoride mineralization takes place mostly in geologically unstable areas and has been associated with rifting and the rise of fluorine from the lower crust or upper mantle. Although the highest fluoride levels are in volcanic rocks, significant concentrations have also been found in Pre-Cambrian rocks and young marine sediments. Heavy clay soils, groundwater and lake water in the vicinity of volcanic areas can take up high levels of fluoride from these rocks (WHO 1984a). The volcanic rocks of the East African Rift System contain higher concentrations of fluoride than analogous rocks in other regions. Some of the highest fluoride levels were recorded in wells in that rift system and the highest values in Lake Nakuru in Kenya (2,800 mg/l) (Gerasimovskiy & Savinova 1969).

Although water is the epidemiologically most important source of fluoride in most areas, considerable exposure risk is also associated with the consumption of fish bones, canned meat, vegetables, grains and other staples, local salt, drinks (especially tea) and air (WHO 1984b; Smet 1990; van Palenstein Helderman *et al.* 1995; Malde *et al.* 1997). In some African and Asian communities, intake of fluoride from food has been found to be higher than from water (Mabeleya *et al.* 1999).

Clinical forms of both dental and skeletal fluorosis are particularly common along the East African Rift system and on the Indian subcontinent (WHO 1984a; Nair & Manji 1982; Tekle Haimanot *et al.* 1987; Teotia *et al.* 1987; Warnakulasuriya *et al.* 1992), although cases are also being reported from the Americas and East Asia. In the early 1980s, more than 260 million people world-wide consumed drinking

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water with fluoride concentrations above 1 mg/l and more than 30 countries, half of them in Africa, had communities with fluoride concentrations above 1.5 mg/l. In the United States, more than 300 community water supply systems reported concentrations above 4 mg/l (Smet 1990).

Drinking water containing optimum levels of fluoride (0.5–1.5 mg/l, depending on climatic conditions and the relative contribution of non-aqueous sources of fluoride to overall fluoride load in individuals) confers protection against dental caries without causing fluorosis. The formula developed by Galagan and Vermillion (1957) to determine optimum fluoride levels in drinking water considers annual mean maximum temperature because water consumption is greater in hot climates. The WHO has set the guideline value at 1.5 mg/l of fluoride, but mild forms of dental fluorosis begin to occur at lower levels. Above 1.5 mg/l, prolonged intake of fluoride can cause dental fluorosis; 3–6 mg/l, skeletal fluorosis; more than 6 mg/l, crippling skeletal fluorosis (WHO 1984b; Brouwer *et al.* 1988).

The public health and economic importance of fluorosis is significant in many endemic areas in view of the occurrence of debilitating skeletal fluorosis in humans and the more recent discovery of pathology in cattle, sheep and other livestock (Baars *et al.* 1987; Shupe *et al.* 1992; Dunipace *et al.* 1995). Crippling skeletal fluorosis is confined to tropical and subtropical areas (WHO 1984b). The maximum allowable fluoride concentration of 1.5 mg/l set by WHO (1984b) has been considered by some researchers to be too high. Critics contend that the high fluid intake in hot tropical areas increases exposure risk, as evidenced by studies showing the development of dental fluorosis from the consumption of water with fluoride concentrations as low as 0.8 mg/l (Brouwer *et al.* 1988). But the inability to adequately reduce fluoride levels in most high-fluoride areas and other factors in fluorosis etiology have rendered these and most other fluoride standards unobtainable. Recognizing its inability to meet the WHO standard, Tanzania set the guideline at 8 mg/l (Schuiling 1990). Other factors in the development of fluorosis at low fluoride levels in drinking water include fluoride intake from other sources and malnutrition. No economic impact studies of fluorosis have been conducted, but the large number of affected persons and disability of workers with crippling disease indicate that economic losses are considerable.

Of the two fluorosis prevention strategies that have been considered by researchers since the 1930s – provision of alternative water sources and defluoridation of drinking water – most efforts have been directed at the latter. Many defluoridation methods developed in the laboratory have not been field-tested. Although several fluorosis prevention methods are technically feasible and can routinely be carried out in central water distribution systems, none of the defluoridation methods are successfully applied on a routine basis (Dahi 1999). Most defluoridation methods are complicated and/or expensive, unproven and unreliable under field conditions in developing countries, use materials of questionable supply and inappropriate technology, are insufficiently effective, and their social acceptability is unknown. Lack of sustained government and community commitments and efficient organizational structures exacerbate the problem (Heidweiller 1990).

The more promising methods which have been field-tested include the granulated bone media and heat-activated bone char, clay and clay pot chip (Bjorvatn *et al.* 1999; Dahi 1999), activated alumina adsorption (Shifera & Tekle Haimanot 1999) and Nalgonda methods (Gumbo & Mkongo 1995). The risk of secondary contamination by metal ions such as aluminium and the search for simple, low-cost methods using local materials have contributed to the use of plant products with defluoridating properties, such as the seeds of *Moringa oleifera* (Stanley & Bulasopramanjan 1999), bone media and clay (Heidweiller 1990).

A major problem in the delivery of water from low-fluoride sources and defluoridated water is the scarcity of piped distribution systems in rural communities and the reliance of households on individual wells, springs and/or surface sources. This situation requires that defluoridation methods be developed that can be applied at individual well and spring sites or household storage tanks and can run without technical supervision at household and community levels for a month or more. Construction of piped networks for the purpose of distributing alternative water supplies, although initially more expensive than defluoridation, promises to be economically feasible during the operation and maintenance phases in the absence of treatment systems (Frencken & Smet 1990).

Community participation has been identified as an important factor in the success and sustainability of community water programmes. Motivating local people to participate in community-based fluorosis prevention efforts has been difficult due to a combination of the chronic nature of fluorosis, lack of awareness of its progressive course and irreversible pathology, and the general deficiency and failure of fluorosis control programmes (Heidweiller 1990; Bregnhoj 1999). Information on the geographic distribution and hydrology of fluoride is extremely important in the development of water resources. Fluoride levels tend to vary considerably in different groundwater and surface water sources as well as aquifers within the same communities. This information can form the basis for developing alternative water sources. For example, mixing water from low-fluoride aquifers with higher-fluoride sources can reduce exposure risk (Schuiling 1990). In the absence of adequate hydrogeological surveys of water sources, high fluoride levels may result in the abandonment of

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wells and entire water supply programmes (Gumbo & Mkongo 1995).

Ethiopia is of particular interest due to the predominance of extensive volcanic basalt flows in the lowland and highland regions that originated from complex tectonic events (Baker *et al.* 1972), low socio-economic level exacerbated by the recent civil war and recurring famine, widespread occurrence of skeletal fluorosis in the Rift Valley and of dental fluorosis in the highlands, and experience with fluorosis control efforts since the 1960s (Kloos & Tekle Haimanot 1993; Shifera & Tekle Haimanot 1999). However, the available data have not been gathered using a standardized method and the geographic distribution of fluorosis and fluoride, especially outside the Rift Valley, is poorly known (Tekle Haimanot *et al.* 1987; Balachew 1990; Kloos & Tekle Haimanot 1993).

The purpose of this paper is to examine the distribution of fluoride in household water sources and fluorosis in different parts of Ethiopia in relation to hydrochemical, demographic and economic parameters, to describe the defluoridation programme at Wonji Sugar Estate and to evaluate prospects for fluorosis control at the national level.

Materials and methods

The fluoride data used in this paper are from unpublished data gathered from 1985 to 1990 by the Ethiopian Water Supply and Sewerage Authority (WSSA) and several academic investigators, and by Sir Alexander Gibb and Partners in 1973, as well as several published reports. The literature on fluorosis in Ethiopia and on control strategies was reviewed. Demographic data are from the 1990 Statistical Abstract (Central Statistical Office 1991) in Addis Ababa.

Results

Spatial distribution of fluoride

Fluoride concentrations above 1.5 mg/l have been reported from all parts of Ethiopia, but the highest levels were found in the Rift Valley, the lowland area with the most recent volcanic activity in the country. In the early 1970s, 124 analyses of Rift Valley lake water showed that 67 (54%) had fluoride concentrations above 2 mg/l and 38 (31%) had levels above 8 mg/l (Lester 1974); 35% of the 152 community water sources tested in the Rift Valley had concentrations of 5 mg/l and higher, compared to only 2.5% of the 118 sources in the highlands. More recently, the largest proportion of fluoride concentrations above 5.0 mg/l in the Rift Valley was found in hot springs (in all 3 hot springs tested), followed by lakes (78%), shallow wells (54%) and boreholes (34%); river and spring sites had levels below 1.5 mg/l (Table 1, Figure 1). A water supply feasibility study by WSSA in 34 towns revealed significantly higher fluoride levels in boreholes (mean 4.7 mg/l) than in shallow hand-dug wells (mean 1.2 mg/l) (WSSA 1983). These differences appear to be due to the more recent volcanic activity of the Rift Valley and generally higher fluoride

Table 1 Fluoride concentrations in 270 water sources in 126 communities in the Rift Valley and Highlands

Fluoride concentration (mg/l)	No. (%) of sources	No. (%) of sources, by type					
		Bore holes	Shallow wells	Springs	Hot springs	Rivers	Lakes
Rift Valley							
0*	4 (3)	3 (3)	0	0	0	0	0
< 1.0	43 (28)	30 (27)	3 (27)	6 (86)	0	3 (33)	1 (11)
1.0–4.9	52 (34)	42 (37)	2 (18)	1 (14)	0	6 (67)	1 (11)
5.0–9.9	24 (16)	20 (18)	2 (18)	0	1 (33)	0	1 (11)
10+	29 (19)	18 (16)	4 (36)	0	2 (67)	0	6 (67)
Subtotal	152 (100)	113 (100)	11 (100)	7 (100)	3 (100)	9 (100)	9 (100)
Highlands							
0*	13 (11)	11 (17)	0	1 (4)	0	1 (5)	0
< 1.0	84 (71)	40 (63)	2 (6)	23 (85)	0	16 (76)	3 (75)
1.0–4.9	18 (15)	9 (14)	1 (33)	3 (11)	0	4 (19)	1 (25)
5.0–9.9	3 (3)	3 (5)	0	0	0	0	0
10+	0	0	0	0	0	0	0
Subtotal	118 (100)	63 (100)	3 (100)	27 (100)	0	21 (100)	4 (100)
Grand total	270	176	14	34	3	30	13

Source: WSSA, unpublished data, Tekle Haimanot *et al.* (1987), Kloos & Tekle Haimanot (1993). *Non-detectable level.

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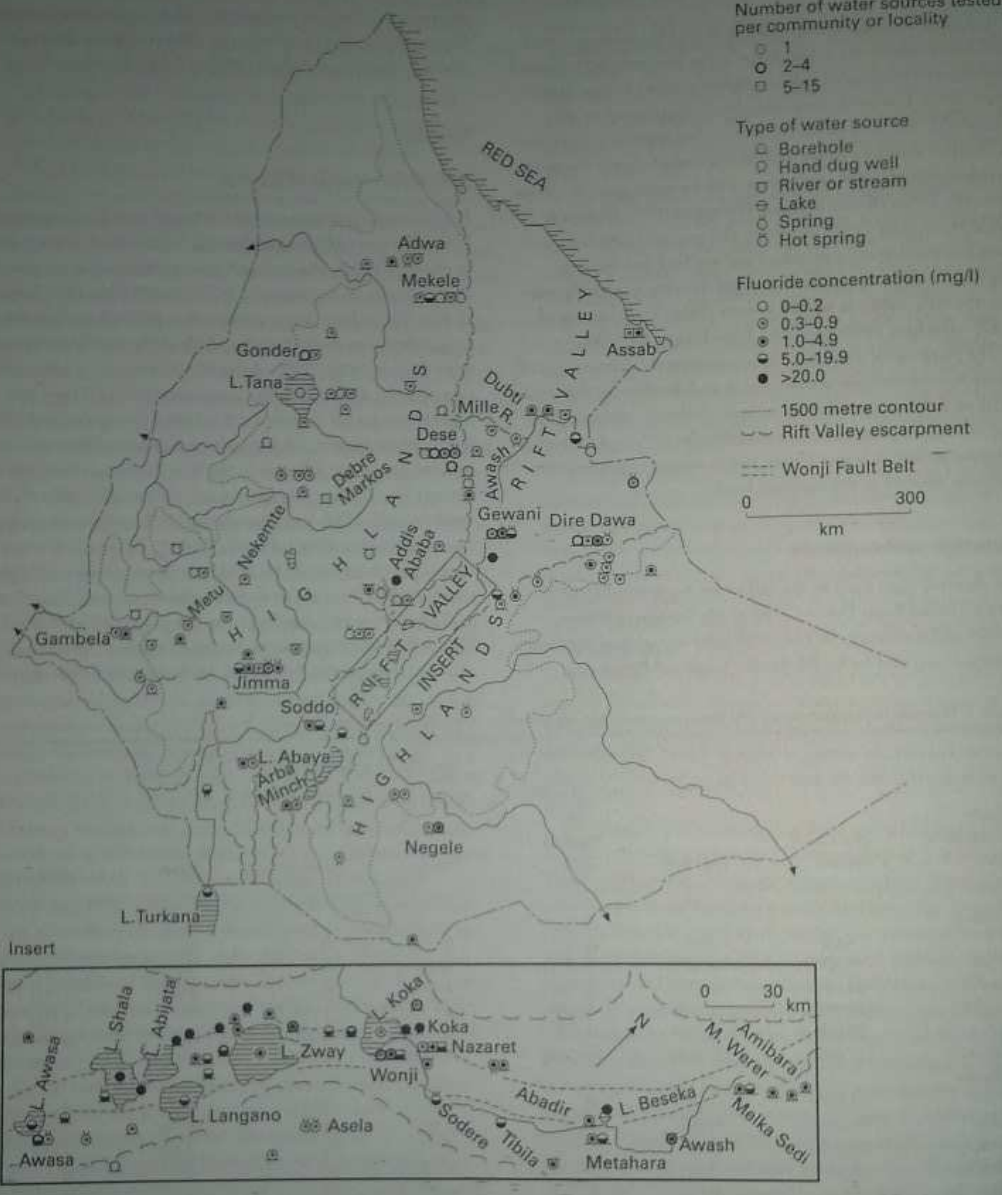


Figure 1 Fluoride concentration in 270 water sources, by type of source. Based on unpublished data by WSSA (for 1985–1991) and Sir Alexander Gibb and Partners (for 1973), and by Tekle Haimanot *et al.* (1987), Solonso (1985), Zewde *et al.* (1977) and Abdo (1978).

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Table 2 Fluoride concentrations in 270 water sources in 126 communities, by type and size of settlements

Fluoride concentration (mg/l)	No. of sources*	9 irrigation schemes	50 villages and towns (< 5000 population)	34 towns (5,000–20 000 population)	23 towns (> 20 000 population)
0	39 (14)	0	1 (2)	23 (34)	15 (13)
< 1.0	107 (40)	0	20 (32)	30 (44)	57 (53)
1.0–4.9	70 (26)	15 (50)	16 (25)	13 (19)	26 (23)
5.0–9.9	30 (11)	10 (33)	9 (14)	2 (3)	9 (8)
10+	24 (9)	5 (17)	17 (27)	0	2 (2)
Total	270 (100)	30 (100)	63 (100)	68 (100)	109 (100)

* Percent in brackets. Source: WSSA, unpublished data, Tekle Haimanot *et al*. (1987), Kloos & Tekle Haimanot (1993).

levels in basaltic rock than in surface sediments (Belachew 1990).

Aside from Lake Shala (177 mg/l), Lake Hertale near Gewani (26 mg/l) and 3 hot springs in the central lakes region (8–20 mg/l), the highest fluoride levels in the Rift Valley have been recorded in boreholes, which typically range in depth from 10 m to 100 m. Nearly all rivers and springs had fluoride concentrations below 1.5 mg/l but many communities in the arid Rift Valley lack surface water sources. Fluoride levels were highest along the Wonji Fault Belt and declined toward the Rift Valley escarpment. The Wonji Fault Belt is a 500 km long belt of many small, recent faults and volcanism on the rift floor extending from Lake Abaya in the southwest to the terminal end of the Awash River in the northeast (Figure 1) (Mohr 1971; Gouin 1979).

Aggregation of fluoride levels for 257 sources in 116 communities in both the highlands and Rift Valley shows that although irrigation schemes had the largest proportion of high-concentration sources, fluorosis-inducing levels were also reported from large towns (Table 2). The Rift Valley population of nearly 1 million in 1976 had doubled by 1990 (Central Statistical Office 1991) due to migration from the overpopulated highlands to the rapidly growing irrigation schemes and towns. Concern over schistosomiasis transmission in irrigation systems caused the larger farms (Wonji, Metahara and Melka Sedi) to provide their labour populations with groundwater, which tended to have significantly higher fluoride concentrations than Awash river water. Fluorosis thus became an occupational disease of farm labourers (Kloos 1985; Kloos & Tekle Haimanot 1993). Large Rift Valley towns, including Nazaret, Awasa and Dire Dawa, also used predominantly groundwater (Figure 1).

The unusually low fluoride levels reported for springs and groundwater in the Rift Valley town of Arba Minch ('Forty Springs') and several communities along the escarpment (Figure 1) are apparently due to runoff from the highlands or seepage from adjacent rivers and Lake Zway. The location of some of these communities within the Wonji Fault Belt indicates the presence of freshwater aquifers that may be used as alternative water sources.

The moderately high fluoride concentrations (1.5–5.0 mg/l) in 24 sources in the highlands (Figure 1, Table 1) indicate that fluorosis is not confined to the Rift Valley as thought earlier. Nevertheless, the more abundant rainfall and surface waters in the affected highland communities and the presence of low-fluoride water sources in each of the 3 highland towns having water with fluoride levels above 5 mg/l can facilitate the development of effective prevention and control programmes. Mean annual rainfall in the highlands ranges from about 800 mm to over 2,400 mm and in the Rift Valley from less than 300 mm to 800 mm (Ethiopian Mapping Authority 1988), but runoff and thus surface water accessibility are even greater in the highlands than these data indicate due to the lower temperatures and evapotranspiration rates than in the Rift Valley.

Analysis of fluoride distribution at the community level revealed the presence of widely varying concentrations in individual water sources and of low fluoride levels in some surface and groundwater sources in the Rift Valley. Alem Tena and Koka villages near Wonji farm, as well as Sodere Resort had boreholes yielding between 6.0 and 22.6 mg/l fluoride in spite of the accessibility of Lake Koka (0.3 mg/l) and the Awash River (0.6 mg/l). Both low-fluoride sources (rivers, streams, springs and shallow wells with 0–1.7 mg/l) and high sources (boreholes up to 5.0 mg/l) were used in the large towns of Mekele, Soddo, Awasa and Jimma and in the small town of Negele (Figure 1) without mixing these waters, rendering some users at higher risk of developing fluorosis.

Seasonal distribution of fluoride

Some of the few longitudinal data gathered on fluoride distribution in Ethiopia by a consulting engineering firm evaluating the suitability of surface waters for large-scale irrigation show seasonal fluctuations in the Awash and Mille rivers and in lakes Hertale and Gedebasa in the northern part

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Table 3 Seasonal distribution of fluoride (mg/l) in selected rivers and lakes in the northern part of the Rift Valley in 1973

	Jan	Feb	Mar*	Apr*	May	Jun	Jul†	Aug†	Sept†	Oct	Nov	Dec
Awash River at Tendaho	1.3	–‡	0.42	–	–	1.5	–	1.2	–	1.4	–	–
Awash R. at Dubti irrigation farm	–	–	–	0.93	–	1.4	1.1	–	–	–	1.4	–
Awash R. at Assaita town	–	–	–	–	2.25	1.4	–	1.2	–	–	1.4	–
Mille River	–	1.4	–	–	–	0.6	0	0.9	–	–	1.4	–
Lake Gedebasa (near Gewani)	–	–	–	0.63	–	–	–	–	–	1.3	–	–

*Time of small rains; † Time of big rains; ‡ No data available. Source: Sir Alexander Gibb & Partners (1973).

of the Rift Valley. Low values generally prevailed in March–April and July–August, during the two rainy seasons, and higher values in the dry periods from October–March and May–June (Gibb *et al.* 1973) (Table 3), indicating a dilution effect of rain water. These results indicate the need for systematic monitoring of fluoride levels during different times of the year for risk analysis and the provision of safe water supplies.

Distribution of fluorosis

The few studies of dental fluorosis in Ethiopia prior to 1985 reported high prevalence rates in several communities in the central part of the Rift Valley (Abdo 1978; Olsson 1979). The mean prevalence of dental fluorosis among 1,549 persons in 23 communities and commercial farms in that part of the Rift Valley was 83.2%, ranging from 32% in Gedemsa Alelu village near Wonji farm, which used exclusively the low-fluoride water (0.3 mg/l) of Lake Koka, to 98.1% in Nazareth town, supplied for more than 20 years with water from several boreholes containing 0.8–4.3 mg/l of fluoride. The beneficial effect of using Awash River water (1.4–1.6 mg/l) was noted in Kuriftu village in Wonji farm, with a 70.0% prevalence of mostly mild forms of dental fluorosis. The degree of dental mottling was fairly closely associated with fluoride concentration, prevalence of fluorosis and length of exposure. The prevalence of the severe form was 48.2% in nearby Jido village, where the population had been using water from a borehole containing 33.6 mg/l fluoride, but only 6.9% in Awash town, supplied mostly by low-fluoride river water (Tekle Haimanot *et al.* 1987). Of the 7 communities studied by Belachew (1990) in the Wonji area, the highest prevalence of mild, moderate and severe dental fluorosis (64%) was in the headquarter village of Wonji scheme, where residents had been using water from boreholes containing 10.8 mg/l fluoride for about 30 years. No such cases were seen in a nearby village, which depended exclusively on Lake Koka water (0.3 mg/l). Dental fluorosis surveys carried out among elementary and high school students in two Rift Valley towns revealed significantly lower rates among students born in non-endemic areas who had recently arrived from the highlands (Abdo 1978; Gilamichael 1986).

Less information is available on the prevalence of skeletal fluorosis. The only area where large numbers of clinical cases have been identified is Wonji farm. Tekle Haimanot *et al.* (1987) described a direct relationship between the development of skeletal fluorosis, fluoride concentration of drinking water and length of exposure, noting that the first clinical cases appeared among agricultural labourers after 10 years of exposure to water with more than 8 mg/l of fluoride. Between 1976 and 1984, Wonji farm retired 530 workers due to disability, 46% of them with a radiological diagnosis of skeletal fluorosis. Of 300 persons subsequently examined, 65% were diagnosed with skeletal fluorosis, 30 of them presenting with the severe, crippling form characterized by complete physical incapacity, cervical myelopathy, radiculomyelopathy and dorso-lumbar radiculomyelopathy (Tekle Haimanot *et al.* 1987).

The higher water consumption of people living in the hot Rift Valley contributes to higher exposure risk than in the nearby highlands. This relationship could not be quantified but casual interviews with farm laborers in both areas by HK indicate that daily per capita fluid intake is up to 3 times higher in the Rift Valley. Mean annual temperatures are 20–38 °C in the Rift Valley (altitude 300–1,800 m) and 10–20 °C in the highlands (1,800–4,600 m), with even greater differences during the hot season (Ethiopian Mapping Authority 1988).

The potential livestock problem

No information is available on fluorosis in livestock in

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Ethiopia. The 1982/83 Agricultural Sample Survey estimated that there were 27 million cattle, 24 million sheep, 18 million goats, 7 million horses and 1 million camels, the largest livestock population in Africa (Ethiopian Mapping Authority 1988). About 40% of the cattle, 75% of the goats, 25% of the sheep and horses, as well as all camels belonged to pastoral nomads in the Rift Valley and other lowland areas (Gedamu 1990). The pastoral nomads indigenous to the Rift Valley, including various Oromo-speaking groups, the Sidamo and the Afar, continue to depend on cattle, camels and small stock as their main source of subsistence. While their livestock and the nomads themselves traditionally drank from surface waters, some herds raised around towns for the rapidly growing urban markets are now given well water, the epidemiological significance of which remains to be studied.

Prevention and control of fluorosis

A combination of lack of resources and low-cost, efficient technology acceptable to the affected populations and bureaucratic bottlenecks have been instrumental in delaying the development of an effective fluorosis control and prevention programme in Ethiopia. Most control efforts were made in Wonji irrigation scheme, where the fluorosis problem was thought to be greatest and where the resources necessary for its control were expected to be generated. Between 1975 and 1977 different surveys found 87–100% of school aged children in different villages with dental fluorosis (Zewde *et al.* 1977; Shifera & Tekle Haimanot 1997).

The defluoridation programme in Wonji, the only one in Ethiopia to date, has been in operation since 1962. The resin activated alumina adsorption technique was used in 1997 but bone char was also used in the past. The major problems – deficiencies in the operation of the defluoridation plants, lack of spare parts and materials, and lack of compliance by the community – resulted in low coverage of the farm's population of 26 000 with defluoridated water and persistently high fluorosis prevalence. Only 2 of the 12 defluoridation plants were in operation in 1997. Even during the 20 years the 12 plants were operating, only 4 plants consistently reduced fluoride levels below 1.5 mg/l. Similarly, fluoride levels varied sharply (in the magnitude of two to twenty-fold) between tests for individual boreholes and between surveys. Thus the lowest levels (all below 1.0 mg/l) were recorded in 1976 and 1979, and the highest rates in 1988 and 1989 (about half of the boreholes with 4–6 mg/l) (Shifera & Tekle Haimanot 1999). These fluctuations indicate not only operational and material supply problems but also reflect the national politico-economic situation during and after the revolution and war. The development of more elaborate Awash River water treatment plants was proposed for the populations of Wonji and Metahara farms but their high cost prevented implementation of this project (McDonald and Partners 1984/85; Belachew 1990). The urgency of either reviving the defluoridation programme or implementing the river water plan was recently expressed by Shifera and Tekle Haimanot (1999).

Currently the Ethiopian government is implementing an extensive centralized piped water supply system in Nazaret town and 7 surrounding rural communities. This project, estimated to cost $115 million, is to supply a population of 162,800 with 41.3 litres of physically and chemically treated water per capita/day by the year 2000. The Ministry of Water Resources, created in 1995 upon the dissolution of the Water Supply and Sewerage Authority, Water Resources Development Authority and Ethiopian Valley Development Authority with the objective of decentralizing the operation and maintenance of water supply activities, is implementing the project. The provision of alternative water sources has significantly reduced the incidence of fluorosis in China and the United States (Smet 1990; Chen *et al.* 1993).

Low-cost defluoridation methods have received increasing attention by Ethiopian research institutions but are not yet technically feasible or culturally acceptable. If successfully developed, these simple methods could benefit especially rural communities that are least likely to obtain piped water supplies in the near future. Particularly the bone-meal and bone-char methods reduce fluoride concentrations efficiently from 6 mg/l to 0.1 mg/l, but they are not acceptable to the general population due to the unacceptable taste of the treated water and to Moslems for religious reasons (Kloos & Tekle Haimanot 1993).

Sengago (1988) refers to a study testing the defluoridation capacity of clay pots and chips from crushed clay pots but these data were not available to us. Bjorvatn and Bardson (1995) and Bjorvatn *et al.* (1997) reported results of laboratory experiments on the effectiveness of lateritic clay. If effective, clay chars, used widely for carrying water in Ethiopia, filters made from fluoride-adsorbing clay, may be developed as simple, feasible methods of fluoride prevention. Development of effective water filtration pottery (to remove turbidity in Sudan, Egypt and elsewhere in North Africa has been dependent on the availability of suitable clay and on relatively highly developed pottery techniques that require kilns rather than open fire (Cembrowicz 1983). Projects using these methods may have to include an education/training component, since pottery as an occupation is pursued mainly by lower socio-economic groups in Ethiopia.

Some rural populations in Ethiopia have traditionally used methods to eliminate turbidity from their household water supplies, including the use of anticoagulant plants and drip containers carved from permeable rocks to control turbidity (Kloos & Tekle Haimanot 1993). The use of lateritic clay, which in its preheated form reduces fluoride at high levels

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(>7 mg/l) to below 1 mg/l, may be explored in Ethiopia, where lateritic clay is abundant in the humid western and southern parts of the country (Murphy 1959). One of the plant materials traditionally used in various African countries to clarify turbid water, the seeds of *Moringa oleifera*, has been shown to reduce in the laboratory fluoride levels from 20 mg/l to less than 1 mg/l at low cost (Stanley & Balasubramanian 1997). It is not known if *M. stenopetala*, which is cultivated as a food plant in Ethiopia (Jahn 1991), has fluoride-absorption properties.

Another little-researched but simple and promising defluoridation method is fishbone charcoal filtration, which has been recommended for use at the household level (Bhargava 1999). The concentration of Ethiopia's lake fisheries in lakes Zway, Langano, Awasa and Abaya in the central and southern Rift Valley would assure an ample supply of fish bones. Health education and technical assistance through community health workers and agricultural extension agents may need to be integrated into any control programme to increase public awareness of the fluorosis problem and encourage the development of this and other potential defluoridation methods in communities lacking alternative water sources.

Discussion

This study confirms earlier reports stating that epidemiologically significant fluoride levels in environmental sources are most widespread in Ethiopia's Rift Valley but indicates that fluorosis may be a public health problem also in some communities outside of the Rift Valley and in livestock. More research is needed on the seasonal distribution of fluoride in surface sources, to determine the usefulness of longitudinal data in evaluating the suitability of surface water sources for domestic use.

Another finding is that commercial agriculture and urban developments in the lowlands since the 1950s and concomitant increase in groundwater use for human consumption appear to have contributed to the spread of the disease. The high fluoride content of boreholes is of particular concern in irrigation schemes and towns in view of the rapidly growing size of the exposed population. Our review revealed various surface and low-fluoride groundwater sources in the Rift Valley, the suitability of which may be evaluated through hydrological studies.

The large-scale government-sponsored community water project in the Nazaret area and organizational changes in the water development agencies, while contributing significantly to preventing fluorosis, cover less than 10% of the Rift Valley population. The magnitude of the task, which extends beyond the Rift Valley and is increased by high population growth rates and a history of halting water development nationwide (Teka 1993), may require that privatization of some water systems or public–private partnerships be considered as well. This approach would be consistent with recent sociopolitical changes in Ethiopia and the trend toward greater privatization of water services in sub-Saharan Africa (Lewis & Miller 1987).

In addition to developing large-scale water supply systems for towns and commercial irrigation farms, there is a need to continue the evaluation of simple, low-cost techniques for use at the household level in semiarid rural areas lacking alternative water sources and piped distribution systems. Health education and community development will have to play major roles in fluorosis risk reduction and active community participation. But their scope needs to be expanded by providing for a role for community groups, local industries and individuals, similar to the control strategies for other water-based diseases (El Karsha & Watts 1997). Strengthening of collaborative projects involving health officials, national water development agencies, research laboratories and medical and social scientists, as well as the communities themselves, may facilitate the development of safe and acceptable water sources on a sustainable basis.

References

Abdo A (1978) A survey of dental fluorosis in Abossa elementary school (abstract). *Ethiopian Medical Journal* **16**, 138.

Baars AJ, Van Beek H, Spierenburg TJ *et al.* (1987) Fluoride pollution in a salt marsh: movement between soil, vegetation and sheep. *Bulletin of Environmental Contamination and Toxicology* **39**, 945–952.

Baker BH, Mohr PA & Williams LAJ (1972) *Geology of the Eastern Rift System of Africa*. Special Paper No. 136 of the Geological Society of America. Washington DC.

Belachew B (1990) *The occurrence and distribution of fluoride and fluorosis in Wonji-Shoa Sugar Estate and adjacent areas*. M.A. thesis, Addis Ababa University.

Bhargava DS (1999) Economical technology of fluoride removal using fishbone charcoal columns at domestic level. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research.

Bjorvatn K & Bardsen A (1995) Use of activated clay for defluoridation of water. In *Proceedings of the First International Workshop on Fluorosis and Defluoridation of Water*. (eds E. Dahi & H. Bregnhoj). International Society for Fluoride Research, Auckland. pp. 40–45.

Bjorvatn K, Bardsen A, Kjellevold M & Tekle Haimanot R (1997) Defluoridation of drinking water by the use of lathyritic clay. *Journal of Dental Research* **76**, Abstract 1273.

Bregnhoj H (1999) Critical parameters in household defluoridation. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research, in press.

Brouwer ID, De Bruin A, Dirks OA & Hautvast JGAJ (1988) Unsuitability of WHO guidelines for fluoride concentration in drinking water in Senegal. *Lancet* **1**, 223–225.

© 1999 Blackwell Science Ltd

Cembrowicz RG (1983) Technically, socially and economically appropriate technologies for drinking water supply in small communities. *Applied Geography and Development* **22**, 74–93.
Central Statistical Office (1991) 1990 *Statistical Abstract*. Central Statistical Office., Addis Ababa.
Chen W, Xu R, Chen G, Zao J & Chen T (1993) Changes in the prevalence of endemic fluorosis after changing water sources in two villages in Guandong, China. *Bulletin of Environmental Contamination and Toxicology* **51**, 479–482.
Dahi E (1999) Contact precipitation a promising method for defluoridation of water. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research, in press.
Dunipace AJ, Brizendine EJ, Zhang W *et al.* (1995) Effect of aging on animal exposure to chronic fluoride exposure. *Journal of Dental Research* **74**, 358–368.
El Katsha S & Watts S. (1997) Schistosomiasis in two Nile delta villages: an anthropological perspective. *Tropical Medicine and International Health* **2**, 846–54.
Ethiopian Mapping Authority (1988) *National Atlas of Ethiopia*. Ethiopian Mapping Authority, Addis Ababa.
Frencken J & Smet J (1990) Notes of the discussion. In *Proceedings of the Symposium on Endemic Fluorosis in Developing Countries: Causes, Effects and Possible Solutions*. (ed JE Frencken), NIPG-TNO, Leiden. pp. 96–98.
Galagan DJ & Vermillion JR (1957) Determining optimum fluoride concentrations. *Public Health Reports* **72**, 491–493.
Gedamu F (1990. Pastoral nomadism and rural development. In *Ethiopia: Rural Development Options*, (ed S. Pausewang) Zed Books, London. pp. 205–212.
Gerasomovskiy VI & Savinova YN (1969) Fluorine contents of volcanic rocks in the rift zone of east Africa. *Geochimie International* **6**, 1124–1128.
Gibb & Partners (1973) *Unpublished data from chemical analyses carried out at the Central Laboratory, Addis Ababa* for Sir Alexander Gibb & Partners, Consulting Engineers, London.
Gilamichael FM (1986) *A study on the prevalence of dental fluorosis among high school students in Nazareth town, Shoa Administrative Region, Ethiopia*. MPH thesis, Department of Community Health, Addis Ababa University.
Gouin P (1979) *Earthquake History of Ethiopia and the Horn of Africa*. International Development Research Centre, Ottawa.
Gumbo FJ & Mkongo G (1995) Water defluoridation for rural fluoride affected communities in Tanzania. In *Proceedings of the First International Workshop on Fluorosis and Defluoridation of Water*. (eds E Dahi & H Bregnhoj), International Society for Fluoride Research, Auckland. pp. 95–100.
Heidweiller VML (1990) Fluoride removal methods. In *Proceedings of the Symposium on Endemic Fluorosis in Developing Countries: Causes, Effects and Possible Solutions*. (ed JE Frencken), NIPG-TNO, Leiden. pp. 51–82.
Jahn SAA (1991) The traditional domestication of a multipurpose tree *Moringa stenopetala* (Bak.f.) Cuf. in the Ethiopian Rift Valley. *Ambio* **20**, 244–247.
Kloos H (1985) Water resources development and schistosomiasis ecology in the Awash Valley, Ethiopia. *Social Science and Medicine* **20**, 609–625.
Kloos H & Tekle Haimanot R (1993) Fluorosis. In *The Ecology of Health and Disease in Ethiopia*. (eds H Kloos & ZA Zein), Westview Press, Boulder and Oxford. pp. 455–461.
Lester FT (1974) Fluoride myelopathy with a review of the literature. *Ethiopian Medical Journal* **12**, 39–49.
Lewis MA & Miller TR (1987) Private-public partnership in water supply and sanitation in Sub-Saharan Africa. *Health Policy Planning* **2**, 70–79.
Mabeleya L, König KG & van Palenstein Helderman WH (1999) Severe dental fluorosis in communities with low fluoride in drinking water–possible explanation. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research, in press.
MacDonald and Partners (1984/85) *Supply of reduced potable water to Wonji-Shoa and Metahara sugar estates*. Ethiopian Sugar Corporation, Addis Ababa.
Malde MJK, Maage A, Macha E & Julshamm K (1997) Fluoride content in selected food items from five areas in Eastern Africa. *Journal of Food Composition and Analysis* **10**, 233–45.
Mohr PA (1971) *The Geology of Ethiopia*. Haile Sellassie I University Press, Addis Ababa.
Murphy HF (1959) *A Report on the Fertility Status of some Soils of Ethiopia*. Experimental Station Bulletin No. 1. Imperial Ethiopian College of Agriculture and Mechanical Arts, Dire Dawa.
Nair KR & Manji F (1982) Endemic fluorosis in deciduous dentition: a study of 1276 children in a typically high fluoride area (Kiambu) in Kenya. *Odontostomatologie Tropicale* **4**, 177–184.
Olsson B (1979) Dental findings in high-fluoride areas in Ethiopia. *Community Dental and Oral Epidemiology* **7**, 51–56.
Schuiling RD (1990) Fluorosis. In *Proceedings of the Symposium on Endemic Fluorosis in Developing Countries: Causes, Effects and Possible Solutions*. (ed JE Frencken), NIPG-TNO, Leiden. pp. 92–94.
Sengago G (1988) Water defluoridation. In *Proceedings of the Symposium on Recent Experiences in Water Resources Development*, (ed S. Alemu). Water Resources Commission, Addis Ababa. pp. 60–67.
Shifera G & Tekle Haimanot R (1999) A review of the defluoridation program of drinking water supplies of an Ethiopian community. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research, in press.
Shupe JL, Bruner RH, Seymour JL & Alden CL (1992) The pathology of chronic bovine fluorosis: a review. *Toxicology and Pathology* **20**, 274–288.
Smet J (1990) Fluoride in drinking water. In *Symposium on Fluorosis in Developing Countries: Causes, Effects and Possible Solutions*. (ed JE Frencken), NIPG-TNO, Leiden. pp. 10–19.
Stanley VA & Balasubramanian MS (1999) Experimental fluoridation by traditional water treatment process using seeds of drumstick. In *Proceedings of the Second International Workshop on Fluorosis and Defluoridation of Water*. International Society for Fluoride Research, in press.
Teka GA (1993) Water supply and sanitation. In *The Ecology of Health and Disease in Ethiopia*, (eds H Kloos & ZA Zein), Westview Press, Boulder and Oxford. pp. 179–190.
Tekle Haimanot R, Fekadu A & Bushra B (1987) Endemic fluorosis

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in the Ethiopian Rift Valley. *Tropical and Geographical Medicine* 39, 209–217.

Teotia SP, Teotia M, Singh DP & Nath M (1987) Deep bore drinking water as a practical approach for eradication of endemic fluorosis in India. *Indian Journal of Medical Research* 85, 699–705.

van Palenstein Helderman WH, Mkasabuni E, Mjengera HJ & Mabelya L (1995) Severe fluorosis in children consuming fluoride containing magadi. In *Proceedings of the First International Workshop on Fluorosis and Defluoridation of Water*. (eds E. Dahi & H. Bregnhoj). International Society for Fluoride Research, Auckland. pp. 15–19.

Warnakulasuriya KA, Balasuriya S, Perera PA & Peiris LC (1992) Determining optimal levels of fluoride in drinking water for hot, dry climates – a case study in Sri Lanka. *Community Dental and Oral Epidemiology* 20, 354–357.

Water Supply and Sewerage Authority (1983) *Thirty-four towns water feasibility study. Report prepared by German Water Engineering for WSSA*. WSSA, Addis Ababa.

Water Supply and Sewerage Authority (1991) *Fluoride test data*. WSSA, Addis Ababa.

WHO (1984a) *Fluorine and Fluorides*. Environmental Health Crueria No. 36. World Health Organization, Geneva.

WHO (1984b) *Guidelines for Drinking Water Quality*, vol. 2, *Health Criteria and Other Supporting Information*. World Heal Organization, Geneva.

Zewde F, Ecclestom BL & Giacometti PI (1977) *The fluoride problem in the Ethiopian Rift Valley*. Awash Valley Authority, Addis Ababa.

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CLINICAL STUDY

Low levels of serum calcidiol in an African population compared to a North European population

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Abstract

Objective: To compare vitamin D status in an African population living at 10 °N with a Norwegian population living at 60 °N.

Design: Serum samples from 30 healthy young Ethiopians and 31 full term pregnant women from Addis Ababa were collected in September, and from 24 healthy Norwegians in March and 23 pregnant women from Oslo in February to June.

Methods: Serum (s) levels of calcidiol and intact parathyroid hormone (iPTH) were measured.

Results: The median values for s-calcidiol were significantly lower in Ethiopians compared with Norwegians (young Ethiopians 23.5 nmol/l vs young Norwegians 81 nmol/l, $P < 0.001$; pregnant Ethiopians 25 nmol/l vs pregnant Norwegians 36 nmol/l, $P < 0.05$) while those for s-iPTH were significantly higher (young Ethiopians 5.7 pmol/l vs young Norwegians 2.4 pmol/l, $P < 0.001$; pregnant Ethiopians 4.8 pmol/l vs pregnant Norwegians 2.8 pmol/l, $P < 0.02$).

Conclusion: In spite of abundant availability of ultraviolet radiation, the population from Addis Ababa had a high rate of biochemical vitamin D deficiency compared with the Norwegian group.

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Introduction

Vitamin D is essential for normal calcium and bone metabolism, and it is derived from oral intake or cutaneous production when the skin is exposed to the ultraviolet B rays contained in sunlight (1). In North America and Europe, the importance of dermal production as a source of cholecalciferol is demonstrated by the seasonal fluctuations in the serum concentration of calcidiol (2). Several studies have shown that people of non-caucasian races moving from a sunny climate to Northern Europe can develop vitamin D deficiency with increased risk of rickets and osteomalacia (3, 4). Differences in serum calcidiol have been demonstrated between white and black people living in the USA (5), but there are few studies on vitamin D status in black people living in Africa. M'Buyamba-Kabangu *et al.* (6) showed that males from Zaïre living in Africa had significantly higher serum (s) calcidiol and non-significantly lower serum intact parathyroid hormone (s-iPTH) than their counterpart who had moved to Belgium. In a study from Nigeria, it was found that pregnant women using veils for religious reasons had significantly lower s-calcidiol levels compared with non-veiled women (7). Addis Ababa is situated at latitude 10 °N, 2700 m above sea-level. We wanted to do a survey on the vitamin D-endocrine system expressed by s-calcidiol and s-iPTH in young, normal Ethiopians and in pregnant Ethiopian women living in Addis Ababa, and compare the values with a Norwegian population living in Oslo at latitude 60 °N at sea-level. As differences in the effect of cholecalciferol could be influenced by polymorphisms in the vitamin D receptor alleles (8), a study of the frequency of a common vitamin D receptor allele in the Ethiopian subjects was also carried out.

Subjects and methods

In Addis Ababa, blood samples were obtained from 102 healthy student nurses aged 19–40 years. The samples were drawn in the first week of September after the period of summer rains. The first 30 serum samples from Ethiopia received in the laboratory (24 men and 6 women, aged 21 (range 20–22) years) were selected for the measurement of s-calcidiol and s-iPTH. For the study of pregnant women, blood samples were drawn

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from 31 healthy full term pregnant Ethiopian women (aged 25 (22–28) years) consecutively entering hospital in August and September. After venepuncture, one aliquot was obtained as an EDTA sample and one as a whole blood sample. The samples containing whole blood were allowed to clot at room temperature, were separated by centrifugation, and serum and EDTA blood were stored at −20 °C. All samples from Addis Ababa were transported by plane frozen on dry ice to the Hormone Laboratory in Oslo for analysis.

In Oslo, blood samples were collected from healthy students and health workers aged 18–35 years in late winter (March) (8 men and 16 women, aged 24 (23–34) years). For the study of pregnant women, blood tests were drawn from 23 full term Norwegian women (aged 28 (22.5–29.5) years) in the period February to June.

The serum concentration of calcidiol was measured using HPLC after extraction and chromatographic separation (9). The intra-assay coefficient of variation (CV) was 10–11%. Serum levels of iPTH were determined in duplicate using a two-site immunoradiometric assay (Allegro intact PTH, Nichols Institute Diagnostics) (9). The intra-assay CV was 6–9%. After extracting DNA from whole blood, a fragment of the vitamin D receptor (VDR) gene was amplified by polymerase chain reaction (PCR) and treated with the endonuclease restriction enzyme BsaMI. VDR alleles were assessed after agarose gel electrophoresis of the digested PCR products and were denoted B to indicate the absence of the restriction site or b in the presence of the restriction site (10).

Results are presented as median and 25 and 75 percentiles. Differences between populations were tested using the Mann–Whitney test. A P value of less than 0.05 was considered statistically significant.

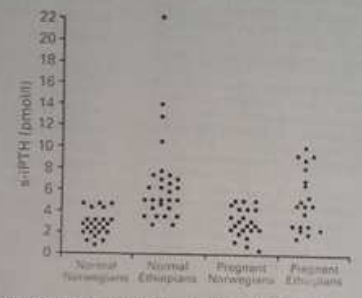


Figure 2 Serum levels of iPTH in normal Norwegians and Ethiopians and in pregnant Norwegians and Ethiopians.

pregnant Norwegians. The normal Ethiopians had significantly lower serum levels of calcidiol (23.5 (18–29) nmol/l vs 81 (67.5–101.5) nmol/l) and higher serum levels of iPTH (5.7 (4.2–7.4) pmol/l vs 2.4 (2.0–3.2) pmol/l) compared with normal Norwegians ($P < 0.001$ for both). Only 2 (8%) of the Norwegians had s-calcidiol below 50 nmol/l, compared with all except one of the Ethiopians (97%). No Norwegians had s-calcidiol below 30 nmol/l, compared with 23 (77%) of the Ethiopians. Seventeen (60%) of the Ethiopians had s-iPTH above the highest Norwegian iPTH value (4.8 pmol/l). Four (14%) of the Ethiopians had values for s-iPTH above 8.5 pmol/l, which is the upper limit of the reference range.

populations were tested after the season of lowest sun exposure. The low vitamin D status expressed as the serum concentration of calcidiol was accompanied by a significantly higher serum level of iPTH in the Ethiopians. The allelic fractions of VDR were not different between the Ethiopians and previous published values from a Norwegian population (11).

The finding of a low vitamin D status in Ethiopians living at 10°N at an altitude of 2700 m was unexpected. It may be explained by clothing habits in Addis Ababa, leaving little skin open to sun exposure, in addition to a low capacity for exposed skin with high melanin content to produce cholecalciferol (12). In addition, while there is no cholecalciferol supplementation of food in Ethiopia, margarine and butter are supplemented in Norway. We do not know whether our findings are applicable to the total Ethiopian population or are the result of the living conditions specific for Addis Ababa.

Low vitamin D status may have a bearing on calcium metabolism and future bone health. We do not, however, have any knowledge on the epidemiology of metabolic bone disease in Ethiopians. As the finding of a low vitamin D status was also made in the full term pregnant women, this could be of importance for the vitamin D status in their newborn children (13). Rickets has been found in as many as 30% of Ethiopian children below 3 years of age (14–16).

In conclusion, in a population from Africa with abundant availability of ultraviolet radiation, low levels of s-calcidiol and increased levels of s-iPTH were found. This might be of clinical importance for disturbances in calcium and bone metabolism both in newborn children and in adults. We do not know, however, whether these metabolic disturbances constitute an important impact on the general health of the population. Further studies are therefore warranted to see if measures should be taken to prevent the low vitamin D status in this African population.

References

1 Reichel H, Koeffler HP & Norman AW. The role of the vitamin D-endocrine system in health and disease. *New England Journal of Medicine* 1989 **320** 980–991.

2 McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. *American Journal of Medicine* 1992 **93** 69–77.

3 Solanki T, Hyatt RH, Kemm JR, Hughes EA & Cowan RA. Are elderly Asians in Britain at a high risk of vitamin D deficiency and osteomalacia? *Age and Ageing* 1995 **24** 103–107.

4 Brunvand L & Haug E. Vitamin D deficiency amongst Pakistani women in Oslo. *Acta Obstetricia et Gynecologica Scandinavica* 1993 **72** 264–268.

5 Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S & Shary J. Evidence for alteration of the vitamin D-endocrine system in blacks. *Journal of Clinical Investigation* 1985 **76** 470–473.

6 M'Buramba-Kabanga JR, Fagard R, Lijnen R, Bouillon R, Lissens W & Amery A. Calcium, vitamin D-endocrine system, and parathyroid hormone in black and white males. *Calcified Tissue International* 1987 **41** 70–74.

7 Okonofua F, Houlder S, Bell J & Davidson P. Vitamin D nutrition in pregnant Nigerian women at term and their newborn infants. *Journal of Clinical Pathology* 1986 **39** 650–653.

8 Dawson-Hughes B, Harris SS & Finneran S. Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 3657–3661.

9 Talch JA, Obrebro H & Haug E. Early postmenopausal bone loss is not associated with a decrease in circulating levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, or vitamin D-binding protein. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 816–843.

10 Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV *et al*. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994 **367** 284–287.

11 Gunnes M, Berg JP, Halse J & Lehmann EH. Lack of relationship between vitamin D receptor genotype and forearm bone gain in healthy children, adolescents, and young adults. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 851–855.

12 Holick SH, MacLaughlin JA & Doppelt SH. Regulation of cutaneous previtamin D_3 photosynthesis in man: skin pigmentation is not an essential regulator. *Science* 1981 **211** 590–591.

13 Brunvand L & Haug E. The estimated free concentration of calcidiol is higher in venous cord blood than in maternal blood. *Scandinavian Journal of Clinical and Laboratory Investigation* 1994 **54** 563–566.

14 US Interdepartmental Commission on Nutrition for National Defence. *1959 Ethiopian Nutrition Survey, Sect. VIII*. pp 62–64.

15 Kettis AA, Bjornesjo KB & Mannheimer IK. The occurrence and clinical picture of the disease in the experience of a paediatric clinic in Addis Ababa. *Ethiopian Medical Journal* 1963 **1** 109–121.

16 Lulseged S. Severe rickets in a children's hospital in Addis Ababa. *Ethiopian Medical Journal* 1990 **28** 175–181.

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MATERNAL AGE, PARITY AND GESTATIONAL AGE ON THE SIZE OF THE NEWBORN IN ADDIS ABABA
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MATERNAL AGE, PARITY AND GESTATIONAL AGE ON THE SIZE OF THE NEWBORN IN ADDIS ABABA

Y. FELEKE and F. ENQUOSELASSIE

ABSTRACT

Objective: To assess birthweight, length and head circumference of live births, and to examine the effect of maternal age, parity and gestational age on birth sizes of the live births.
Design: A prospective study.
Setting: Tikur Anbessa, Zewditu, Gandhi memorial and St. Pauls hospitals, in Addis Ababa, Ethiopia.
Subjects: Four thousand two hundred and six consecutive live births from July 1996 to January 1997.
Main outcome measures: Weight, length and head circumference of the newborns.
Results: Among the 4206 consecutive live births, 4047 were singleton and 159 were twins. Two thousand one hundred and sixty (51.7%) were males and 2046 (48.6%) were females. The mean birthweight of singleton live births was 3065 ± 465 g, with modal group of 3000-4000 g. The incidence of low birthweight was 9.1%. The mean length for all singleton live births was 48.6 ± 3.3 cm and modal group of 46-52 cm. The mean head circumference was 34.4+2.9 cm; modal group 31-37 cm. The incidence of low birthweight of the newborns was significantly higher for females, younger maternal age, primiparas and pre-term babies. On the multivariate analysis, gestational age and sex of the newborn respectively had significant effects on birthweight, length and head circumference of the neonates controlling for the other variables. On the other hand parity and maternal age had significant effects only on the birthweight of the neonates.
Conclusion: This study has provided information on the effects of some of maternal characteristics on the size, particularly length and head circumference of newborns which were not given emphasis on previous birthweight studies. We suggest proper recording and analysis of birthweight, length and head circumferences be given importance for monitoring and evaluating maternal and child health programmes.

INTRODUCTION

Anthropometric data on neonates provide useful information and at any given time indicate the development status of the child(1). Birthweight is recognised as a reliable indicator of intrauterine growth and one of the major factor determining child survival and future growth(2-4).

Low birthweight and prematurity are important contributors to neonatal mortality and morbidity especially in developing countries, where these conditions are common(5).

Different studies have reported the distribution of birthweight for Ethiopian babies ranging from 2950g to 3243g and low birthweight rate of 8.0%-12.3%(6-12). However little is known about the size of head circumference and length of the Ethiopian neonates. No emphasis was given to measurements of head circumference and length at birth. However, several studies have showed head circumference to have significant association with microcephaly at the early childhood and a large number of other studies have documented a strong correlation between size at birth and subsequent height, although the repeated incidence of catchup growth varied within time and between countries(13,14). Niklasson *et al*(15) showed that infants with growth hormone deficiency had normal length at birth(15).

The aforementioned studies indicate that attention need be given to birth length and head circumference when size at birth is evaluated.

The main objectives of this study were to assess birthweight, length and head circumference of live births at the four referral hospitals in Addis Ababa, Ethiopia, and to examine the effects of maternal age, parity, and gestational age to birthweight height and head circumference of the live births.

Amir sultan (MD), 2014

MATERIALS AND METHODS

The study was conducted at the four referral hospitals, Tikur Anbessa, Zewditu, Gandhi Memorial and St. Pauls, in Addis Ababa, Ethiopia from July 1996 to January 1997. Birth sizes of a total of 4206 consecutive live births were measured. The following information were included in the questionnaire; age of the mother, parity, gestational age, address, sex, Apgar score, weight, length, head circumference of the neonate and type of delivery. The data were recorded by midwives who were orientated about the purposes and techniques.

The naked infant was weighed to the nearest 10gm on seca infant scale. The scale was checked periodically. The maximum occipito frontal head circumference was measured to the nearest 0.1 cm with non-stretch tape. Infant length was measured to the nearest 1 cm. The duration of gestational age was calculated from the mothers last menstrual period. The gestational age was classified as unknown if the date of the last menstrual period could not be ascertained.

Preterm refers to infants less than 37 completed weeks, term infants between 38-42 weeks and post term infants greater than 42 weeks. Low birthweight refers to infants less than 2500gm(16). Parity was determined taking into consideration all the number of pervious pregnancies resulting in either live births or still births after 28 weeks of gestational age.

Student's t-test was used to compare differences between mean values. Multiple linear regression was used to determine the effects of maternal age, parity and gestational age on the size of the newborns.

RESULTS

Among the 4206 consecutive live births, 4047 were singletons and 159 were twins. Two thousand one hundred and sixty (51.7%) were males and 2046 (48.6%) were females. There were 3105 (74.9%) normal deliveries and 1043 (25.1%) abnormal deliveries. Caesarean sections were done in 495 (11.8%), forceps in 185 (4.4%), vacuum in 249 (5.9%), breech in 92 (2.8%) and other assisted deliveries in 22(0.5%).

Among the 159 twins, 76 were males and 83 were females. The mean±SD weight for twins was 2542 ± 4579. 51.6 were below or equal to 2500g. Their mean ± SD length was 46.6 ± 3.2 cm while their mean ± SD head circumference was 33.2 ± 2.2cm. 159 twins were excluded from the rest of the analysis.

Among the 4047 singletons, 4010 live births were included because of the adequate information, their mean birthweight was 3065 ± 465g, with modal group of 3000-4000g ranging between 800-5200g. The incidence of low birthweight was 9.1% and 2.3% weighed more than 4000g. The mean length for all singleton live births was 48.6 ± 3.3cm; median, 49cm and modal group of 46-52cm. The mean head circumference was 34.4 ± 2.9cm, median 34cm, modal group 31-37cm.

Table 1

Effect of sex, maternal age, parity, gestational age on weight, length and head circumference of the neonates

Factor	Weight			Length		Head circumference	
	No.	Mean (SD)	LBW	No.	Mean (SD)	No.	Mean (SD)
Sex							
Male	2069	3111±471	8.1%	1971	48.9±3.3	1931	34.5±8.4
Female	1939	3014±452	10.1%	1827	48.4±3.3	1795	34.3±8.3
		P<0.001	P<0.05		P<0.001		P<0.05
Maternal age							
<19	483	2904±432	13.9%	463	48.1±3.2	455	33.9±3.5
20-24	1246	2992±428	10.7%	1183	48.6±3.0	1166	34.1±2.4
25-29	1255	3105±472	7.7%	1181	48.7±3.8	1152	34.5±3.1
30-34	626	3158±482	7.3%	599	48.8±3.6	590	34.6±2.8
≥35	397	3213±466	5.0%	370	49.0±3.0	361	34.9±2.9
		P<0.01	P<0.001		P<0.001		P<0.01
Parity							
One	1767	2054±433	11.7%	1676	48.4±3.0	1636	34.0±2.8
Two	896	3091±445	6.9%	846	48.7±3.6	850	34.5±3.0
Three	541	3160±488	8.3%	502	48.8±3.2	495	34.1±3.1
Four	309	3172±435	8.1%	297	49.0±3.3	292	34.8±3.0
Five	171	3282±435	2.3%	159	48.9±4.7	155	35.1±3.0
Six	104	3229±576	8.7%	98	48.9±3.0	95	34.9±3.0
>Seven	179	3248±491	4.5%	171	48.8±4.0	167	34.6±2.0
		P<0.001	P<0.01		P<0.05		P<0.05
Gestational age							
<37	417	2878±516	19.1%	393	48.0±3.3	384	33.7±2.8
38-42	2932	3087±448	7.4%	2801	48.7±3.4	2760	34.4±2.9
>42	237	3206±475	5.5%	222	49.3±2.6	218	39.5±2.2
		P<0.001	P<0.001		P<0.001		P<0.001

Table 2

Estimates of the regression coefficient in multiple linear regression of the variables of interest over weight, length and head circumference of the neonates.

Variable	Weight β (95% CI)	Height β (95% CI)	Head circumference β (95% CI)
Mothers age (in years)	8.11 (4.78,11.44)	0.02 (-0.01,0.05)	0.04 (0.02,0.06)
Parity	28.81 (18.90,38.72)	0.03(-0.05,0.10)	0.02(-0.05,0.08)
Gestational age (in weeks)	50.09 (12.67,57.42)	0.20 (0.14,0.26)	0.14 (0.09,0.18)
Sex female	-100.98 (-130.26,-71.69)	-0.44(-0.67,-0.22)	-0.19(-0.38,-0.001)

Table 1 shows that mean birthweight, length and head circumference were significantly lower for females than for males. More than 10% of the females had low birthweight as compared to 8.1% of males. Mean birthweight, and the incidence of low birthweight respectively increased with maternal age. Babies of mothers younger than 19 years of age had the lowest mean birthweight (2904 ± 432) and the highest incidence of low birthweight (13.9%).

Birthweight of the neonates increased significantly ($p<0.0001$) with parity. Babies of zero parity had the lowest mean birthweight (2054 ± 433) and the highest incidence of low birthweight (11.7%).

Also as shown in Table 1, the length and head circumference of the neonates increased significantly with maternal age, parity and gestational age.

Table 2 shows the estimate of coefficients using multiple linear regression over birthweight, head circumference and length of the neonates. Maternal age had significant effect on birthweight and head circumference adjusting for the other variables but the effect of maternal age on length of the newborn disappeared when adjusted. Controlling for maternal age, gestational age and sex of the neonate, the effect of parity remained significant only on birthweight. On the other hand, both gestational age and sex of the neonate had significant effects on birthweight, length and head circumference of the newborns respectively.

Birthweight, length and head circumference of the newborns increased significantly with gestational age adjusting for sex, maternal age and parity. Similarly, birthweight, length and head circumference were significantly lower for females than for males controlling for maternal age, gestational age and parity.

DISCUSSION

The mean length of Ethiopian infants was lower by 9 cm as compared to Saudi singleton infants which was 49.5±2.0 cm and similarly the head circumference of Ethiopian infants was also lower (34.4 ± 2.9cm versus 34.5 ± 1.5cm)(17). On the other hand, the mean birthweight, length and head circumference of Brazilian infants were lower than the Ethiopian infants by 352 gm, 1.1 cm, 1.7cm, respectively(18).

As in other birthweight studies in Ethiopia, the mean birthweight for male babies was found to be higher than that of the female babies and females had significantly higher incidence of low birthweight (6-12). Our study showed that babies born of mothers aged 19 years and below had birthweight of 100g-300g lower than those mothers older than 19 years. Similarly, the low birthweight rate is significantly higher in this age group. When the other variables are controlled in the multivariate analysis, the effect of maternal age on birthweight is statistically significant. Data from some reports are in agreement with these findings(10,12,14).

Many studies have shown the impact of parity on birthweight(8,9,11,12) and the same was observed in this study. Mean birthweight was considerably lower for primigravidae and the proportion of low birthweight correspondingly higher. This could be explained by the fact that there is a general agreement that pregnancy outcomes are more favourable for multiparae than primiparae.

The major determinant of birthweight in this study was gestational age. Gestational age was found to be a strong, independent predictor of birthweight. The mean birthweight increased on average by 50g per week after controlling for maternal age, parity and sex of the baby. Similar findings were reported by Serenius *et al.*(17) from Saudi and Gebre-Medhin *et al.*(8) from Ethiopia, except that weight increased only up to 41 weeks, which was not detected in our study. The mean birthweight after 42 weeks gestation in our study was higher than that of 41 weeks of gestation.

This study has provided information on the effects of some of maternal characteristics on the size, particularly length and head circumference, of newborns which were not given emphasis on previous birthweight studies. It is suggested that birthweight, length and head circumference be given importance as essential indicators for monitoring and evaluating maternal and child health programmes.

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REFERENCES

1. Nelson, W.E. Text book of paediatrics, 14th ed. Growth and development 13-14 Saunders, Philadelphia, 1992.
2. Ramankuttyp, Tikreeti R.A.S., Rasaam, K.W., Al-Thamery, D.M., Yacoub A.A.H. and Mahmood D.A. A Study on birthweight of Iraqi Children. *J. trop. Paediat.* 1983; **29**:5-10.
3. Oni, G.A. The effects of maternal age, education and parity on birthweight in a Nigerian Community. The comparison of results from bivariate and multivariate analyses. *J. trop Paediat.* 1986; **32**:295-300.
4. Hardy, J.B. Birthweight and subsequent physical and intellectual development. *N. Engl. J. Med.* 1973; **289**:973-974.
5. Villar, J. and Belizan, J. The relative contribution of prematurity and fetal growth retardation to low birthweight in developing and developed societies. *Amer. J. Obstet. Gynaec.* 1982; **143**:793-98.
6. Hofvander, Y. A survey of 3000 children examined at the "Mobile Child Health Centre" in Addis Ababa in 1962. *Ethiop. Med. J.* 1963; **1**:156.
7. Young, P.N. Birthweights of hospital-delivered infants in Addis Ababa and Gondar. *Ethiop. Med. J.* 1968; 6:15.
8. Gebre-Nedhin, M., Gurovsky, S. and Bondstam, L. Association of maternal age and parity with birthweight, sex ratio, still births and multiple births. *J. trop. Paediat.* 1976; **22**:99-102.
9. Zein, A., Gebre-Kidan, K., Eshete, N. and Haile, N. *et. al.* Birthweight of hospital delivered neonates in Gondar, North Western Ethiopia. *Ethiop. Med. J.* 1985; **23**:59-63.
10. Green, A. Changes in birthweight distribution from 1973 to 1982 in Addis Ababa. *Bull. Wld Hlth. Org.* 1987; **64**:711-714.
11. Shiferaw, T. Some factors associated with birthweight in Jima, Southwestern Ethiopia. *Ethiop. Med. J.* 1990; **28**:183-190.
12. Nadebo, T. A two year retrospective study of birthweight in Sidamo regional Hospital. *Ethiop. Med. J.* 1990; **32**:255-257.
13. Skull, S.A., Ruben, A.R. and Walker, A.C. Malnutrition and microcephaly in Australian aboriginal children. *Med. J. Aust.* 1997; **166**:412-14.
14. Karlberg, J., Albertsson, W.K., Baber, F.M., Low, L.C. and Yeung, C.Y. Born small for gestational age: Consequences of growth. *Acta Paediat.* suppl 1996; **417**:8-13.
15. Niklasson, A., Karlberg, P. and Albertsson, W.K. The normal weight for length in new born infants in whom growth hormone deficiency was later diagnosed. *Acta Paediat.* 1994; **83**:192-5.
16. Kramer, M.S. Determinant of low birthweight: methodological assessment and meta-analysis. *Bull. Wld. Hlth. Org.* 1987; 65:663-737.
17. Serenius F., Edressee A.W. and Swailem A.R. Size at birth of infants in a Saudi maternity hospital. *Acta paediatr. Scand.* (suppl) 1988; **346**:44-56.
18. Paine, P.A., Pasquali, L. and Joaquim, M.C.M. Effects of birthweight and gestational age upon growth in Brazilian infants. A longitudinal study. *J. trop. Paediat.* 1983; **29**:11-17.

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PREVALENCE AND CLINICAL FEATURES OF TUBERCULOSIS IN ETHIOPIAN DIABETIC PATIENTS

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ABSTRACT

Objective: To determine the prevalence and clinical characteristics of tuberculosis (TB) in diabetic patients.

Design: This study was a cross-sectional survey based on the retrospective analysis of data on tuberculosis in diabetic patients.

Settings: The study was conducted at Endocrinology and Metabolism Unit of the Department of Internal Medicine, Faculty of Medicine, Addis Ababa, Ethiopia.

Subjects: Seventy eight cases of tuberculosis among 1352 diabetic patients were included from September 1989 to 1996.

Main outcome measures: Clinical evaluation, chest x-ray, acid fast bacilli (AFB) in sputum or measures tissue and histopathologic characteristic of biopsy specimens.

Results: Seventy eight cases of TB were identified among 1352 diabetic patients giving a prevalence of 5.8%. Among 1352 diabetic patients, 713 (52.7%) were males, 639 (43.3%) were females, 619(45.8%) were IDDM and 733 (54.2%) were NIDDM. The mean age of the 71 TB patients whose records could be retrieved was 34.0±11.9 years, 42(59%) were males, 29 (41%) were females. Fifty four (76.1%) were IDDM and 17(23.9%) were NIDDM, 17 of the IDDM had clinical characteristics of malnutrition-related diabetes mellitus (MRDM), 36 (56%) of 71 patients were admitted for management. The three most common symptoms of tuberculosis were fever (80.5%), sweating (80.4%) and cough (70.5%). Twenty six (36.6%) of 71 cases were positive for AFB and six (8.5%) were seropositive for HIV. Fifty six (78.9%) had pulmonary, eight (11.2%) had extrapulmonary and seven (9.8%) had disseminated TB. Forty eight of 53 abnormal chest x-ray showed unilateral involvement. Thirty eight of 41 (53.6%) had completed TB treatment, relapse occurred in seven (9.8%), eight (11.2%) are currently on treatment, 13 (18.3%) were lost to follow up, four (5.6%) defaulted and three (4.2%) died. The relative risk of developing TB in IDDM and NIDDM patients was being 26 times and seven times than the general population respectively.

Conclusion: The prevalence of tuberculosis in the diabetic population is high and this warrants a prospective study to determine association between pulmonary tuberculosis and diabetes.

INTRODUCTION

Tuberculosis has long been a major cause of morbidity and mortality in Ethiopia. According to the Ministry of Health report of 1995(1), tuberculosis accounted for 3.7% of outpatient morbidity, 9.4% of hospital admissions and 27% of hospital deaths(1,2). The group mainly affected is that between 15 and 45 years. In such an environment with underdeveloped health care facilities and a high prevalence of tuberculosis, diabetics could be expected to be at an even greater risk of contracting the disease than non diabetics because of their generally poorer state of health engendered by inadequate diabetic control.

Available information on the prevalence of tuberculosis in diabetics in sub-Saharan Africa shows that it is common and its prevalence is significantly higher in diabetics than in the general population(3-6). A report from this country by Lester *et al*(7) in 1976 showed a prevalence of 13% in 404 diabetics. Another report by Lester in 1984(8) showed a higher prevalence level of 3.9% in 847 patients. These recent prevalence levels in African diabetics are comparable to those reported in American diabetics in the pre-insulin era(5) suggesting that the current high prevalence of tuberculosis in African diabetics is related to the high prevalence of tuberculosis in the population and the shortcomings of diabetics care in Africa than to an inherently higher susceptibility of African diabetics to tuberculosis.

The purpose of this study on tuberculosis in Ethiopian diabetics was to determine its current prevalence and clinical characteristics, as seen in patients attending the diabetic clinic of the Endocrinology and Metabolism unit of the Department of Internal Medicine, because the earlier report on this topic from Ethiopia in 1984(8) antedated the HIV epidemic.

MATERIALS AND METHODS

A review of the records of 1352 patients seen at the endocrinology and metabolism unit of the Department of Internal Medicine, Medical Faculty, Addis Ababa University, from September 1989 to 1996 revealed 78 cases had developed tuberculosis. The records of 71 of them could be retrieved for analysis. Diagnosis of pulmonary tuberculosis was based on clinical evaluation plus chest x-ray and/or detection of acid fast bacilli (AFB)) in the sputum. Extrapulmonary tuberculosis was diagnosed on the basis of clinical evaluation and demonstration of acid fast bacilli in tissue or body fluid or the characteristic histopathology in a biopsy specimen. The patients were seen at the diabetes clinics where follow up occurs at intervals of eight to 20 weeks, after the initial period of stabilisation, and every seven to 15 days during pregnancy.

RESULTS

Among the 1352 diabetic patients, seventy eight cases of TB were identified, giving a prevalence of 5.8%. Seven hundred and thirteen (52.7%) were males, 639 (47.3%) females; 619 (45.8%) IDDM and 733 (54.25%) were NIDDM. Among the 71 patients, 54 (76.0%) had IDDM and 17(23.9%) NIDDM. Seventeen of the IDDM patients, had clinical characteristics commensurate with those of malnutrition-related diabetes mellitus (MRDM)(9). The age, sex and type of diabetes in patients diagnosed with tuberculosis is shown in Table 1. The mean age of the 71 patients with tuberculosis was 34±19 years (range 14-85 years)

Table 1

Age and sex distribution of diabetic patients with tuberculosis

	IDDM (N = 54)						NIDDM (N = 17)							
	M	%	F	%	Total		M	%	F	%	Total		Grand Total	
Age in years					No	%					No	%	No	%
14-19	2	3.7	0	-	2	3.7	0	-	0	-	0	-	2	2.8
20-29	18	33.3	8	14.8	26	48.1	0	-	0	-	0	-	26	36.6
30-39	11	20.3	8	14.8	19	35.2	1	5.8	1	5.8	2	11.8	21	29.6
40-49	3	5.6	1	1.9	4	7.4	2	11.8	3	17.6	5	29.4	9	12.8
50-59	1	1.9	1	1.9	2	3.7	2	11.8	6	35.3	8	47.0	10	14.1
60+	1	1.9	0	-	1	1.9	1	5.8	1	5.8	2	11.8	3	4.2
Total	36	66.7	18	33.4	54	100.0	6	35.3	11	64.5	17	100	71	100

Table 2

Clinical characteristics of tuberculosis in diabetic patients

Presenting complaint	Yes		No		No information	
	No.	(%)	No.	(%)	No.	(%)
General	57	80.5	5	7.0	9	12.7
Fever	57	80.5	7	9.9	7	9.9
Sweating	57	80.5	5	7.0	9	12.7
Weight loss	5	7.0	40	56.3	26	36.6
Contact with chronic cougher						
Pulmonary	54	76.1	9	12.7	8	11.3
Cough	12	16.9	0	-	59	83.1
Chest pain	4	5.6	67	94.4	-	-
Haemoptysis						
Extra pulmonary	1	1.4	-	-	-	-
Swelling of wrist joint	1	1.4	-	-	-	-
Abdominal pain and diarrhoea	1	1.4	-	-	-	-
Hematuria	1	1.4	-	-	-	-
Lymphadenopathy						

Clinical characteristics of patients with tuberculosis are shown in Table 2. Fifty six (78.9%) patients had pulmonary TB, eight (11.2%) had extrapulmonary TB and seven (9.9%) had disseminated tuberculosis. The duration of symptoms of tuberculosis varied from two weeks to one year. The most frequent symptoms of tuberculosis were fever, sweating and cough which occurred in 57 (80.5%), 57 (80.5%) and 54 (76%) patients respectively. The duration of diabetes at the time of diagnosis of tuberculosis in diabetic patients ranged from onset to 25 years.

Among those with extrapulmonary tuberculosis, one patient presented with swelling and pain in the wrist joint. Synovial biopsy showed histopathology consistent with tuberculous arthritis. The patient completed his antituberculosis therapy, but two years later developed smear positive pulmonary tuberculosis and was found to be HIV positive.

A patient who presented with persistent pyuria and later found to have an echodense mass in the right kidney was admitted to the surgical ward with the diagnosis of hypernehproma. He subsequently developed cough, sweating and pleural effusion whose analysis and cytology were consistent with tuberculosis. Urine culture for AFB was not done. Both pleural effusion and renal mass resolved following antituberculosis treatment.

Two other patients presented with productive cough, fever, and sweating. Their physical findings and chest x-rays were normal but sputum specimens were positive for AFB. At the time of this study, both of them had completed treatment and were doing well during last follow up visits. Twenty six (46.4%) of 56 pulmonary tuberculosis patients were smear positive for AFB. Erythrocyte sedimentation rate was normal in two (both of whom had extrapulmonary tuberculosis), elevated in 64 patients, unrecorded in five of 71 patients. The mean fasting blood glucose (FBG) in 59 of 71 patients at the time of diagnosis of tuberculosis (those for whom the information was available) was 301.3mg/dl. Only six of the 59 patients had FBG less than 140mg/dl.

Chest x-ray reports were available in 58 of the 71 patients, while findings were normal in five, and abnormal in 53 of the patients showing unilateral single or multiple lobe involvement in 48 and bilateral involvement in five. Among 53 chest x-rays, pulmonary parenchymal involvement consisting of interstial, alveolar infiltration or consolidation was found in 33 of 53 chest x-rays, pleural effusion in seven, interstitial infiltrate in three, pneumothorax in two, cavitary lesion with infiltration in five, atelectasis in three, lung absecess in one. Four of abnormal chest x-rays showed honey combing appearance with interstitial infiltrate which was suggestive of bronchiectasis in four patients. Six of the 71 patients were HIV positive. Patients were treated with three to five antituberculosis drugs(INH, rifampicin, ethambutol, pyrazinamide and streptomycin) for six to 12 months. Thirty six (50.7%) patients required admission to the hospital for control of diabetes and management of their tuberculosis treatment.

Outcome of treatment in patients with tuberculosis: Thirty eight (53.6%) out of the 71 diabetic patients with tuberculosis had completed antituberculosis treatment. Four (5.6%) patients defaulted while relapse occurred in seven (9.8%) patients out of which four had bronchiectasis. Six (8.5%) patients were still on treatment while three patients had died. In two of the deaths, the cause of death was not related to tuberculosis. One died of hepatic coma, the second of sepsis and the third patient developed evidence of intracranial space occupying lesion while she was on antituberculosis treatment though the cause remained undetermined since no autopsy was done.

The mean duration of follow up after completion of treatment in 58 patients was 20±15.2 months (range of one month to six and a half years), 13 (18.3%) patients were lost to follow up after initiation of antituberculosis medication.

DISCUSSION

The tuberculosis prevalence of 5.8% in our patients was higher than the 3.9% previously reported from this country by Lester in 1984(8). This may be due to an increasing incidence of tuberculosis in the community and poor control of diabetes which increases the risk for infections in general. Tuberculosis was more common in IDDM than in NIDDM and in the former group more common among MRDM diabetics (which is a most socioeconomically underprivileged group)(9). The mean ±SD age of our diabetic patients with tuberculosis was 34±19 years as compared to our diabetic patients which was 41±14.4 years. A study done by Vucinie et al.(11) from Yugoslavia, showed that most tuberculosis diabetic patients were IDDM but more than 40 years old while the highest prevalence of tuberculosis was in those aged 60-70 years (40%). The relative risk for our IDDM and NIDDM respectively being 26 and seven times that in the general population(10).

The most frequent symptoms were fever, sweating and cough (80.4%, 76% and 80.4%) compared to the report by Vucinie *et al*(11) which showed fever and weight loss in 82% and 72%, respectively. In this report, 8.4% of diabetic patients with tuberculosis were seropositive for HIV which could be one of the reasons why the prevalence of tuberculosis is increasing in diabetic patients. There was no strong association between development of tuberculosis and duration of diabetes in our study but there was a significant association between development of tuberculosis and degree of hyperglycaemia because 90% of tuberculosis patients were severely hyperglycaemic (the mean fasting blood glucose level of 301.3 (112.9 mg/dl) at the time of diagnosis of tuberculosis. The mean FBS of those without tuberculosis was 191.2±60mg/dl (p value <0.05)

In our study, 81.6% of the patients developed

tuberculosis within ten years of the diagnosis of diabetes as compared to the study done by Turner(12) which showed 62% developing tuberculosis in similar time period. In our patients, radiographic appearance at the start of treatment showed a unilateral involvement in 82.2% and bilateral involvement in 17.8% as compared to the study done by Luntz(13) which showed 43% and 57% respectively.

Ninety per cent of our patients had a high ESR and multiple lobe involvement which was similar to the report by Ekim *et al*(14). Relapse rate in our study was ten per cent as compared to the study done by Turner, in which relapse rate was 36%(12). This could be attributable to the improved combination antituberculosis treatment in our cases. The combination of three to five antituberculosis drugs (ethambutol, INH, pyrazinamide, streptomycin and rifampicin) were given to the patients based on the national policy of tuberculosis management(1,2). However, in situations of scarcity of drugs, the anti TB drug regimen was appropriately modified.

In general tuberculosis was diagnosed in diabetic patients at an advanced disease stage and 50% of the patients required inpatient management. The young IDDM patients were mainly affected. In order to justify these facts, it is advisable to do a prospective study and also determine the serology status of HIV in the diabetic patients.

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REFERENCES

1. National Tuberculosis and Leprosy Control Programme, Ministry of Health, Ethiopia. 1997; 1st Edit. 9-27.
2. Guideline for the National Tuberculosis Control Programme In Ethiopia, Addis Ababa, 1992.
3. Mugusi, F. Diabetes and pulmonary tuberculosis in Tanzania (Dissertation). Dar es Salaam, Tanzania, University of Dar es Salaam, 1987.
4. Mugusi, F., Swal A.B.M., Alberti K.G.M.M and McLarty D.G. Increased prevalence of diabetes mellitus in patients with pulmonary tuberculosis in Tanzania. *Tubercle* 1990; 71:271-276
5. McLarty, D.G., Pollitt, C. and Swali, A.B.M. Diabetes in Africa. *Pract. Diabetes Digest.* 1992; 3:35-40.
6. Adetuyibi A. Diabetes in the Nigerian African Review of Longterm complications. *Trop. geogr. Med.* 1976; 155-168.
7. Lester, F.T., Abdulkadir, J., Lalson, D. and Qana'a P. Diabetes Mellitus Clinical features in 404 Ethiopians. *Ethiop. Med. J.* 1976; 14:185-197.
8. Lester, F.T. Tuberculosis In Ethiopian Diabetics. *Ethiop. Med. J.* 1984; 22:129-133.
9. Abdulkadir, J., Mengesha, B., Weldegebriel, Z., Keen, H., Worku, Y., Gebre, P., Bekele, A., Urga, K. and Tadesse, A.S. The Clinical and hormonal (C-peptide and glucagon) profile and liability to ketoacidosis during nutritional rehabilitation in Ethiopian patients with malnutrition-related diabetes mellitus. *Diabetologia.* 1990; 33:222-227.
10. Demissie, M., Gebeyehu, M. and Berhane, Y. Primary resistance to antituberculosis drugs in Addis Ababa, Ethiopia. *Int. J. Tuberc. Lung. Dis.* 1997; 1:64-67.
11. Vucinic, V., Duric, O., Zugic., V. and Videnovic, J. Tuberculosis in patients with diabetes mellitus Tubercle and lung disease 1995; 76:39.
12. Warmick, M.T. Pulmonary tuberculosis and diabetes mellitus. *Quart. J. Med.* 1957; 26:31-41.
13. Luntz, G.R.W.N. Management of Tuberculos diabetic *Brit. Med. J.* 1957; 2:1082-1086.
14. Ekim, N.N., Cilli, A., Guz, G. and Gazi A.M. Pulmonary

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ORIGINAL ARTICLE

Effects of HIV Co-infection and Chemotherapy on the Urinary Levels of Nitric Oxide Metabolites in Patients with Pulmonary Tuberculosis

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The presence of nitric oxide (NO) and its role as a factor in host defence against intracellular pathogens in human macrophages is controversial. We measured the metabolites of NO (nitrite (NO_2^-) and nitrate (NO_3^-)) in urine from Ethiopian patients suffering from tuberculosis. The urinary level of NO_2^-/NO_3^- in a group of healthy Ethiopians was 1020 ± 471 μM ($n = 22$). Untreated HIV negative patients with active pulmonary tuberculosis (1574 ± 588 μM, $p < 0.01$, $n = 12$) and household contacts to tuberculosis patients (1949 ± 812 μM, $p = 0.006$, $n = 7$) had significantly higher levels of urinary NO_2^-/NO_3^- than the control group. Untreated HIV positive patients with pulmonary tuberculosis did not have increased levels of urinary NO_2^-/NO_3^- (1101 ± 614 μM, $n = 6$). Some of the HIV negative untreated patients with pulmonary tuberculosis (1710 ± 519 μM, $n = 6$) were followed up after treatment and showed a reduction in the levels of urinary NO_2^-/NO_3^- 1 week after treatment (945 ± 599 μM, $p < 0.05$). We conclude that HIV negative patients with active pulmonary tuberculosis have increased urinary levels of nitric oxide metabolites with a reduction following specific anti-tuberculous chemotherapy.

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INTRODUCTION

Tuberculosis caused by Mycobacterium tuberculosis is a global health problem of escalating proportions. In 1990, 8 million new cases were reported, 95% of them in developing countries where co-infection with HIV is common (1). Most individuals infected with M. tuberculosis do not develop clinical disease, which indicates effective defence mechanisms within the host.

Evidence is accumulating from in vitro experiments and animal models that nitric oxide (NO) produced by macrophages is an important factor in host defence against intracellular pathogens such as M. tuberculosis (2–5), M. leprae (6, 7) and Leishmania (8, 9). The presence of NO and its role as a factor in host defence in humans is controversial (10–13), but active expression of the enzyme responsible for the production of NO (inducible NO-synthase (iNOS)) has been detected in blood mononuclear cells from patients with rheumatoid arthritis and malaria (14, 15), neutrophils from patients with bacterial urinary tract infection (16) and alveolar macrophages from patients with tuberculosis (17). Inducible NO synthase (iNOS) is responsible for the synthesis of NO and L-citrulline from L-arginine by activated macrophages. NO is highly unstable and decays to its stable end products nitrate and nitrite, which are eliminated in the urine (18).

In 1981 it was shown by long-term metabolic studies that endogenous synthesis of nitrate occur in humans. The amounts excreted in urine were significantly higher than could be accounted for by intake of food or air (NO_2) (19). Bacillus Calmette-Guerin (BCG) infection of mice resulted in a large increase in urinary nitrate production over the course of infection and iNOS-inhibitor administration to the animals prior to BCG-infection blocked BCG-induced nitrate excretion (20). INOS-inhibitor administration to mice infected by virulent M. tuberculosis also resulted in increased mortality, bacterial burden and pathological tissue damage (3). High levels of nitrate and nitrite have been observed in serum from patients with gastroenteritis (21), malaria (15) and in urine from children with coeliac disease (22). We have also recently shown that leprosy patients in reversal reaction have increased urinary levels of NO metabolites (23).

The aim of this study was to investigate the levels of nitric oxide metabolites in urine from patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Materials

The materials and suppliers were: Aspergillus nitrate reductase (Boehringer Mannheim, Freiburg, Germany); NADPH as the tetrasodium salt (Boehringer Mannheim, Freiburg, Germany); sulphanilic acid (Merck, Darmstadt, Germany); sodium nitrite (Merck, Darmstadt, Germany); sodium nitrate (Sigma Chemical Co., St Louis, USA); N-(1-naphtyl) ethylenediamine (Sigma Chemical Co., St Louis, USA); phosphate buffered saline (PBS).

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Collection of patient samples

The diagnosis of active tuberculosis was made when a patient was acid fast bacilli (AFB) positive by microscopy, and had X-ray findings and a clinical picture suggestive of active tuberculosis. Patients ($n = 18$, 10 M, 8 F, average age 26 ± 8 y, age range 16–42 y) were previously untreated for tuberculosis, investigated for HIV-status by serology (Enzygnost anti-HIV 1/2 plus, Behring Diagnostic Inc., Germany) and had no clinical signs of any other co-infection. The household contacts of tuberculosis patients ($n = 7$, 5 M, 2 F, average age 32 ± 13 y, age range 16–51 y) were apparently healthy individuals who were family members and close contacts to open tuberculosis-cases. These patients were sputum negative for tuberculosis by culture, HIV negative by serology and had no pathological findings on X-ray. The control group for urinary levels of NO_2^-/NO_3^- comprised 22 healthy Ethiopian individuals ($n = 22$, 13 M, 9 F, average age 36.4 ± 9 y, age range 20–55 y). The urine samples were all collected in the early morning after an overnight fast and were stored at $-20°C$ until analysed. The study was approved by the ethics committee at Armauer Hansen Research Institute and ALERT Hospital, Addis Ababa, Ethiopia.

Analysis of nitrite and nitrate

The sum of the nitrate and nitrite concentration in urine was determined as described by Giliam et al. (24). The urine samples were diluted in PBS. Nitrate in the samples was reduced to nitrite by incubating the sample with 10 µl nitrate reductase from Aspergillus (10 U/ml) and 10 µl nicotinamide adenine dinucleotide phosphate (reduced NADPH; 1mM) for 2 h at 37°C. The nitrite level was then determined by Griess reaction. The final reaction volume of 1 ml contained 728 µl HCl (0.45 M), 86 µl sulphanilic acid (2 mg/ml), 86 µl N-(1-naphtyl) ethylenediamine (1 mg/ml) and 100 µl of the diluted urine sample. The urine samples were then analysed in an ELISA multiwell reader (Titertec Multiscan Plus) at 542 nm.

Statistics

Statistical evaluation was done using the Mann-Whitney U test for comparison between the different patient groups. The effect of treatment on the levels of NO_2^-/NO_3^- in patients with tuberculosis was compared using the Wilcoxon signed rank test. Data is presented as mean ± standard deviation.

RESULTS

The control group comprised apparently healthy Ethiopian individuals. The mean urinary excretion of NO_2^-/NO_3^- in this group of 22 individuals was found to be 1020 ± 471 µM (range 198–1907). To investigate the levels of NO metabolites in patients with active pulmonary tuberculosis, morning urine was collected. We found that untreated patients with active pulmonary tuberculosis had significantly higher levels of urinary NO_2^-/NO_3^- compared with the control group (1416 ± 622 µM, $p < 0.05$, $n = 18$). These samples were boiled for 30 min before analysis to kill any viable mycobacteria in the urine. This treatment did not affect the concentration of NO_2^-/NO_3^- (data not shown). To elucidate whether co-infection with HIV had any influence on the levels of NO_2^-/NO_3^- in patients with active pulmonary tuberculosis, HIV screening was performed. In the group of patients with pulmonary tuberculosis 33.3% (6/18) was HIV positive by serology. The HIV/tuberculosis co-infected patients did not have significantly different levels compared with the control group (1101 ± 614 µM, $n = 6$). A highly significant difference, however, was observed in the HIV negative patients with active tuberculosis (1574 ± 588 µM, $p < 0.01$, $n = 12$). In the group of household contacts of tuberculosis patients the mean difference was nearly two times higher than the control group (1949 ± 812 µM, $p = 0.006$, $n = 7$). These apparently healthy individuals were close contacts of patients with severe tuberculosis. The data from the various patient groups are presented in Fig. 1.

Some of the untreated HIV negative patients with active pulmonary tuberculosis were followed up 1 week and 4 weeks after treatment to investigate the effect of anti-tuberculous treatment on the levels of nitric oxide metabolites (Fig. 2). Patients were treated within 1 d after the first sample was obtained with a standard 4-drug regimen consisting of rifampicin, isoniazid, pyrizinamide and ethambutol. Rifampicin, excreted in the urine, does not interfere with the measurements of NO_2^-/NO_3^- (data not shown). A group of 6 individuals from the HIV negative patients with pulmonary tuberculosis (1710 ± 519 µM) was selected randomly. A decrease in the urinary NO_2^-/NO_3^- level compared with that before treatment was observed 1 week after treatment (945 ± 599 µM, $p < 0.05$) and this level remained stable 4 weeks after treatment (888 ± 770 µM, $n = 5$). In the group of HIV/tuberculosis co-infected patients (1125 ± 791 µM, $n = 4$) the urinary levels of NO_2^-/NO_3^- remained at low levels 1 week (969 ± 553 µM, $n = 4$) and 4 weeks (740 ± 531 µM, $n = 3$) after treatment.

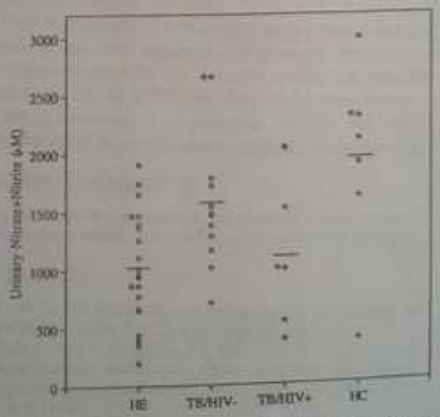


Fig. 1. Urinary levels of nitrate and nitrite (the mean is represented by bold lines) in: healthy Ethiopian individuals (HE, $n = 22$), untreated HIV negative patients with active pulmonary tuberculosis (TB/HIV−, $n = 12$), untreated HIV positive patients with active pulmonary tuberculosis (TB/HIV+, $n = 6$) and household contacts to patients with active pulmonary tuberculosis (HC, $n = 7$).

Amir sultan (MD), 2014

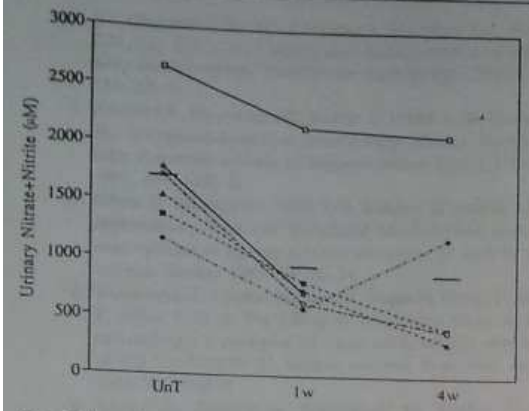


Fig. 2. The effect of anti-tuberculous treatment on the urinary levels of nitrate and nitrite (the mean is represented by bold lines) in untreated HIV negative patients with active pulmonary tuberculosis (UnT, $n = 6$) 1 week (1w, $n = 6$) and 4 weeks (4w, $n = 5$) after treatment.

DISCUSSION

Our results show increased urinary levels of the NO metabolites NO_2^-/NO_3^- in household contacts to patients with tuberculosis and in patients with active pulmonary tuberculosis with a reduction following anti-tuberculous treatment. The most likely explanation for this is the endogenous production of NO as a host response to M. tuberculosis infection that has been suggested by others (2, 3, 17). The metabolites of NO, nitrite and nitrate, have been frequently used as an indirect measurement of the production of NO in vivo (15, 21, 22, 25). In humans the sources of NO_2^-/NO_3^- are mainly food and endogenous synthesis (19) and dietary intake may contribute greatly to the excretion of NO_2^-/NO_3^-. Therefore, the urinary levels of these metabolites as a measure of NO production should be interpreted with caution. We have chosen to use an overnight fast as suggested by others (26, 27) followed by early morning urine samples. Although dietary intake and renal function could differ between individuals in our study, it is unlikely that these factors are responsible for the increased levels of NO metabolites when different groups are compared. Moreover, the levels of NO_2^- NO_3^- in the control group in our study (1020 ± 471 μM, $n = 22$) are similar to those reported in a group of healthy Europeans (1016 ± 516 μM, $n = 14$) (24) and in healthy Swedish children (1174 ± 844 μM, $n = 53$) (22). Thus, the mean urinary excretion of nitrate and nitrite does not seem to differ significantly between healthy individuals from Europe and Ethiopia despite their diet being different.

Untreated patients with active pulmonary tuberculosis had slightly elevated urinary levels of NO_2^-/NO_3^- in the urine. However, when this group was divided into subgroups according to HIV status, only the HIV negative patients with tuberculosis had significantly increased levels of NO metabolites in the urine. Interestingly, the group of household contacts of tuberculosis patients had increased urinary levels of NO_2^-/NO_3^-. The reason for this might be that the induction of NO-synthesis is highest during the very early stage of the disease even before symptoms appear and the patients present at the hospital. Household contacts of tuberculosis patients with severe tuberculosis from the same area as the group of household contacts in this study show high interferon gamma production in response to M. tuberculosis derived antigens, indicating an intense subclinical response following M. tuberculosis exposure (28).

The seroprevalence of HIV among the patients with pulmonary tuberculosis in this study was 33.3%. This is in accordance with previous reports that the average seroprevalence in tuberculosis patients in Africa is 40% (range 20–73%) (29). It has been reported that HIV infection induces NO production in vitro (30) and in vivo (31). In this study we found high levels of NO metabolites in HIV negative patients with active pulmonary tuberculosis, but not in patients co-infected with tuberculosis and HIV. In HIV/tuberculosis co-infected patients, lack of substrates for NO production like L-arginine could be an explanation for this.

The prompt decrease of NO metabolites as a result of chemotherapy would argue that it is linked to tumour necrosis factor alpha (TNF-α) production, the main pyrogen in tuberculosis. The decay of nitric oxide metabolites in the urine coincides with the normalization of body temperature as a result of specific treatment in patients with tuberculosis (32). It has also been shown that TNF-α can induce production of NO (33).

If NO is important in host defence against tuberculosis in humans it has been suggested that there are implications for immunotherapy by the cytokine profile that increase synthesis of NO by iNOS (34). In both of these diseases, multidrug resistance is an emerging problem and iNOS induction could be an adjuvant to available chemotherapy.

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REFERENCES

1. Kochi A. The global situation and the new control strategy of the World Health Organization. Tubercle 1991; 72: 1–6.
2. Chan J, Xing Y, Magliozzo RS, Bloom BR. Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 1992; 175: 1111–22.
3. Chan J, Tanaka K, Carrol D, Flynn J, Bloom BR. Effects of nitric oxide synthase inhibitors on murine infection with Mycobacterium tuberculosis. Infect Immun 1995; 63: 736–40.
4. Wei Xiao-qing, Charles IG, Smith A, Ure J, Feng Gui-jie, Huang Fang-ping, et al. Altered immune responses in mice lacking inducible nitric oxide synthase. Nature 1995; 375: 408–11.

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5. Xie Qiao-wen, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, et al. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science 1992; 256: 225–8.
6. Adams LB, Franzblau SG, Vavrin Z, Hibbs Jr JB, Krahenbuhl JL. L-arginine-dependent macrophage effector functions inhibit metabolic activity of Mycobacterium leprae. J Immunol 1991; 147: 1642–6.
7. Khare S, Bhutani LK, Rao DN. Release of reactive nitrogen intermediates from the peripheral blood-derived monocytes/macrophages of leprosy patients stimulated in vitro by tuftsin. Leprosy Review 1997; 68: 16–24.
8. Vouldoukis I, Riveros-Moreno V, Dugas B, Ouaaz F, Becherel P, Debre P, et al. The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the FcεRII/CD 23 surface antigen. Proc Natl Acad Sci 1995; 92: 7804–8.
9. Stenger S, Thüring H, Röllinghoff M, Bogdan C. Tissue expression of inducible nitric oxide synthase is closely associated with resistance to Leishmania major. J Exp Med 1994; 180: 783–93.
10. Granger DL, Hibbs Jr JB. High-output nitric oxide: weapon against infection? Trend Microbiol 1996; 4: 46–7.
11. Denis M. NO or no NO? J Leukoc Biol 1994; 55: 682–4.
12. Schoedon G, Schneemann M, Walter R, Blau N, Hofer S, Schaffner A. Nitric oxide and infection: Another view. Clin Infect Dis 1995; 21 Suppl 2: S152–7.
13. Liang Yan R, Vandivier RW, Suffredini AF, Danner RL. Human polymorphnuclear leukocytes lack detectable nitric oxide synthase activity. J Immunol 1994; 153: 1825–34.
14. St Clair EW, Wilkinson WE, Lang T, Sanders L, Misukonis MA, Gilkeson GS, et al. Increased expression of blood mononuclear cell nitric oxide synthase type 2 in rheumatoid arthritis patients. J Exp Med 1996; 184: 1173–8.
15. Anstey NM, Weinberg JB, Hassanali MY, Mwaikambo ED, Manyenga D, Misukonis MA, et al. Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. J Exp Med 1996; 184: 557–67.
16. Wheeler MA, Smith SD, Garcia-Cardena G, Nathan CF, Weiss RM, Sessa WC. Bacterial infection induces nitric oxide synthase in human neutrophils. J Clin Invest 1997; 99: 110–6.
17. Nicholson S, Bonecini-Almeida M, Lapa e Silva JR, Nathan C, Xie Qiao-wen, Mumford R, et al. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J Exp Med 1996; 183: 2293–302.
18. Änggård E. Nitric oxide: mediator, murderer and medicine. Lancet 1994; 343: 1199–206.
19. Green LC, De Luzuriaga KR, Wagner DA, Rand W, Istfan N, Young VR, et al. Nitrate biosynthesis in man. Proc Natl Acad Sci USA 1981; 84: 6369–73.
20. Granger DL, Hibbs JB, Broadnax LM. Urinary nitrate excretion in relation to murine macrophage activation. J Immunol 1991; 146: 1294–302.
21. Dykhuizen RS, Masson J, McKnight G, Mowat ANG, Smith CC, Smith LM, et al. Plasma nitrate concentration in infective gastroenteritis and inflammatory bowel disease. Gut 1996; 39: 393–5.
22. Sundqvist T, Laurin P, Fälth-Magnusson K, Magnusson K-E, Stenhammar L. Significantly increased levels of nitric oxide products in urine of children with celiac disease. JPGN 1998; 27: 196–8.
23. Schön T, Gebre N, Sundqvist T, H/Mariam HS, Engeda T, Britton S. Increased levels of nitric oxide metabolites in urine from leprosy patients in reversal reaction. Lepr Rev 1999. In press.
24. Giliam MB, Sherman MP, Griscavage JM, Ignarro LJ. A spectrophotometric assay for Nitrate Using NADPH oxidation by Aspergillus nitrate reductase. Anal Biochem 1993; 212: 259–65.
25. Ellis G, Adatia I, Yazdanpanah M, Makela SK. Nitrite and nitrate analyses: a clinical biochemistry perspective. Clin Biochem 1998; 31: 195–220.
26. Leone AM, Francis PL, Rhodes P, Moncada S. A rapid and simple method for the measurement of nitrite and nitrate in plasma by high performance capillary electrophoresis. Biochem Biophys Res Commun 1994; 200: 951–7.
27. Rhodes P, Leone AM, Francis PL, Struthers AD, Moncada S, Rhodes PM. The L-arginine: nitric oxide pathway is the major source of plasma nitrite in fasted humans. Biochem Biophys Res Commun 1994; 209: 590–6.
28. Demissie A, Ravn P, et al. T-cell recognition of extracellular mycobacterial antigens by tuberculosis patients and their household contacts. Infect Immun 1999. In press.
29. Garay, SM. Tuberculosis and the human immunodeficiency virus infection. In: Rom WN, Garay SM. Tuberculosis. New York: Little, Brown and Co., 1996: 443–65.
30. Bukrinsky MI, Nottet HSLM, Schmidtmayerova H, Dubroysky L, Flanagan CR, Mullins ME, et al. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: Implications for HIV-associated neurological disease. J Exp Med 1995; 181: 735–44.
31. Groeneveld PHP, Kroon FP, Nibbering PH, Bruisten SM, van Sweiten P, van Furth R. Increased production of nitric oxide correlates with viral load and activation of mononuclear phagocytes in HIV-infected patients. Scand J Infect Dis 1996; 28: 341–5.
32. Barnes P, Chan LS, Wong SF. The course of fever following treatment of tuberculosis. Tubercle 1987; 68: 255–60.
33. Drapier JC, Wietzerbin J, Hibbs JB Jr. Interferon-γ and tumor necrosis factor induce the L-arginine-dependent effector mechanism in murine macrophages. Eur J Immunol 1988; 18: 1587–92.
34. Ellner JJ. The immune response in human tuberculosis: implications for tuberculosis control. J Inf Dis 1997; 176: 1351–9.

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Review

Nuclear Medicine : Part I. Introduction, Fundamentals and Scope of Applications

Yohannes Jorge, B. Pharm, M.Sc.*

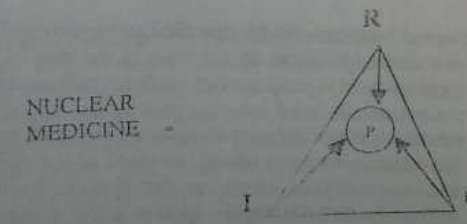
Summary

Nuclear medicine is playing vital diagnostic and therapeutic role in contemporary medicine. Although the dimension of its diagnostic application predominates its therapeutic utility , the latter is also exhibiting significant development recently. The current versatile application of nuclear medicine is attributed to its non-invasiveness, relative cost effectiveness, easiness and rapidity of the procedures, comprehensive relation with other medical disciplines and its being the sole option to rule out the functional disorders of our body. The strength of nuclear medicine lies in the use of radiopharmaceuticals to study function. It can be classified as In-vitro and In vivo uclear Medicine on the basis of the style of its application. The in-vivo nuclear medicine is composed of Radiopharmacy, Medical Physics and Clinical Nuclear Medicine. The in-vitro Nuclear Medicine is composed of Radio-immunoassay (RIA) and Immuno-radiometric assay (IRMA). Nuclear medicine as a discipline of medicine is a compound made of many active ingredients including Medicine, Pharmacy, Nuclear Physics, Biochemistry ; making its application broad and comprehensive. The basics of nuclear medicine and scope of its applications globally and at Ethiopian level is reviewed in this paper. Its diagnostic and therapeutic role will be reviewed in subsequent publications of this journal. [J Ethiop Med Pract 1999 ;1 (2) :110-125]

Introduction

Nuclear medicine is the application of radioactive materials (Radiopharmaceuticals) to the diagnosis and treatment of patients and the study of human disease.[1] The field can be symbolized by a triangle with, Radiopharmaceutical(R), Instrument (I), and Clinical/ biomedical problems (C) at the three corners, and with the patient (P) at the center.

If one of these is missing, the triangle cannot exist. Some texts also put nuclear medicine as the triangle composed of the radiopharmaceutical, imaging (scintigraphy) and clinical interpretation. Therapeutic nuclear medicine can be defined as the use of radioactive compounds for the treatment of disease.[1,2] In diagnostic nuclear medicine radiopharmaceuticals (radionuclides) which are emitting electromagnetic radiations (gamma rays, X-rays) are solely employed due to their penetrative capacity that makes them detected by external instruments (detectors). In therapeutic Nuclear medicine radionuclides emitting beta particles, are used in order to enable the deposit of maximum radiation energy at the vicinity of their localization that can devastate proliferating malignant and other abnormally hyperactive cells.[2,3,4] Nuclear medicine is probably the best clinical example of the integration of medical and scientific disciplines (physics, chemistry, radiopharmacy, computer sciences etc.) and applied routinely on daily basis for the care of an individual patient.



Clinical nuclear medicine like radiology and ultrasound should be problem oriented and problem solving and indeed it is! The strength of nuclear medicine lies in the use of radiopharmaceuticals to

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study function rather than structure of organs (tissues) and is well encapsulated by three often quoted aphorisms:

A. Nuclear medicine is to Radiology what physioclogy is to anatomy
B. Biochemical and functional changes occur in disease before structural changes can be identified
C. How things function is more important to the patient than how they look.

The important features of nuclear medicine studies are shown on table 1.

Nuclear medicine is one of the fast growing disciplines of medicine at present and its clinical role in diagnostic and therapeutic medicine is dramatically rising. Consequently, it is clearly mentioned in the recently published texts of various disciplines of medicine, pharmacy, biochemistry, physiology, microbiology and other biomedical sciences. It is recommended as standard techniques of solving basic research and clinical oriented problems. As a result, a greater proportion (>50%) of patients visiting the hospitals in advanced nations are passing through nuclear medicine procedures.

The scope of nuclear medicine has grown tremendously in the past two decades. The specialty has acquired a greater autonomy in relation to other

Table 1. The important features of Nuclear Medicine studies.

1. The radioactive tracers (radioisotopes/ radionuclides) are handled physiologically and biochemically in the same way as stable element.
2. Radiopharmaceuticals (radioactive tracers) are given in such small quantities that they do not alter the physiological processes and consequently allow observation of the function under investigation without alteration of that function.
3. They are non- invasive, requiring in most instances no more than an intravenous injection.
4. There is a negligible morbidity and virtually non-existent mortality.
5. The radiation dose is similar to that given in an equivalent radiological investigation and often far less in spite of the general misconception.
6. It is cost effective and the procedures are simple & fast.
7. Both anatomic as well as functional (physiologic) studies can be carried out in the patient with the single administered dose (1,3,4,5,6,7).

imaging modalities, since it permits, the study of both morphology and function in a single step. The use of small amounts of drugs devoid of pharmacologic action, coupled with redionuclides which emit radiation detected by modern gamma-cameras, allows the study of organ and tissue function without interfering with that function. Computer programs has also developed greatly the acquisition of digitized images of the internal organs and the quantification of their functional status. From the viewpoint of the patient, the techniques performed are simple and require only an intravenous injection or the oral administration of a radiopharmaceutical. There are no adverse reactions. The Nuclear medicine physician then utilizes his/her techniques in order to try to answer the questions posed by the Clinician, such as 1. Is there any alteration present? 2. If so what ? 3. What is its extension or to what extent? 4. Is it possible to determine the etiology of the alterations? 5. Is it possible to determine the degree of activity of the alterations ?

However, the applications and utilities of the advanced diagnostic and therapeutic Nuclear medicine services have remained highly limited in developing countries including Ethiopia. Despite this fact however, some developing countries in Asia, Latin America and Africa are showing significant development in the application of Nuclear medicine technology nearly in pace with advanced Nations. For example ,

1. In Asia:- India, China, South Korea and most other South East Asian Countries.
2. In Africa:- South Africa, Egypt, Morocco, Algeria, Tunisia.
3. In Latin America:- Brazil, Mexico, Argentina and most Latin American Countries.

Therefore, unreserved efforts and focal attention should be paid to develop Nuclear Medicine in the least developed countries in order to make their patients beneficiaries of this high technology imaging diagnostic modality as well as its therapeutic utility.

Classification

Nuclear Medicine can be classified as In-vitro and In vivo nuclear medicine on the basis of the style of its application. In in-vitro nuclear medicine radionuclides are not administered into the patient and biological substances associated with diseases are analyzed in the test tubes (laboratory) taking the blood of the patient and separating its serum or the plasma. In vitro nuclear medicine is highly applied in the diagnostic procedures of clinical biochemistry, clinical endocrinology, in the diagnosis of gynecological -

obstetrics complications and in the detection of the commencement of malignant tumours at its early stage. Good examples are Radioimmunoassay (RIA) and Immuno-radiometric-assay (IRMA).

In the in -vivo nuclear medicine radiopharmaceuticals (radioisotope labeled drugs or pharmaceuticals) are administered into the patient at optimized quantity. Then the scintipictures or images of the internal organs are taken with the appropriate high technology imaging instrument called gamma - camera after giving ample time for specific organ localization of the administered radiopharmaceutical.

For example, the administered Radiopharmaceuteal depending on its nature can concentrate in liver, kiney, heart, bone, spleen, brain etc and imaging of one of these is carried out in order to rule out/evaluate their functional and /or organic (anatomic) damage (disorder). Taking the pictures of internal organs by the instrument (detector) located outside the patient is possible due to the earlier administered radiopharmaceutical into the patient which has specifically localized in an organ and emits radiation (electromagnitic rays) from where it is, which is detected by the instrument. Then, the instrument changes the ray emitted and detected from the corresponding organ (s) into visible pictures. The obtained picture (image) can have normal or abnormal anatomical morphology or physiologic (functional) status which is influenced by pathologic condition. The pictures obtained are called scintigraphic pictures or scans and the technique is called "nuclear scintigraphy or nuclear medicine scanning". Forexample, Brain scintigraphy or brain scan, bone scan, liver scan, cardiac scan, renal scan etc. [6,7,9]

Nuclear Medicine can also be classified as diagnostic and therapeutic on the basis of the purpose of its application i.e either for diagnosis or therapy.

In-vitro Nuclear Medicine and Scope of its applications.

As the name indicates the diagnostic procedure here does not involve administration of the appropriate radionuclide (radioisotope) into the patient. Rather the patient blood sample is analyzed in the test tube to determine hormones, tumor markers, etc of that particular patient with respect to that particular disease. For example, in thyroid abnormality, serum level concentrations of T_3, T_4 and TSH are evaluated (assayed / measured) in the laboratory by taking the blood sample of the patient. Deviation from normal values (concentrations) is an indication of the a disease i.e Hyper-or hypo-secretions of the hormones. Accordingly, the pertinent biological substance(s) (hormones, enzymes etc) intended to be assayed is labeled with the suitable radionuclide. In this case ^{125}I is exclusively and extensively used at present for its in-vitro labeling due to its suitable physico-chemical and radionuclidic properties compared to other existing radioisotopes. The two well known and well standardised in-vitro radionuclidic procedures are Radio-immunoassay (RIA) and Immuno-radiometric assay (IRMA). RIA is defined as the competitive protein binding assay in which radionuclide labeled antigen (Ag) competes with the unlabelled Ag in the patient blood sample for the limited quantity of the antibody (Ab) presented for the immunological reaction. Here the unlabelled Ag is the hormone to be measured in the patient; for example in the case of thyrotoxicosis or hypothyroidism it can be T_3, T_4 and the corresponding labeled Ag would be either ^{125}I labeled T_3 or T_4 prepared in-vitro with similar (identical) chemical and immunologic properties like the unlabeled Ag (T_3, T_4, TSH) from the patient blood. If the concentration of the Ag in the patient sample is too high like in the case of thyrotoxicosis less of the labeled Ag (^{125}I-T_4) will react with the given Ab and more of the unlabelled Ag (T_4) will consume the presented Ab in order to form Ag- Ab complex. The competition of this immunologic reaction takes place in the solution form in the same test tube where these three substances (unlabelled-Ag, labeled Ag and specific Ab) are added together in mixture.

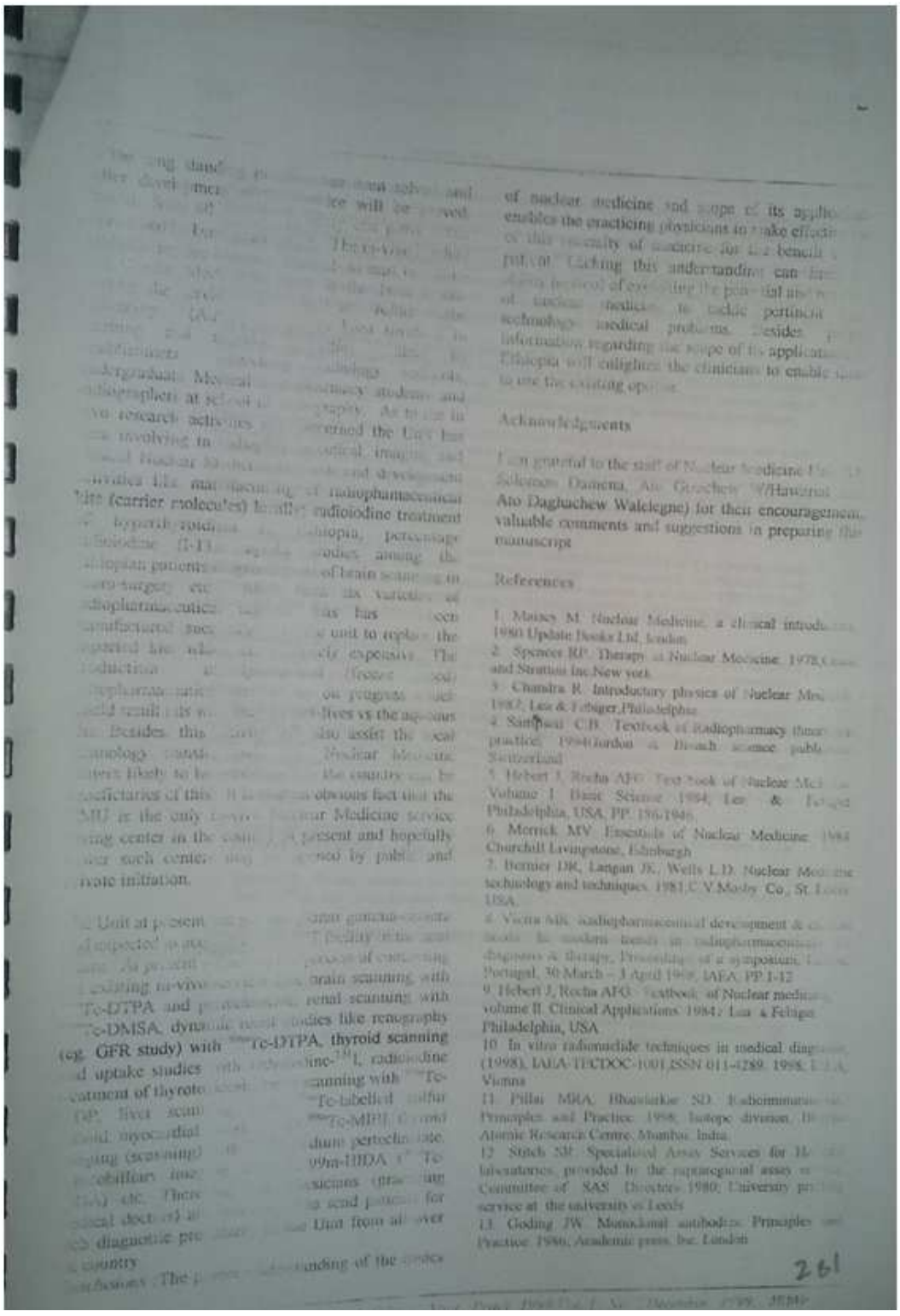
IRMA is the modified version of RIA. The basic difference lies in that in RIA the Ag is labeled with ^{125}I whereas in IRMA the Ab is labeled with same radionuclide. Apart from that both are the radionuclidic in vitro techniques. However, IRMA being the modified version is more sensitive and specific than RIA since it is reagent limited assay technique in addition to employing the highly specific monoclonal antibodies ($MoAb_s$) in the determination of some disease related hormones in the patient's blood. [10,11,12,13] Otherwise, both RIA and IRMA are highly specific and sensitive, precise, cost-effective (relatively cheap), with negligible radiation hazard to the operator, accessible (easily available) and versatile compared to most other in vitro diagnostic modalities. Many disease related biological (biochemical) substances as indicated above which exist in extremely minute quantities (in micro– and nano-grams) in the patient blood which cannot be measured using the conventional clinical chemistry techniques can be measured successfully employing RIA and IRMA techinques. Indeed, the advent of RIA techinques has revolutionized the in vitro diagnostic medicine.

In Vivo Nuclear Medicine and Scope of Its applications

In Contemporary utility is predominantly diagnostic. However, its therapeutic application is also developing dramatically particularly in treatment of various neoplasm. The in vivo Nuclear Medicine can also be classified as imaging and Non-imaging studies. The imaging application has already been defined earlier. The non-imaging applications is like measuring the radioiodine uptake of thyroid gland, measuring vitamin B_{12} absorption in the terminal ileum by Schilling absorption test, bile acid breath test, measurement of glomerular filtration rate (GFR), measurement of gastro-intestinal blood loss, determination of red blood, RBC or plasma volume, etc using radiopharmaceutical. In this case the quantity of the radiopharmaceutical administered into the patient is distributed in the particular organ-system which is affected by pathology is measured. For example in thyrotoxicosis the orally administered ^{131}I-Sodium Iodide (Radio-active Iodine) concentrates in greater proportion compared to normal thyroid gland and the neck region of such patient gives greater radio-activity counts compared to the normal. This role of Nuclear Medicine is applicable to many other organs and organ-systems like liver, spleen, blood and blood components, Iron and vit. B_{12} in-vivo Pharmakinetic determination etc. It differs from In-Vitro Nuclear Medicine techniques in such a way that the radionuclide (Radiopharmaceutical) is administered into the patient in the in-vitro technique whereas it does in the in-vivo non imaging technique. Apart from that all the assay procedures in the in-Vitro method is carried out in the laboratory taking the blood sample of the patient whereas in the in-Vivo technique the radiopharmaceutical distribution in the patient is measured, though no imaging of the organ is done.

Nuclear Medicine in Ethiopia

In-vitro Nuclear Medicine : RIA technique was started in Ethiopia in the beginning of 1984 under the assistance of International Atomic Energy Agency (IAEA) and the routinely conducted procedures were determination of thyroid hormones (T3, T4 and TSH) in the beginning and later, fertility hormones like prolactin, LH and FSH were added. Since then the work has been carried out on the on and off basis due to merely depending on the external assistance and lack of National budget allocated for the purpose. This is also true in the case of in-vivo Nuclear Medicine services. Nevertheless, it is possible to say that some important work has been accomplished specially in the determination of thyroid hormones in serving patients coming from all [illegible] the country as the Nucl[illegible] Black Lion Hospital remain[illegible] Medicine Unit of [illegible] the only such service [illegible] section so far [illegible] service has been [illegible] medical colleges [illegible] Hospital [illegible] [illegible] [illegible]



of nuclear medicine and scope of its applic
enables the practicing physicians to make effectiv
of this specialty of medicine for the benefit
patient. Lacking this understanding can
chance (error) of exploiting the potential
of nuclear medicine to tackle pertinent
technology medical problems. Besides
information regarding the scope of its applicat
Ethiopia will enlighten the clinicians to enable
to use the existing options.

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References

1. Maisey M. Nuclear Medicine, a clinical introduc
1980 Update Books Ltd. London
2. Spencer RP. Therapy in Nuclear Medicine. 1978, C
and Stratton Inc.New york
3. Chandra R. Introductory physics of Nuclear Me
1987, Lea & Febiger,Philadelphia
4. Sampath C.B. Textbook of Radiopharmacy (theo
practice). 1994Gordon & Breach science publi
Switzerland
5. Hebert J, Rocha AFG. Text book of Nuclear Me
Volume I. Basic Science 1984, Lea & Febiger
Philadelphia, USA. PP. 156-1946.
6. Merrick MV. Essentials of Nuclear Medicine. 1984
Churchill Livingstone, Edinburgh
7. Bremer DR, Langan JK, Wells LD. Nuclear Me
technology and techniques. 1981,C V.Mosby Co., St. Lo
USA.
8. Vieira MA. radiopharmaceutical development &
needs for modern trends in radiopharmaceutic
diagnosis & therapy. Proceedings of a symposium,
Portugal, 30 March – 3 April 1998, IAEA. PP.1-12
9. Hebert J, Rocha AFG. Textbook of Nuclear medici
volume II. Clinical Applications 1984, Lea & Febiger
Philadelphia, USA
10. In vitro radionuclide techniques in medical diag
(1998), IAEA-TECDOC-1001,ISSN 011-4789. 1998, I.A.E.A.
Vienna
11. Pillai MRA, Bhanushkar SD. Radioimmunoa
Principles and Practice. 1998, Isotope division, B
Atomic Research Centre, Mumbai, India.
12. Stitch SR. Specialized Assay Services for H
laboratories, provided by the supraregional assay s
Committee of SAS Directors 1980, University pr
service at the university of Leeds
13. Goding JW. Monoclonal antibodies: Principles
Practice. 1986, Academic press, Inc. London

261

NOTES TO CONTRIBUTORS

1. The *Ethiopian Medical Journal* publishes papers containing original observations or research of relevance to clinical medicine and public health (usually up to 2,000 words in length, with not more than six tables or illustrations). The Journal also publishes case reports; letters to the Editor (not more than 400 words, signed personally by all authors); news items; abstracts; reviews; teaching articles; brief communications; and other material relevant to health and medicine in Ethiopia, e.g., Proceedings of the Annual Conference of the Ethiopian Medical Association. Teaching articles and reviews will be submitted only by invitation of the Editorial Board. Monographs of the Journal: the decision on sharing publication expenses between the author(s) and the Journal will be at the discretion of the Editorial Board.

2. All contributions should be submitted to the Chairman of the Editorial Board with a statement signed by each author that the paper has not been published elsewhere in whole or in part, and is not submitted elsewhere while offered to the *Ethiopian Medical Journal*. This dose not refer to abstracts of oral communications at symposia or other proceedings. Papers accepted become the copyright of the *Ethiopian Medical Journal*.

3. Manuscripts should be submitted in triplicate, typed on one side of A4 paper in double spacing with a left-hand margin of 3cm. On a single separate sheet, there must be the title of the paper, with key words for indexing if required, and each author's full name and professional degrees, department where work was done, present address of any author if different from that where work was done, and the name and full postal address of the author to whom correspondence should be sent. Each table, complete with title and footnotes, should be on a separate page.

4. The language of the Journal is English. The usual format must include an **ABSTRACT** (on separate sheet) not exceeding 200 words; an introductory statement of purpose and background; **MATERIALS AND METHODS** (including sufficient information to permit repetition of experimental work) or **PATIENTS AND METHODS; RESULTS; DISCUSSION; ACKNOWLEDGEMENTS, REFERENCES, TABLES, LEGENDS FOR ILLUSTRATIONS.**

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    1. Knaack RV, Wright W, Leithead CB, Kidan TG, Plorde JJ. Penicillin versus tetracycline in the treatment of louse-borne relapsing fever. *Ethiop Med J* 1972;10:15-8.

    2. Wilheim DL. Chemical mediators. In: Zweifach BW, Grant L, McCluskey RT, eds. The Inflammatory Process 2nd ed. New York, Academic Press, 1972:251-3.

    A more extensive list of examples (and of standard abbreviations) can be obtained from the secretary of EMJ on request.

6. Tables should be typed in triplicate on separate sheets and given serial Arabic numbers. They should be comprehensive without reference to the text. Unnecessary and lengthy tables and figures are discouraged. Units should appear in parentheses in captions but not in the body of the table. Statistical procedures, if not in common use, should be detailed in the METHODS section or supported by references.

Can J Gatroenterol 1995;9(Issue No.1):1-4.

49. Alter MJ, Coleman PJ, Alexander WJ, *et al.* Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *J Amer Med Asso* 1989;262:1201-5.
50. Hoofnagle JH, Di Biceglie MD. The treatment of chronic viral hepatitis. *New Eng J Med* 1997;336:347-56.
51. Reichard O, Norkrans G, Fryden A, *et al.* Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavarin for chronic hepatitis C. *Lancet* 1998;351:83-7.
52. Peynard T, Marcellin P, Lee SS, *et al.* Randomised trial of interferon alpha-2b plus ribavarin for 48 weeks or for 24 weeks versus interferon alpha-2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998;352:1426-32.
53. McHutchison JG, Gordon SC, Schiff ER, *et al.* Interferon alpha-2b along or in combination with ribavarin as initial treatment for chronic hepatitis C. *New Eng J Med* 1998;339:1485-92.
54. Mauss S, Heintges T, Adams O, *et al.* Treatment of chronic hepatitis C with interferon-alpha in patients infected with human immunodeficiency virus. *Hepatogastroenterol* 1995;42:528-34.
55. Depew W. Hepatitis update: Hepatitis C in special clinical conditions. *Can J Gastroenterol* 1997;11(Issue No.12):1-4.
56. Alter J. The cloning and clinical implications of HGV. *New Eng J Med* 1996;334:1536-7.
57. Linnen J, Wagens J Jr, Zhang-Keck Z-Y, *et al.* Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996;271:505-8.
58. Stark K, Bienzle U, Hess G, *et al.* Detection of hepatitis G virus genome among injecting drug users, homosexual and bisexual men, and blood donors. *J Infect Dis* 1996;174:1320-3.
59. Takagi H, Kakizaki S, Satoh K, *et al.* Prevalence of hepatitis G in liver disease. *Can J Gastoenterol* 1999;13:823-6.
60. Okamoto H, Nishizawa T, ukita M. A novel unenveloped DNA virus (TT virus) associated with acute and chronic non-A to G hepatitis. *Intervirol* 1999;42:196-204.
61. Kao JH, Chen W, Hsiang SC, *et al.* Prevalence and implication of TT virus infection: minimal role in patients with non-A-E hepatitis in Taiwan. *J Med Virol* 1999;59:307-12.
62. Thoelen S, Van Damme P, Leenfvaar-Kuypers A, *et al.* The first combined vaccine against hepatitis A and B: an overview. *Vaccine* 1999;17:1657-62.

(Accepted 3 April 2000)

hospital personnel in hyperendemic areas require immunization against hepatits B virus (HBV) infection? Is vertical transmission of HBV infection common in this group? *Ethiop Med J* 1989;27:101-5.

16. Taylor PE, Stevens CE, Rodriguez deCS, Rubenstein P. Hepatitis B virus and human immunodeficiency virus: possible interactions. In:Zuckerman AJ, Ed: Viral Hepatitis and Liver Disease. New York: Alan R. Liss, 1988,pp.198-200.
17. Tsega E, Nordenfelt E, Hansson BG, Mengesha B, Lindberg J, Chronic liver disease in Ethiopia: a clinical study with emphasis on identifying common causes. *Ethiop Med J* 1992;30 (No.2 suppl):1-33.
18a. Beasely RP, Hwang L-Y, Lin C-C, Chien C-S. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. *Lancet* 1981;2:1129-32.
18b. Beasely RP. Hepatitis B virus as the etiologic agent in hepatocellular carcinoma-epidemiolgic consideration. *Hepatol* 1982;2:215-65.
19. Prevention of liver cancer. *World Health Org Tech Rep Ser* 1983;691:1-30.
20. Chang M-H, Chen C-J, Lai M-S, *et al.* Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. *New Eng J Med* 1997;336:1855-9.
21. Tsega E, Tafesse B, Horton J, Nordenfelt E, Wolde-Hawariat G, Hansson BG, Lindberg J. Immunogenicity, reactogenicity and comparison of two doses of recombinant DNA yeast-derived hepatitis B vaccine in Ethiopian children. *Trop Geog Med* 1990;43:220-7.
22. Tsega E, Tafesse B, Nordenfelt E, Wolde-Hawariat G, Hansson BG, Lindberg J. Immunogenicity of hepatitis B vaccine simultaneously administered with the expanded programme on immunization (EPI). *J Med Virol* 1990;32:232-5.
23. Tsega E, Horton J, Nordenfelt E, Hansson BG, Tafesse B, Wolde-Hawariat G, Lindberg J. Antibody levels in Ethiopian children five years after vaccination with two different doses of hepatitis B vaccine: Is there a need for booster vaccine? *Can J Gastroenterol* 1998;12:57-60.
24. Lai C-L, Wong BC-U, Yeoh E-K, Lim W-L, Chang W-K, Lin H-J. Five-year follow-up of prospective randomized trial of hepatitis B recombinant DNA yeast vaccine vs plasma-derived vaccine in children: Immunogenicity and anamnestic responses. *Hepatol* 1993;18:763-7.
25. Whal M, Hermodisson S. Intradermal, subcutaneous or intramuscular administration of hepatitis B vaccine: side effects and antibody response. *Scand J Infect Dis* 1987;19:617-21.
26. Bryan JP, Sjogren M, Iqbal M, *et al.* Comparative trial of low-dose, intradermal, recombinant and plasma-derived hepatitis B vaccine. *J Infect Dis* 1990;162:789-93.
27. Di Biceglie AM. Long-term outcome of interferon-alpha treatment for chronic hepatitis B. *Hepatol* 1995;22:565-567.
28. Lok ASF, Wu PC, Lai CL, *et al.* A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterol* 1992;102:2091-7.
29. Wong JB. Interferon for chronic hepatitis B or C infection: cost and effectiveness. *Acta Gastroenterol Belg* 1998;61:238-42.

284

30. Wong JB, Koff RS, Tine F, Pauker SG. Cost-effectiveness of interferon-alpha 2b treatment for hepatitis Be antigen-positive chronic hepatitis. *Ann Intern Med* 1995;122:664-75.
31. Dienstag JL, Perrillo RP, Schiff ER, *et al.* A preliminary trial of lamivudine for chronic hepatitis B infection. *New Engl J Med* 1995;25:1657-61.
32. Lai C-L, Chien R-N, Leung NWY, *et al.* A one-year trial of lamivudine for chronic hepatitis B. *New Engl J Med* 1998;339:61-8.
33. Nordenfelt E, Hansson BG, Al-Nakib B, Al-Kandari S, Al-Nakib W. Frequency of delta infection in Kwait. *J Infect Dis* 1983;339:61-8.
34. Nordenfelt E. Epidemiology of hepatitis delta virus and non-A, non-B hepatitis. *Scand J Infect Dis* 1990;69(suppl.):49-53.
35. Rizzetto M, Purcell RH, Gerin JL. Epidemiology of HBV-associated delta agent: geographical distribution of anti-delta and prevalence in polytransfused HBsAg crriers. *Lancet* 1980;1:1215-9.
36. Craxi A, Raimondo G, Longo G, *et al.* Delta agent infection in acute hepatitis and chronic HBsAg carriers with and without liver disease. *Gut* 1984;25:1288-90.
37. Rapicetta M, Hailu K, Ponzetto A, *et al.* Delta hepatitis virus infection in Ethiopia. *Eur J Epidemiol* 1988;4:185-8.
38. Zhang YY, Tsega E, Hansson BG. Phylogenetic analysis of hepatitis D virus indicating a new genotype I subgroup among African isolates. *J Clin Microbiol* 1996;34:3023-30.
39. Wu JC, Lee SD, Govindarajan S, *et al.* Sexual transmission of hepatitis D virus infection in Taiwan. *Hepatol* 1990;11:1057-61.
40. Di Biceglie AM, Hoofnagle JH: Chapter 2: Chronic viral hepatitis. *Hepatology: A textbook of liver disease.* 3rd Edition: In:Zakim D, Boyer TD, eds. WB Sunders Inc., Philadelphia, 1996 pp.1299-1329.
41. Van der Poel CL, Cypers HT, Reesink HW. Hepatitis C virus six years on. *Lancet* 1994;344:1475-9.
42. Esteban JI, Lopez-Talavera JC, Genesca J, *et al.* High rate of infectivity and liver disease in blood donors with antibodies to hepatitis C virus. *Ann Int Med* 1991;115:443-9.
43. Alter MJ, Masr EE. The epidemiology of viral hepatitis in the United States. *Gastroenterol Clin North Am* 1994;23:437-55.
44. Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antiboides in southern African Blacks with hepatocellular carcinoma. *Lancet* 1990;335:873-4.
45. Chen DS, Kuo GC, Sung J-L, *et al.* Hepatitis C virus ihfection in an area hyperendemic for hepatitis B and chronic iiver disease. The Taiwan experience. *J Infect Dis* 1990;162:817-22.
46. Stevens CE, Taylor PE, Pindych J, *et al.* Epidemiology of hepatitis C virus. A preliminary study in volunteer blood donors. *J Amer Med Asso* 1990;263:49-53.
47. Tsega E, Nordenfelt E, Hansson BG. Hepatitis C virus infection and chronic liver disease in Ethiopia where hepatitis B infection is hyperendemic. *Trans Roy Soc Trop Med Hyg* 1995;89:171-4.
48. Sherman M. hepatitis update: How significant is hepatitis C infection in Canada?

complete and advanced laboratory set-up should be established in either the national laboratory or the blood transfusion centre to facilitate follow-up of national vaccination programmes, applied and basic research in all the areas of viral hepatitis.

REFERENCES

1. Tsega E, Krawczynski K, Hansson BG, *et al.* Outbreak of acute hepatitis E virus infection among military personnel in northern Ethiopia. *J Med Virol* 1991;34:232-6.
2. Lednar WM, Lemon SM, Kirkpatrick JW, *et al.* Frequency of illness associated with hepatitis A virus infection in adults. *Am J Epidemiolog* 1985;122:226-33.
3. Vento S, Garofano T, Renzini C, *et al.* Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *New Eng J Med* 1998;338:286-90.
4. Tsega E, Nordenfelt E, Mengesha B, Hansson BG, Tsega M, Lindberg J. Age-specific prevalence of hepatitis A virus antibody in Ethiopian children. *Scand J Infect Dis* 1990;22:145-8.
5. Tsega E, Mengesha B, Hansson BG, Lindberg J, Nordenfelt E. Hepatits A, B and Delta infection in Ethiopia: A serologic survey with demographic data. *Amer J Epidemiol* 1986;123:344-51.
6. Tsega E, Hansson BG, Krawezynski K, Nordenfelt E. Acute sporadic viral hepatitis in Ethiopia: Causes, risk factors, and effects on pregnancy. *Clin Inf Dis* 1992;14:961-5.
7. Werzberger A, Mensch B, Kuter B, *et al.* A controlled trial of a formalin-activated hepatits A vaccine in healthy children. *New Eng J Med* 1992;327:453-7.
8. Westblom TU, Gudipati S, DeRousse C, Midkiff BR, Belshe RB. Safety and immunogenicity of an inactivated hepatitis A vaccine. *J Infect Dis* 1994;169:996-1001.
9. Conrad ME, Lemon SM. Prevention of endemic icteric viral hepatitis by administration of immune serum gamma globulin. *J Infect Dis* 1987;156:56-63.
10. Tsega E, Krawczynski K, Hansson BG, Nordenfelt E. Hepatitis E virus infection in pregnancy in Ethiopia. *Ethiop Med J* 1993;31:173-81.

11a. Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet* 1995;345:1025-6.

11b. Joshi YK, Babu S, Sarin S, *et al.* Immunoprophylaxis of epidemic non-A, non-B hepatits. *Indian J Med Research* 1985;81:18-9.

12. Tsega E, Mengesha B, Nordenfelt E, Hansson BG, Lindberg J. Prevalence of hepatitis B virus markers among Ethiopian blood donors: Is HBsAg screening necessry? *Trop Geog Med* 1987;39:336-40.
13. Maynard JE. Hepatits B: global importance and need for control. *Vaccine* 1990;8(Suppl):S18-S20.
14. Tsega E, Tsega M, Mengesha B, Nordenfelt E, Hansson BG, Lindberg J. Transmission of hepatitis B virus infection in Ethiopia with emphasis on the importance of vertical transmission. *Int J Epidemiol* 1988;17:91-6.
15. Tsega E, Horton J, Nordenfelt E, Hansson BG, Wolde-Hawariat G, Lindberg J. Do

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blood transfusion, injecting drug users (IDU) and, less often, by high risk sexual behaviour (57,58). However, the role of HGV in acute and chronic hepatitis remains to be fully defined. At present, HGV does not appear to be an important cause of clinical liver disease (59).

TT-virus: TT-virus is a novel DNA virus (named after the initials of the first patient from whom the virus was isolated) reported from patients with post-transfusion hepatitis in Japan (60). Since then, this virus has been reported from different countries in association with HCV, other known hepatotropic viruses and in healthy adults (61). The role of this virus in the pathogenesis of acute and/or chronic liver disease is yet to be elucidated.

CONCLUSION

It is quite obvious that there is an urgent need for drastic socioeconomic improvement and public health education about personal and environmental hygiene to control HAV and HEV infections. Mass vaccination against HAV is not practical at the present time since almost all Ethiopian children under 14 years of age have acquired protective levels of anti-HAV-IgG following asymptomatic or subclinical infections occurring from early childhood. In the future, the combined hepatitis A and hepatitis B vaccines (62) may become a part of the EPI. However, it is prudent to give hepatitis A vaccine to patients who are infected with HCV and are non-immune to HAV. One hopes that the production of HE vaccine for mass active immunization will take place soon, especially for women in third world countries who are in their reproductive years.

In the case of HBV infection, it is ethically unjustifiable to further delay the implementation of universal immunization of infants and adolescents as part of the EPI, especially with the current epidemic of HIV-1/AIDS in the country. Future generations will never pardon us if we continue to allow more and more Ethiopian children to be infected with this virus and HDV when the means of prevention is available. Blood and blood products must be screened before transfusion. Public education should focus on the avoidance of unsterilized syringes and needles, tattooing, ear-piercing, scarification, circumcision, tonsillectomy and similar unhygienic cultural practices.

How about the millions of HBV carriers who act as reservoirs of infection? Interferon alpha 2B and lamivudine therapy should be offered to patients during the active or immune-intolerant phase (active replication of HBV with detectable HBV-DNA and HBeAg) while preventive education should be given to the rest of the carriers.

The high prevalence and serious sequelae of HCV infection must be appreciated by all physicians, other health workers and the general public through institutional and mass educational programmes. Epidemiological studies should continue with emphasis on elucidating modes of transmission other than transfusion of blood and blood products. Blood and blood products should be screened for HCV, along with HBV and HIV, before transfusion. The universal precaution measures should be practiced strictly in all health provision centres. All patients who are HCV positive, except those with advanced cirrhosis, should be treated with a combination of interferon and ribavarin.

Laboratory facilities for the diagnosis of hepatitis A-E viruses must be available in some tertiary care centres and a more

infection, HCV (38%) is more common than HBV (28%) in patients with chronic hepatitis, cirrhosis and HCC. Thus, HCV infection appears to be a more common cause of chronic liver disease and HCC than HBV infection in this Ethiopian population (17,47).

Transmission of HCV is via the parenteral route (transfusion, intravenous drug use, tattooing, circumcision, body piercing, using contaminated instruments such as hypodermic needles, long-term haemodialysis and high-risk sexual behaviour) and transplantation of infected organs. Sexual transmission is very low, intrafamilial spread is not known and vertical transmission occurs in only about 5% of cases, being greater when the maternal HC viral load is high and in those infected with HIV. The virus has been detected in breast milk but no transmission via breast-feeding has been reported (43,48,49). The exact mode of transmission in Ethiopia is not known and the role of the above modes of transmission requires further studies.

Blood and blood products should be screened before transfusion, risky cultural practices such as tattooing, scarification, tonsillectomy, circumcision and the use of hypodermic needles and syringes outside health institutions should be discouraged through public health education, and, in all health institutions, the universal precaution guidelines should be strictly applied.

Efforts to develop vaccine have been unsuccessful because of high mutation of the HCV, difficulties in establishing stable culture of the virus in a cell line and the initial difficulties in identifying the actual virus (the molecular structure was known in 1989 but the virus, as a whole, was not identified until 1996). Patients with chronic hepatitis C infection and non-immune to HAV (negative for anti-HAV-IgG) should be given hepatitis A vaccine to prevent early and fulminant hepatitis with high mortality (3).

Since HC vaccine is unlikely in the near future, current emphasis is on early detection and treatment of chronic hepatitis C infection. Until recently, interferon alpha 2B monotherapy was the only choice available but with less than 25% sustained biochemical and virological response (normal ALT and negative HCV-RNA 6 months after the end of 6 months of interferon treatment) (50). As of 1998, however, multicentre, controlled, randomised and double blind trials have confirmed that a combination of interferon alpha 2B 3 million units thrice a week subcutaneously plus ribavarin 1000-1200 mg per os daily in two divided doses administered for 24-48 weeks result in 50%-57% sustained virological response, those with genotype 2 or 3, low levels of HCV-RNA, females, younger than 40 years of age, and those with minimal histolgic evidence of fibrosis responding better and earlier (51-53). This combination is, therefore, the current choice of treatment of chronic hepatitis C infection without advanced cirrhosis. Patients with chronic HCV infection and co-infection with HIV, respond less to interferon monotherapy or to the above combination therapy when compared to those without HIV (54-,55). In North America, end-stage liver disease due to HCV accounts for a significant number of patients with indications for liver transplantation at the present time.

Hepatitis G: Two independent groups discovered and sequenced an agent with limited homology to HCV in 1995 and 1996. These agents were called GBV-C and hepatitis C virus (HGV), respectively. They were later found to be variants of HGV, an RNA virus and a member of the Flaviviridae, transmitted commonly by

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HDV occurs world-wide with gross geographic differences, some of the highest frequencies being in the Mediterranean region and the Middle East (33-35). Acute HDV infection may occur simultaneously with HBV infection but HDV infection superimposed on chronic HBV infection is more common and is associated with rapid course and higher morbidity (36). In Ethiopia, three subjects were found positive for HDV infection in a population survey of 500 people. Thirty-one of the 500 people surveyed were positive for HBsAg (5). Only one HDV carrier was found among 485 blood donors with 50 HBsAg positive (12). This prevalence was lower than that of HDV infection reported among Ethiopian military recruits (37). On the other hand, the prevalence of HDV infection among 92 HBsAg carrier Ethiopian patients with chronic liver disease (chronic hepatitis, cirrhosis and HCC) was 23 (25%) (17). Thus, in Ethiopia, there is a significant difference in the prevalence of HDV infection between healthy carriers and those with chronic liver disease. These findings indicate that HDV is endemic in Ethiopia and most likely plays significant role in chronic liver disease associated with the HBsAg carrier state. A recent phylogenetic analysis of HDV has shown that Ethiopian isolates form a novel group, IC, within HDV genotype I (38). Whether this novel genotype influences the clinical or therapeutic outcome of HDV infection is yet to be shown.

The mode of transmission of HDV in Ethiopia is not known. However, parenteral transmission (e.g. blood transfusion, injecting drug use, etc.), sexual exposure to prostitutes, and, in some endemic areas, non-parenteral modes of transmission have been reported (35,39). There are no specific measures available to prevent HDV infection in HBsAg carriers.

Treatment with interferon alpha 2B has been tried but the results are less than satisfactory (40). However, vaccination against HBV is the most logical way of preventing HDV infection.

Hepatitis C: Hepatitis C virus (HCV) is an RNA virus of the Flaviviridae family, classified into six distinct major genotypes. These genotypes vary in their geographic distribution, type 1, with subtypes A and B, being the most prevalent world-wide, and appear to dictate the natural history and outcome of therapeutic response (41). The virus can undergo significant mutations over time within the host. In contrast to HBV which causes progression to chronic infection in only a minority of infected adults, more than 80% of patients exposed to HCV will remain chronic carriers (42). It is an important cause of acute and chronic hepatitis, cirrhosis and HCC (41-43).

The prevalence of antibody to HCV (anti-HCV), determined by second generation enzyme-linked immunosorbent assay (ELISA-2) and further confirmed by second generation recombinant immunoblot assay (RIBA-2), in healthy adult Ethiopian blood donors is 1.4%, similar to reports in many countries (41,44-46). On the other hand, the prevalence of anti-HCV among Ethiopian patients with chronic hepatitis, cirrhosis of the liver and HCC (21%, 36% and 46%, respectively) are significantly higher than that in healthy blood donors, indicating an aetiological association between HCV and these liver diseases. As mentioned above, the association between HBV infection and chronic liver disease is high, but there remains a significant number of patients negative for HBV infection but positive for HCV infection and that both active infections do not commonly co-exist in the same patient. In terms of current

HCC are strongly in favour of aetiological association (17-19). The most convincing proof comes from a nation-wide hepatitis B vaccination programme in Taiwan which resulted in significant reduction of the HBV carrier state and the incidence of HCC in children (20).

Thus, it is now generally accepted that implementation of a policy of universal immunization of infants, adolescents, or both against hepatitis B, preferably integrated into the EPI, should take place in all countries. It is particularly important and urgent that countries like Ethiopia, hyperendemic for HBV infection and with high prevalence of HCC and HIV, should implement such a programme. The safety and efficacy of the available vaccines in Ethiopian infants and children, either alone or integrated into EPI, have been shown to be as good as anywhere (21,22). It was also shown that these Ethiopian vaccinees were protected against HBV infection for at least 5 years (23).

As summarised above, there are ample clinical and epidemiological data on HBV infection, obtained from research conducted within Ethiopia and in collaboration with international institutions, to convince Ethiopian health providers that the implementation of universal immunization of infants against HBV is long overdue. Should cost be a deterrent to the implementation of such a policy? It is clear that the prevention of HBV infection by universal immunization of infants and adolescents or both is more cost-effective than treating (often with repeated hospitalizations with long-term bed occupancy) acute and chronic hepatitis, cirrhosis and HCC caused by the same virus. Lower doses of HB vaccine have been shown to achieve the same protective level of antibodies as the standard adult doses (21,23,24-26). Furthermore, there is no need to use hepatitis B immune globulin along with HB vaccine in infants born to Ethiopian mothers since vertical transmission of HBV is not common in this population (14,15,20,22).

What should be done with chronic HBV carriers (HBsAg positive for more than six months)? Interferon or lamivudine treatment works best during the immune-tolerant or active phase of HBV infection (HBV-DNA and HBeAg detectable in serum). Interferon alpha 2B administered in doses of 10 million units three times a week or 5 million units daily subcutaneously for 16 weeks will seroconvert a replicative to a non-replicative phase in 30% to 50% of patients (27,28). Interferon therapy for patients with chronic hepatitis B is reported to be cost-effective (29,30). Several antiviral agents have been tried in recent years and, of these, lamivudine (2-deoxy-3-thiacytidine) is found most effective. Given orally as a single dose of 100 mg daily, it suppresses the activity of HBV by inhibiting the reverse-transcriptase of the virus and improvement occurs in the liver histology. The side effects of lamivudine are better tolerated than those of interferon (27,28). Unfortunately, after discontinuation of therapy, there is often recurrence of viral activity and about 10% to 15% of patients will develop a mutant virus after 9-12 months of treatment which results in increase in the serum HBV-DNA levels and progression of hepatitis (31,32). Famciclovir, gancyclovir, adefovir and lobucavir have been tried but these antiviral agents are found to be less effective than lamivudine. A combination therapy of interferon and lamivudine is being tried presently.

Hepatitis D: Hepatitis D virus (HDV) is a defective, hepatotropic RNA virus which depends on the presence of HBV for its expression and replication. Infection with

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Amir sultan (MD), 2014

Thus, since there is no vaccine or immune prophylaxis against HEV at the present time, emphasis must be on public health education about personal and environmental hygiene, especially protection of water supplies from faecal contamination, adequate chlorination and/or boiling of drinking water.

Parenterally transmitted hepatitis

Hepatitis B: Hepatitis B virus (HBV), a DNA virus belonging to the hepadna group, has a variable distribution world-wide. The hepatitis B surface antigen (HBsAg) carrier state and the overall HBV marker prevalence are 0.1% to 0.5% and less than 5%, respectively, in western Europe and north America, while they are 5% to 20% and 20% to 95%, respectively, in sub-Saharan Africa (5,12). It is estimated that, world-wide, over 2,000 million people are infected with HBV, that there are over 300 million carriers of the virus and more than 2 million deaths occur every year as a direct result of HBV infection (13). In Ethiopia, population based epidemiological surveys indicate that the prevalence of HBsAg carrier state is 8% to 12%, the overall HBV marker prevalence is 72% to 79%, that infection with HBV occurs at an early age with the HBV marker prevalence increasing gradually with age, and that the male-to-female ratio of the HBsAg carrier state is 2:1 (5,12).

The main mode of transmission of HBV is percutaneous from blood and blood products, the most important being blood transfusion. Less common means of transmission include unsterilized syringes and needles, tattooing, ear piercing, scarification, circumcision, etc. However, in the majority of Ethiopians with past or present HBV infection, there is no known exposure to the above and the modes of transmission in Ethiopia remain unknown and, therefore, require further studies. A two year controlled study of 54 Ethiopia families and a survey of hospital staff with their offspring indicate that, unlike in south-east Asia, vertical transmission of HBV infection plays a minor role and that horizontal transmission is the major route within family settings (14,15). While traditional practices such as circumcision, tonsillectomy, tattooing, etc. cannot be ignored as important vehicles for the transmission of HBV infection, it should be emphasised that the risk of HBV infection has increased three fold in those infected with the human immunodeficiency virus (HIV) (16), a condition which exists in modern Ethiopia in epidemic proportion.

Acute viral hepatitis, chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma (HCC) account for 12% of hospital admissions and 31% of mortality on medical wards in Ethiopia (4). Hepatocellular carcinoma, 95% of which occurs in association with cirrhosis, is the commonest tumor in medical in-patients in Ethiopia. It follows an aggressive course with rapid deterioration and death resulting within a few weeks after diagnosis (17). The risk of acquiring HCC in HBV carriers is estimated to be 100 times higher than in non-carriers (18a,18b). For this reason and the significantly high prevalence of HCC in the world, HBV is believed to be second only to tobacco among the known carcinogens (19). While a direct cause and effect relationship is difficult to demonstrate, the clinical and epidemiological data, the high prevalence of HBV markers in the general population and in each type of chronic liver disease, and the progression from one stage to the next with obvious histological overlap between chronic hepatitis, cirrhosis and

carrier state and hence, does not cause chronic liver disease and confers life long immunity. Infection with HAV at a younger age is usually asymptomatic or subclinical but severity increases with age: children under 5 years of age have a case fatality rate of 1.5 per 1000 and patients over 50 years of age have a case fatality rate of 27 per 1000 (2). Fulminant hepatitis due to HAV alone is rare but a recent study suggests that it is common when HAV superinfection occurs in patients with chronic hepatitis C virus (3). Serological surveys from different parts of the world have shown varying prevalence of IgG-specific antibodies to HAV (anti-HAV-IgG), depending on socio-economic status and other factors. An age-specific prevalence study of HAV antibody in Ethiopia revealed that 50% of 959 subjects, 89% of whom were children under 15 years of age, were positive for anti-HAV-IgG before 5 years of age, increasing rapidly with age to become universally positive after 15 years of age (4). In another seroepidemiological study of adult subjects from five different provinces of Ethiopia, 84% were positive for anti-HAV-IgG(5). These data show that HAV infection in Ethiopia is widespread, that it occurs early in life, and, therefore, environmental and socio-economic factors play a major role in its transmission. As expected, HAV is an uncommon cause of acute sporadic hepatitis (6) and very unlikely to cause epidemic outbreaks of hepatitis (4).

Two types of vaccine against HAV (Havrix, SmithKline Beecham; Vaqta, MSD), given with a booster dose after a primary vaccination, have been shown to give protective levels of anti-HAV antibodies in over 90% of vaccinees (7,8). If most HAV infections occur early in life and the clinical presentation at this age is usually subclinical or asymptomatic, there is no need for a mass vaccination programme against HAV with or without the Expanded Programme of Immunisation (EPI) in present day Ethiopia. Emphasis should be on the improvement of socio-economic and sanitary conditions. However, patients with chronic hepatitis C should be tested for anti-HAV and, if found negative or non-immune, they should receive active immunisation against HAV to prevent fatal, fulminant hepatitis (3). Regular immunoglobulin, administered in a dose of 0.06 mL/Kg body weight before or immediately after exposure to HAV, is 80% to 90% effective as a prophylaxis (9). Thus, those in close contact with HAV-infected patients, especially children under 15 years of age, should be offered immunoglobulin prophylaxis. There is no known specific treatment for HAV.

Hepatitis E: Sporadic and epidemic forms of hepatitis E virus (HEV) infection are also common in Ethiopia as in many developing countries. A study of 110 consecutive Ethiopian patients presenting with acute sporadic viral hepatitis revealed that 36 (33%) were due to HEV (6). Epidemic outbreaks were only due to HEV (1). The clinical course of HEV infection is generally mild, short and self-limited. However, its occurrence in pregnancy is associated with high maternal and perinatal morbidity and mortality (1,6,10).

A recent study has shown that HEV is commonly transmitted from infected mothers to their newborn (vertical transmission) with significant perinatal morbidity and mortality (11a). Unlike HAV infection, it is still not known if antibodies after HEV infection will provide lifelong immunity. Immune globulin prepared from previously infected local donors is said to be protective against HEV infection, but this approach is impractical and of uncertain value (11b).

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REVIEW ARTICLE

EPIDEMIOLOGY, PREVENTION AND TREATMENT OF VIRAL HEAPATITIS WITH EMPHASIS ON NEW DEVELOPMENTS

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INTRODUCTION: There are a large number of viruses, such as cytomegalovirus, Epstein-Barr, Herpes simplex, mumps, varicella, yellow fever, etc., known to cause inflammatory disease of the liver, but the term viral hepatitis generally refers to the five well described hepatotropic viruses which are divided into enteral and parenteral groups based on their mode of transmission. Hepatitis A and E viruses are enterically transmitted by the faecal-oral route and do not exist in a chronic carrier state. Hepatitis B, C and D viruses are parenterally transmitted, occur both in the acute and chronic forms, and, when they persist in a chronic carrier state, they serve as a reservoir for infection and give rise to chronic hepatitis, cirrhosis and hepatocellular carcinoma. Hepatitis G virus has recently been described but its significance in the causation of human liver disease is yet to be established. Also, the most recently described TT virus in patients with post-transfusion hepatitis awaits further studies.

Acute sporadic and epidemic viral hepatitis are common world-wide, mostly in the developing countries, including Ethiopia, and account for high morbidity and mortality, especially among pregnant women. Chronic infection with hepatitis B virus is a significant problem on a global scale, affecting over 300 million people. Hepatitis C virus infection is probably the most common cause of chronic viral hepatitis, end-stage liver disease and hepatocellular carcinoma in the world, especially in sub-Saharan Africa, including Ethiopia. Therefore, this article will review and highlight the relevant epidemiological, preventive and therapeutic aspects of viral hepatitis with emphasis on new developments and recent data obtained from Ethiopian studies.

Enterically transmitted viral hepatitis

Enterically transmitted viral hepatitis, caused by hepatitis A virus (HAV) and hepatitis E virus (HEV), is common world-wide, mostly in developing world. The common est mode of transmission being the faecal-oral route and person-to-person contact, both HAV and HEV are well known causes of sporadic acute hepatitis and outbreaks of epidemic hepatitis (1).

Hepatitis A: Hepatitis A virus, a ribonucleic acid (RNA) virus belonging to the family picornaviridiae, causes mild and self-limited disease, does not result in chronic

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NEONATAL CONGENITAL HYPOTHYROIDISM SCREENING IN ADDIS ABABA, ETHIOPIA
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NEONATAL CONGENITAL HYPOTHYROIDISM SCREENING IN ADDIS ABABA, ETHIOPIA

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ABSTRACT

Objectives: To determine the prevalence of congenital hypothyroidism, establish reference levels for thyroid stimulating hormone (TSH), and determine the cut-off points of TSH level for neonatal screening in congenital hypothyroidism.
Design: A cross-sectional study.
Setting: Ghandhi Memorial, Zewditu, Tikur Anbessa and St Paulo's referral hospitals in Addis Ababa, Ethiopia, from July 1996 to January 1997.
Subjects: Four thousand two hundred and six consecutive newborns.
Main outcome measures: TSH and T4 values using standard TSH level estimation approach.
Results: The overall mean TSH value was 9.6 mIU/l with standard deviation of 7.8 mIU/l. Transient hypothyroidism occurred in 3.6% of the neonates. No true case of congenital hypothyroidism was identified. The mean(SD) sampling time was 12.8(6.7) hours and the recall rate 4.8%. Most neonates (98.6%) were screened at or less than 24 hours of age. Twins had lower mean TSH value of 7.9(SD=6.4)mIU/l than single neonates with 9.6(SD=7.8)mIU/l, while females had significantly lower mean TSH value of 9.4(SD=7.9) mIU/l than males of 9.8 (SD=7.6)mIU/l. The mean TSH level decreased significantly as sampling time increases, the mean TSH level for neonates older than 24 hours is significantly lower than the mean TSH level for those with 24 hours old or less. No significant difference was found in the mean TSH levels in relation to maternal age, parity and gestational age. No significant correlation was found between TSH value and birth weight (r=0.02), height (r=0.03),and head circumference (r=0.02) of the neonates. The most appropriate TSH level cut-off point was found to be at 29.4mIU/l. The reference range (the range between 2.5th and 97.5th percentiles) for serum thyrotrophin value was higher in the age range of 6 to 12 hours and then declined.
Conclusion: This pilot study has highlighted important findings, however larger studies should be conducted to assess the magnitude and associated factors of congenital hypothyroidism because congenital hypothyroidism represents one of the most common preventable causes of mental retardation.

INTRODUCTION

Congenital hypothyroidism represents one of the most common preventable causes of mental retardation (1). The prevalence and/ or incidence of permanent primary congenital hypothyroidism is approximately 1:3800 to 4000 livebirths worldwide (1-7). At birth the weight of the brain is 25% that of the adult brain. Pickering and Fisher have shown that half of the postnatal brain growth is completed by six months of age (2,8). Thyroid hormones play an essential role during the critical stage of brain growth and development. Therefore, congenital thyroid hormone deficiency can be a cause for impairment of brain development. Early recognition and treatment of hypothyroidism can prevent the neurological and mental deficiencies secondary to thyroid hormone deprivation in early life (2,9).

Since the pioneer development of a pilot screening programme for congenital hypothyroidism in Quebec, Canada and Pittsburgh in USA in 1979, new-born screening for congenital hypothyroidism has become routine in

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western countries and many countries in eastern Europe, South America, Asia and Africa (1-9).

Iodine deficiency is a major cause of thyroid diseases resulting in physical and mental disorders. To prevent iodine deficiency at an early stage it is emphasised to screen out biochemical hypothyroidism at the neonatal stage (10). In Africa there is a widespread deficiency of iodine and endemic goitre occurs in many areas. Iodine deficiency disease (IDD) is one of the top ten priority problems in Tanzania, affecting 65% of adolescents. In the Sudan the prevalence of IDD reaches up to 84% in some inland mountainous areas (11-13). The Ethiopian Nutrition Institute had undertaken a systematic survey of 70% of the country covering randomly selected sites stratified by region and altitudes and found prevalences of goitre from 1.9% to 58.9% in urban areas and 1.2% to 73.6% in rural areas. The population with higher goitre rates had low urinary iodine excretion rates (14). Zewde *et al*(15), studied the severity and extent of IDD in Ethiopia, and goitre prevalence rate in school children and household were found to be 30.6% and 18.7%, respectively.

A raised thyroid stimulating hormone (TSH) level indicates a likely diagnosis of congenital hypothyroidism but does not provide a reliable guide about the severity of the disorder. Plasma thyroxine provides information on severity of congenital hypothyroidism. Newer and more sensitive TSH assay techniques such as the Enzyme Linked Immunoassay (ELISA), Immuno Radiometric Assay (IRMA), Chemiluminescent assay and fluroimmunoassay offer an advantage of using TSH for screening programmes(1,5,7,16). Early diagnosis and treatment of congenital hypothyroidism before two months of age usually results in mental development within the normal range (7,17). Klein and associates showed that when hypothyroid infants were treated before three months of age, 70% attained an IQ above 85, whereas when treatment was delayed for three to seven months, 85% had definite mental deficiency (2,17-20).

Although a large sample size is required to address a health problem whose worldwide prevalence and/ or incidence of permanent primary congenital hypothyroidism is very low, there has been no report on congenital hypothyroidism from Ethiopia. Therefore it is pertinent to perform a pilot study with the objective of determining the prevalence of congenital hypothyroidism, establish the reference intervals for TSH in normal neonates and determine the cut-off point of TSH value for neonatal screening for hypothyroidism.

MATERIALS AND METHODS

A pilot survey for neonatal congenital hypothyroidism was conducted in infants born at the four referral hospitals (Ghandi Memorial, Zewditu, Tikur Anbessa and St Paulo's) in Addis Ababa, Ethiopia from July 1996 to January 1997.

A total of 4206 consecutive new-borns were screened for congenital hypothyroidism after obtaining informed consent from each mother to whom the purpose of the study had been explained.

Midwives were instructed on the method of blood collection by the heel prick techniques and application of blood in the three holes of Guthrie cards. The specimens were collected just before discharge of the neonates from the hospital ranging from six hours to seven days after birth. The blood was applied on one side of the filter paper to saturation. After drying, the specimens were stored in a sealed bag at -20°C until assayed at the Nuclear Medicine Unit, Department of Internal Medicine, Medical Faculty, Addis Ababa University.

TSH assay was chosen as the primary screening method because, it is a one step method, the time required for TSH assay was short and the dried blood spots on Guthrie cards were easier to handle and were more safely transportable. TSH was determined in the dried blood spots by IRMA method(21). The Assay is a simple two-site solid-phase IRMA for the measurement of TSH in dried blood spots. TSH antibody coated beads, and ^{125}I-labelled TSH monoclonal antibody were utilised in the assay system (NETRIA, The Royal Hospitals NHS Trust, St. Bartholomew's Hospital, 51-53 Bartholomew Close, London ECIA 7BE).

Polystyrene assay tubes (12x75mm) were labelled in duplicate for standards, quality control pools, patient samples and total tubes. Six millimeter discs were punched from each standard, labelled tubes. All tubes were vortex mixed after 300□l ^{125}I-anti-TSH monoclonal antibody was added into respective tubes. Then each tube received one coated bead and was left rotating overnight at room temperature. All tubes were washed by adding 2ml wash buffer to remove unbound radio labelled tracer. These were aspirated and the filter discs removed. The washing and aspiration steps were repeated twice and the tubes were counted for 150 seconds in the RIASTAR 5410 gamma counter to determine the amount of bound radio labelled TSH tracer. Data from the gamma counter was processed using the programme supplied with counter. Four parameter logistic curve fitting procedure was used to generate a standard curve and results of patient samples were read from the standard curve (21).

Infants with TSH values greater than 25mIU/l were recalled as recommended by co-ordination meeting of AFRA-V (13). The paediatrician did physical examination for evidence of congenital hypothyroidism and serum was collected for T4 and TSH and determined by RIA and IRMA Methods. T4 determination was done as follows: polystyrene assay tubes (12x75mm) were set up in duplicate, standards, quality control pools and patient sera (25µl each) were pipetted into respective tubes; then 50µl of ^{125}I labelled Thyroxin (125I-T4/ANS) was added in all tubes followed by 50µl anti-T4 (no anti-T4 in the non-specific Binding tubes). Donkey anti-Sheep Serum (50µl) was also added followed by 50µl buffer. Tubes were vortex mixed and incubated for two hours at room temperature. After two hours incubation 500µl 4% Polyethylene Glycol 600 (PEG) was added, vortexes and centrifuged at 1000g for about 30 minutes. The antibody bound and the free fractions were separated by decanting the supernatant and the radioactivity associated with the antibody bound fraction counted in the RIASTAR 5410 gamma counter for 60 seconds. Four parameter logistic curve fitting procedure was used to calculate the results of patient samples.

Accordingly, infants with TSH greater than 7mIU/l and T4 less than 58nmol/l were diagnosed as having congenital hypothyroidism (22), and infants with TSH greater than 7mIU/l but normal T4 were considered as compensated

congenital hypothyroidism (sub clinical congenital hypothyroidism). The identified infants were transferred to the paediatric department and followed by a paediatrician.

In accordance with the recommendation of the International Federation of Clinical Chemistry, the ninety fifth scatter range i.e. the range between 2.5th and 97.5th percentiles were calculated as reference range (23).

Statistical analysis: Analysis of variance (non parametric Kruskal Wallis when found appropriate) was used to compare more than two means, while Student's t-test(non parametric Mann-Whitney two sample test when found appropriate) was applied for only two means. Scatter plots and correlation coefficients were applied to asses relationship between weight, height and head circumference of the new borns and TSH values. Data were analysed using STATA (Intercooled V. 5.0. Stata Corporation).

RESULTS

There was a total of 5965 livebirths in the four referral hospitals during the study period. Among these, blood spot tests were collected from 4206 neonates which accounted for 73.7% of the livebirths. The remaining 22.3% were not included in the screening because of early neonatal death, early discharge before six hours of age and those neonates delivered at Gynaecology out-patient department (OPD) and also those referred to Ethio Swedish Paediatrics Hospital did not appear for screening. Out of 4206 neonates 2160 (51.7%) were males and 2046 (48.6%) were females.

The mean sampling time (age of neonate at the time of discharge) was 12.8±6.7 hours. The recall rate was 4.8%. Four thousand one hundred and thirty one (98.6%) neonates were screened at or less than 24 hours of age. Four mothers had been on propylthiouracil, 12 mothers had a history of thyroidectomy for goitre. None of the neonates had goitre or umbilical hernia, 40 neonates had cold extremities, three had oedema of the extremities and seven neonates had congenital malformations (three had clubfoot, one had club foot with hypotonia, one had absence of toes of the lower extremities, one had absence of fingers of upper extremities, another one had closed urethral office).

The mean TSH level was 9.6 mIU/l with 95% confidence interval (CI) 9.3-9.8. Transient hypothyroidism occurred in 3.6% of the neonates. No true case of congenital hypothyroidism was identified. TSH was less or equal to 25mIU/l in 4004 (95.2%) neonates but higher than 25mIU/l in 202 (4.8%) neonates. About 3811 (90.6%) neonates had TSH levels less or equal to 20mIU/l. Among 202 neonates, 152 (75.2%) came for repeat examination of TSH and T4. The remaining babies died in the neonatal period or early infancy. Some infants were untraceable either due to wrong address or migration.

The TSH returned to normal and T4 was normal in 150 neonates. But TSH was high and T4 was normal in two neonates (a male and a female with initial TSH values of 26.9mIU/l and 30.9mIU/l respectively). These two neonates were identified as compensated congenital hypothyroidism. Table 1 shows that twins had slightly

Table 1

Mean ± SD TSH values in 4206 live births from the four referral hospitals

Variable	No. of neonates	Mean (SD) TSH in MIU/l	F-value	p-value
Age of the mother (yrs)				
<19	492	9.7(7.7)		
20-24	1279	9.6(7.8)		
25-29	1311	9.7(7.8)		
30-34	675	9.2(7.8)		
35+	423	9.8(7.7)	0.43	0.7884
Sex of neonates				
Male	2160	9.8(7.6)		
Female	2046	9.4(7.9)	2.73[1]	0.01
Parity				
1	1819	9.5(7.5)		
2.	929	9.5(8.0)		
3.	564	9.8(8.2)		
4.	336	8.6(7.6)		
5.	181	10.2(7.8)		
6.	110	9.6(7.1)		
7+	193	9.8(8.3)	0.89	0.5029
Gestational age (wks)				
<38	344	9.6(8.1)		
38-42	3134	9.6(7.8)		
>42	248	9.0(6.7)		
unknown	44.8	9.5(7.7)	0.48	0.6690
Birth weight(kg)				
<2500	437	9.1(7.3)		
>2500	3737	9.6(7.8)	1.01[1]	0.3132
Delivery outcome				
Singleton	4017	9.6(7.8)		
Twin	159	7.9(6.4)	1.89[1]	0.0587
Sampling time (age in hours)				
6-12	2349	9.8(7.9)		
13-24	1750	9.4(7.6)		
>24	75	8.0(7.6)	8.48[2]	0.014

[1] Mann-Whitney z-value [2] Kruskal Wallis Chi-squared value

lower mean TSH value (7.9mIU/l, 95% CI 7.2-9.3) than single tons (9.6mIU/1, 95% CI 9.4 - 9.9) while females had significantly lower mean TSH value (9.4mIU/l 95% CI 9.0 - 9.7) than males (9.8mIU/1, 95% CI 9.5-10.1). The mean TSH value decreased significantly as sampling time increases. The mean TSH value for neonates older than 24hrs was significantly lower than the mean TSH values in relation to low birthweight, maternal age, parity and gestational age. No significant correlation was found between TSH and birthweight (r=0.02), TSH and height (r=0.03), and, TSH and head circumference (r=0.02) of the neonates (Figure 1)

Figure 1a

Scatter plot of weight versus TSH value of neonates screened

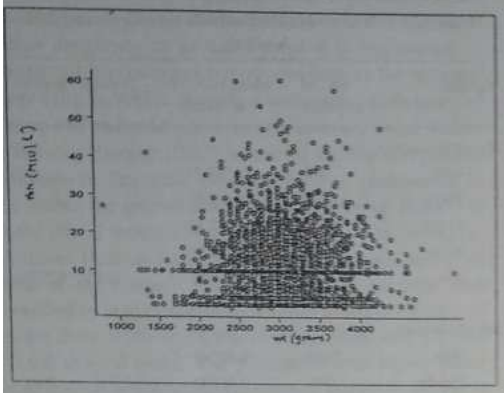


Figure 1b

Scatter plot of height versus TSH value of neonates screened

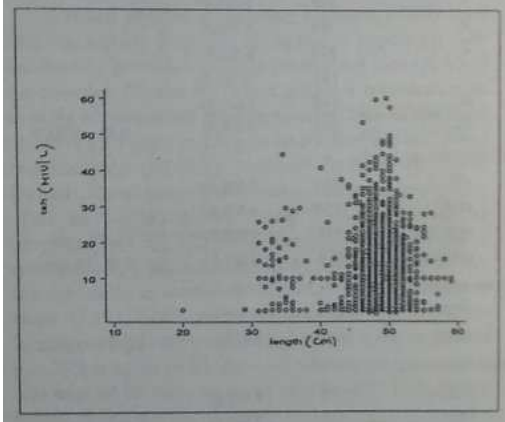


Figure 1c

Scatter plot of headcf versus TSH value of neonates screened

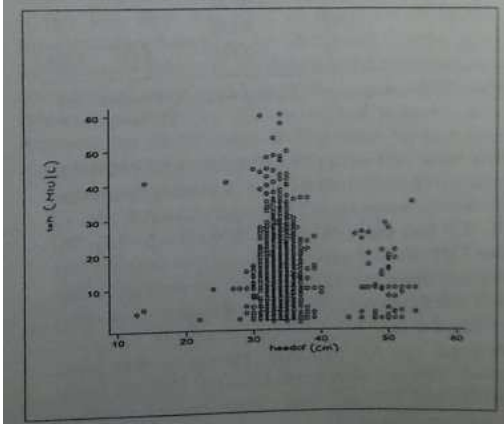


Table 2

Reference range for thyrotrophin (TSH value in MIU/l) of neonates

Sampling time*	Number of neonates	Median value	Reference 2.5-97.5	Minimum value	Maximum value
6-12	2349	10.0	1.0-29.8	0.3	60.4
14-24	1750	10.0	0.8-27.8	0.3	57.8
>24	75	9.2	1.0-30.8	1.0	36.1

*Age of neonate in hours

The TSH cut-off value was found to be 29.4mIU/l. From this survey, a reference range (the range between 2.5th and 97.5th percentiles) were determined for serum concentration of thyrotrophin in normal neonates at different age groups (Table 2), where serum thyrothrophin value was higher in age range of six to 12 hours and then declined.

DISCUSSION

In the present pilot survey of neonatal screening for hypothyroidism at four referral hospitals in Addis Ababa over six months period, 73.7% of the livebirths were screened. Two compensated cases of congenital hypothyroidism were identified among 4206 neonates. No definitive case of congenital hypothyroidism was identified.

While a high prevalence of congenital hypothyroidism is expected in areas where iodine deficiency (IDD) is common (14,15), this study identified no neonate with congenital hypothyroidism. The incidence of congenital hypothyroidism was reported to be 1:4000 in England, 1:3600 to 1:4148 in America and 1:7000 births in Quebec(2,3). In Africa, from pilot survey reports on the third co-ordination meeting of African regional co-operative Agreement (AFRA-V) in 1996, the prevalence in Cairo was 1:3519, Tanzania 1:800, Morocco 4:3300 and in Tunisia 5:17659 respectively (22).

Several reasons could be given for the absence of congenital hypothyroidism in neonates in this study. First, and foremost could be that the number of screened neonates might not be sufficient enough to find reacted congenital hypothyroidism. Secondly, about 25% of the neonates with TSH values higher than 25mIU/l, could not be traced because of change of address or probably due to early neonatal or infant deaths. Infants were recalled within three weeks to six months of initial TSH determination which could have contributed for change of address of the infants. Some parents declined to bring infants even back after repeated explanation probably due to other reasons such as fear of HIV testing.

Transient hypothyroidism frequently results from intra-uterine exposure to anti thyroid drugs, maternal anti-thyroid antibodies or endemic iodine deficiency (1). From our report in addition to the above possible reasons, 98.6% of the samples were collected within six to 24 hours of birth a period where there is rapid rise and fall in serum TSH levels. Because of the release of stored pituitary TSH (25), which might have contributed to high prevalence of

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transient hypothyroidism of 1:21 in our report as compared to other reports from North America of 1:50000 and Europe of 1:200 to 1:8000 (1).

The recall rate was 4.8% as compared to 0.05%, which was recommended by American Academy of Paediatrics Section on Endocrinology and Committee on Genetics and American Thyroid Association Committee on Public Health (1). The average sampling time in this study was 12 hours, which is much lower as compared to the average sampling time (age of the neonate in hours) of 24 hrs reported by a study from Tunis (22). Most of the neonates (98.6%) were discharged from the hospital within 24 hours of age.

The higher TSH value in singletons may be most likely due to the rapid rise and fall in serum TSH and may represent release of stored pituitary TSH in newborns(25). However, clearly more specific studies would be needed to confirm this supposition. Similarly, this study showed that females had significantly lower TSH values than males. Again, more studies on sex specific congenital hypothyroidism are needed to confirm this difference.

Our study further showed that 3811 neonates (90.6%) had TSH values equal or less than 20mIU/l, and one of the compensated congenital hypothyroid neonates had TSH of 26.9mIU/l, while the other one had 30.9mIU/l. The TSH cut-off point for TSH values for congenital hypothyroidism screening was found to be 29.4mIU/l. The reference ranges were determined for serum concentration of thyrotropin in normal neonates at different age groups. Serum thyrotropin value was higher in age range of six to 12 hours and then declined. Similar findings were reported by Widemann *et al* (24). The number of neonates in the age range greater than 24 hours is small for comparison. Although it is very difficult to establish reference range from such a pilot study, it is assumed that this study will be helpful as a first step in generating hypotheses for larger studies.

To our knowledge this is the first report of neonatal hypothyroidism screening in Ethiopia which has highlighted some important findings. Larger studies should however be conducted to assess the magnitude of the problem and factors associated with congenital hypothyroidism because it represents one of the most common preventable causes of mental retardation.

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REFERENCES

1. A.A.P. section on Endocrinology and Committee on Genetics, and American Thyroid Association Committee on Public Health. New born screening for Congenital Hypothyroidism. Recommended guidelines *Paediatrics* 1993; **91:** 14th edition.
2. Dussault, J.H., Coulombe, P., Laberge, C., Letarte, J., Guyda, H. and Khoury K. Preliminary report on a mass screening program for neonatal hypothyroidism. *J. Paediat.* 1975; **86:** 670-674.
3. Grant, D.B. and Smith, I. Survey of neonatal screening for primary hypothyroidism in England, Wales and Northern Ireland 1982-84. *Brit. Med. J.* 1988; **296:** 1355-1358.
4. Hanna, C.E., Kranz, P.L. and Skeels, M.R. *et al.* Detection of congenital hypopituitary hypothyroidism. Ten year experience in the North West Regional Screening Program. *J. Paediat.* 1986; **109:** 959-964.
5. Hulse, J.A., Grant, D.B. and Clayton, B.E. *et al.* Population screening for congenital hypothyroidism. *Brit. Med. J.* 1980; **280:** 675-678.
6. Verkerk, P.H., Buitendijk, S.E. and Pauline, S., Verloove-Vanhorick. Congenital and the cut off for thyrotropin measurement. Recommendations from Netherlands. *Amer. J. Publ. Hlth.* 1993; **83:** 868-871.
7. Fisher, D.A., Calf, T. Second International conference on Neonatal Thyroid Screening: A progress note. *J. Paediat.* 1983; **102:** 653-654.
8. Nelsons, Textbook of paediatrics. Congenital Hypothyroidism. 1992. 14th ed. 1414-1421.
9. Carr, E.A. Jr, Beierwaltes, W.H., Raman, G., Daolson, V.N., Tanton, J., Betts, J.S., Stanbaugh, R.A., Spafford, N.R. and Tanner, K. The effect of Maternal thyroid function on fetal thyroid function and development. *J. Clin. Endocrinol. Metab.* 1959; **19:** 1.
10. A.M., Ishaque, F., Moslem and S., Khalil Ullah, *et al.* Neonatal hypothyroidism screening in Bangladesh (in press)
11. McGill, P.E., Taylor, J.R. and Sporry, A. A new focus of endemic goiter in Mwezi, Tanzania, East Africa. *East Afr. Med. J.* 1970; **47:** 66-72.
12. Hanegreof, T.H.A. and McGill, P.E. Prevalence and geographic distribution of endemic goiter in East Africa. *East Afr. Med. J.* 1970; **47:** 61-65.
13. Third Coordination Meeting of the Project African Regional Cooperative Agreement (AFRA V) Tunis, 1995.
14. Nekatebeb, H. Goiter problems in Ethiopia. *Amer. J. Clin. Nutr. suppl.* 1993; **57:** 315 S-316.
15. Zewdie, Welde-Gebriel. Micro nutrient deficiencies in Ethiopia and their inter-relationships Printing Grafisch Service Centrum, LUW p41-56.
16. Grant, D.B. Congenital hypothyroidism. Optimal management in the light of 15 years experience of screening. *Arch. Dis. Child* 1995; **72:** 85-89.
17. New England congenital hypothyroidism collaborative. Effects of Neonatal Screening for hypothyroidism. Prevention of Mental retardation by treatment before clinical manifestations. *Lancet* 1981; **ii:** 1095.
18. Tilloston, S.L., Fuggie, P.W., smith, I., Ades, A.E. and Grant, D.B. Relation between biochemical severity and intelligency in early treated congenital hypothyroidism: threshold effect. *Brit. Med. J.* 1994; **309:** 440-444.
19. LaFranchi, S.H., Hanna, C.E. and Krainz, P.T. *et al.* Screening for congenital hypothyroidism with specimen collection at two time periods, results of the north west regional screening program. *Paediat.* 1985; **76:** 734-40.
20. Rovert, J., Ehrlich, R. and Sorbara, D. Intellectual out come in children with fetal hypothyroidism. *J. Paediat.* 1987; **110:** 700-704.
21. Coated bead Immuno Radiometric Assay (IRMA) for human TSH applied to dried blood spots, NETRIA 1996.
22. Report on the Co-ordination meeting of AFRA (African Regional cooperative agreement) V RAF/6/007, 1996, Addis Ababa, Ethiopia.
23. International Federation of Clinical Chemistry Scientific Committee, Clinical section expert panel on theory of reference values and International Committee for standardization in Haematology, Standing committee on reference values (1987) approved recommendation (1987) on the theory reference values. Part 5, Statistical treatment of collected reference values. Determination of reference limits. *J. Clin. Chem. Clin. Biochem.* **25:** 645-656.
24. Wiedemann, G., Jonet Mentzel, L. and Panse, R. Establishment of reference ranges for thyrotrophin, triiodothyronine, and thyroxine and Free thyrotine in neonates, Infants, children and Adolescents. *Eur. J. clin. chem. clin. biochem.* 1993; **31:** 277-288.
25. Fisher, D.A. and Odell, W.D. Acute Release of thyrotropine in the newborn *J. clin. investigation* 1969; **48:** 1670-7.

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MATERNAL MORTALITY AT THE RIFT VALLEY PROVINCIAL GENERAL HOSPITAL, NAKURU
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MATERNAL MORTALITY OCCURRING AT THE RIFT VALLEY PROVINCIAL GENERAL HOSPITAL, NAKURU

E.A. JUMA and F.N.O. ODIYO

ABSTRACT

Objective: To evaluate the factors that led to maternal mortality over a five-year period at the Rift Valley Provincial General Hospital, Nakuru.
Design: A retrospective survey based on secondary data.
Setting: Department of Obstetrics and Gynaecology, Rift Valley Provincial General Hospital, Nakuru, between 1994 and 1998.
Subjects: Evaluation of 109 deaths where records were available.
Results: The average maternal mortality was 3.16/1000 live births. Twenty eight (26%) occurred in teenagers. Sixty two deaths (58%) accounted for those aged (14-24 years). Thirty four per cent of sixty two deaths occurred in nulliparous and primiparous patients. The commonest cause of death was haemorrhage and ruptured uterus (44%). Most deaths (53%) occurred within 24 hours of admission. Of these, 36((62%) came directly from home in labour or ill or where deliveries were being attempted by non-medical persons.
Conclusion: Most of the deaths evaluated were preventable. Management of the major causes of death include blood (haemorrhage and ruptured uteri) transfusion. With poor blood transfusion services and the risk of HIV, this calls for alternatives to direct blood transfusion. Referral systems need to be overhauled and referring health institutions should get properly staffed. Despite good antenatal clinic attendance and availability of resources most patients still deliver at home.

INTRODUCTION

Maternal and perinatal mortality rates are critical indicators of the quality of care in any health institution(1). Maternal deaths are indeed a tragedy in any societybut with proper management virtually all are preventable(2). A lot has been said and written on maternal mortality and ways of improving it the world over. Women in the developing world have a much greater risk of pregnancy-related death than do women in the developed world(3). Knowing that there exists a huge disparity in maternal mortality rates between developed and developing countries and that the majority of maternal deaths are avoidable, a retrospective study of maternal deaths occurring at the Rift Valley Provincial General Hospital (PGH) in Nakuru between 1994 and 1998 was carried out. The difficulties notwithstanding, Mahler and Rosenfield, in 1985 noted "although accurate data on mortality are difficult to compile, the magnitude of the problem is such that the absence of exact measures of incidences should not inhibit the design of health policies and programmes to combat this problem"(4).

MATERIALS AND METHODS

One hundred and nine maternal deaths occurring at the Provincial General Hospital Nakuru, between 1994 and 1998 whose records were available were included in this study.

Three main sources of data from PGH records department were used. The annual hospital statistical returns enabled us to know how many stillbirths, neonatal and maternal deaths occurred at the maternity ward. All deaths occurring in the gynaecology ward were lumped together and it was impossible to tell which of these were actual maternal deaths due to abortions, ectopic pregnancies and molar pregnancies. From the files of patients and the Hospital Matron's daily reports, we were able to get only 109 maternal deaths for evaluation including those due to abortions. Patients' records were irretrievable or non-existent as most files were simply never returned to the records department. Those remained were more often than not, poorly coded and therefore irretrievable. The Matron's daily reports though not as detailed as the patients' files gave useful information that was evaluable.

RESULTS

Figure 1 shows maternal and neonatal mortality rates and stillbirth rates for the years 1994-1998. The average maternal mortality rate was 3.16/1000 livebirths, these excluding deaths due to abortions, ectopic pregnancies and molar pregnancies.

A total of one hundred and nine maternal deaths were evaluated. The average age was 25 years with a range of 14-45 years. Those aged 14-19 years were 28(26%), 20-24 years 34(32%), 25-29 years 17(16%), 30-34 years 15(14%), >35 years 13(12%). Age was unknown for three women. Figure 2 shows age distribution of the deceased.

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Leprosy

Redda Tekle Haimanot and Zenebe Melaku

Leprosy is a unique infectious disease with a prolonged incubation period and a predilection for skin and nerves. The involvement of nerves by the primary infection as well as the immunologically mediated reversal reactions result in impairment of nerve function and severe disabilities. The introduction of the World Health Organization Multi Drug Therapy over the last two decades has produced dramatic changes in the management and control programmes of leprosy. A recent important contribution to the understanding of leprosy pathogenesis has been the elucidation of the molecular basis for the entry of *Mycobacterium leprae* into the Schwann cell and the peripheral nerve. Leprosy still remains the commonest cause of peripheral neuropathy in developing countries. Curr Opin Neurol 13:317–322 © 2000 Lippincott Williams & Wilkins

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Abbreviations

BCG	Bacillus Calmette-Guerin
BI	bacteriological index
ENL	erythema nodosum leprosum
MB	multibacillary
MDT	multiple drug therapy
NFI	nerve function impairment
PB	paucibacillary

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Introduction

Mycobacterium leprae affects the skin and nerves. However, a majority of the damage falls on nerves which results in serious disabilities. As the commonest cause of peripheral neuropathy, leprosy is still important in developing countries where the disease is endemic. A spectrum of clinical manifestations of *M. leprae* are encountered depending on the immune status of the host. Leprosy reactions that are associated with fluctuations in the immune status of the patient occur suddenly and without warning. Left untreated these reactions can exacerbate the neural damage and functional impairment. The introduction of the World Health Organisation Multiple Drug Therapy (WHO/MDT) using clofazamine, dapsone and rifampicin has shortened the effective treatment period for leprosy and has encouraged the discharge of old cases as well as increased the number of newly registered cases. It has therefore significantly contributed towards the goal of eliminating the disease by the year 2000. However, a great deal remains to be done because there are many patients in endemic countries who are not being reached. This review presents current concepts of immunopathogenesis, clinical manifestations, diagnosis and management of the disease with emphasis on the peripheral nerve involvement.

Epidemiology

The global prevalence of leprosy in 1999 was estimated to be around 1.4 per 10 000 population with 731 369 leprosy cases having been registered for treatment in 82 countries. Compared with 1998 figures the number of new cases has remained constant. The majority of the global leprosy burden is concentrated in the intertropical belt where leprosy is a public health problem in 24 countries out of which 11 endemic countries represent 90% of the prevalence and detection worldwide. The target prevalence for these endemic countries is 4.5 per 10 000 population which is 4.5 times the elimination level (< 1 case per 10 000 population) [1••]. According to two recent prospective studies the prevalence of nerve function impairment in leprosy ranges from 14 to 20% [2,3•]. In preliminary familial linkage analysis studies susceptibility to leprosy has been linked to the NRAMP1 gene which controls innate susceptibility to mycobacterial infections in mice [4].

Immunopathogenesis

Although it is well documented that the Schwann cells are the main target of *M. leprae* infection, the molecular basis of this tropism, and how the organism gains entry

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into the nerve and then in to the Schwann cells, is not known. Rambukkana *et al* [5,6] have identified a glycoprotein which binds to the surface of *M. leprae* which is in turn bound by a molecule on the Schwann cell surface, thereby providing a potential mechanism for internalization of the bacilli by Schwann cells. *M. leprae* binds specifically to the G-domain, which is located at the C-terminal end of the α-2 chain of laminin-2. The study revealed that *M. leprae* bound to the G domain of laminin-2 is in turn bound to α-dystroglycan on the Schwann cell surface. α-Dystroglycan bound to laminin-2/*M. leprae* is associated with the transmembrane molecule β-dystroglycan. At an intracellular binding site, α-dystroglycan binds to dystrophin, an intracellular protein which in turn binds to actin [7]. These four proteins (laminin-2, α-dystroglycan, β-dystroglycan, dystrophin) thus provide a bridge between *M. leprae* and the internal environment (the cytoskeleton) of the Schwann cell. Rambukkana *et al.* observed clustering of the α-dystroglycan/*M. leprae* complexes subsequent to binding on the cell surface of the Schwann cell, which presumably leads to the internalization of the bacilli into a cytoplasmic vacuole and establishment of Schwann cell infection.

Concerning the question of how *M. leprae* gains access to the peripheral nerve, it is likely that infected blood monocytes from broken skin and mucous membranes transport the bacilli in to the naive nerve during the normal trafficking of macrophages through the peripheral nervous system. This type of trafficking occurs in all individuals as a low, steady state monocyte exchange between nerves and blood [8]. However, in an *M. leprae* infected individual it is possible that an infected macrophage might enter a nerve and be trapped there. Experimental study suggested that this may involve adhesion events between *M. leprae* (*M. leprae*-parasitized macrophages) and the endothelial cells of the vasa nervorum [9]. When the load of replicating organisms ultimately leads to destruction of the cell, the bacilli would be released into the endoneural space. The mycobacteria would then be bound by laminin α-2, which would tether them to the α-dystroglycan on the surface of myelinating Schwann cells. Internalization would then occur. The infected Schwann cells would degenerate, increasing recruitment of macrophages into the nerve [10]. Because α-2 laminin and the α-dystroglycan are restricted to peripheral nerves, *M. leprae* infection would not occur in the central nervous system.

The axonal damage in leprosy occurs as a result of the release of pharmacological mediators produced by these infiltrating inflammatory cells and the resulting granuloma formation within the nerve [11]. There is also some damage caused by pressure on the nerves resulting from perineural oedema as nerve decompression has a beneficial effect [12].

The elucidation of the molecular mechanism of the infection of the peripheral nerve by *M. leprae* by Rambukanna *et al.* [5,6] will have a very useful implication on treatment of nerve damage in leprosy. It may also open the door for further research in leprosy neuropathology and other neuropathies. A recent review of the pathogenesis of leprosy with special emphasis on immunologic mechanisms is offered by Abulafia and Vignale [13••].

Clinical manifestations

The clinical manifestation of the disease is dependent on the immune status of the patient. The spectrum of the clinical presentation therefore ranges from the lepromatous leprosy end of low host resistance to that of the tuberculoid leprosy end of high host resistance and borderline types that fall in between the two poles. For chemotherapeutic purposes the disease is classified into two groups. The first is multibacillary (MB), consisting of lepromatous leprosy, borderline lepromatous, borderline borderline leprosy, and smear positive-borderline tuberculoid leprosy. The other is paucibacillary (PB) consisting of indeterminate, tuberculoid leprosy, and smear negative-borderline tuberculoid. The nerve damage in lepromatous leprosy although widespread tends to occur late in the course of the disease and is less severe. On the other hand, as a result of the intense host response nerves at the tuberculoid end are affected early and more severely. Nerve damage results in varying degrees of nerve function impairments that lead to deformities. A prospective cohort study of patients without nerve damage at registration showed that sensory and motor loss increased with age and both were higher among multibacillary patients [14].

Primary neuritic leprosy presents as a peripheral neuropathy with either pure sensory or combined sensory and motor nerve dysfunction. The characteristic clinical skin lesions of leprosy are absent [15]. Two good histological studies, however, demonstrate that there is a cutaneous component and nasal mucosal involvement in primary neuritic leprosy and the disease is not totally confined to nerves [16,17].

Reactions in leprosy are very serious complications that are a consequence of dynamic changes in the immune status of the patient. Type 1 upgrading reversal reaction which is associated with an increase in cell mediated immunity is most commonly seen in borderline cases. Type 2 reaction or erythema nodosum leprosum (ENL) is a systemic inflammatory response to the deposition of extravascular immune complexes and occurs in lepro-

matous and borderline lepromatous leprosy. These reactions are more common during treatment. The risk for ENL has a linear relationship with increasing bacteriological index and an inverse relationship to increasing age [18].

Multibacillary patients have been found to have a prevalence of reaction and nerve function impairments, needing treatment nearly seven times higher than paucibacillary cases and males nearly double that of females. The most commonly affected nerves are the posterior tibial, followed by ulnar [3]. According to a study from Nepal, risk factors for Type-1 reaction include facial patches, enlarged ulnar nerve, positive bacteriological index, lesions in more than two parts and seropositivity to antiphenolic glycolipid-1 (PGL-I) [19]. Lockwood and Sinha, in their comprehensive literature review concerning reactions in pregnancy, found that Type-1 reversal reactions were particularly likely to occur during postpartum. Type-2 (ENL) reaction occurs throughout pregnancy and during lactation and may be severe and recurrent [20].

Early detection of reversal reaction is extremely crucial if one is to avoid the catastrophic resultant nerve damages. Some metabolites have been studied as possible markers of reactions. Neopterin, a product of gamma-interferon-activated macrophages, was found to be significantly higher in reactional states [21]. Schon *et al.* found that the reversal reaction is associated with increased urinary levels of nitric oxide metabolites [22]. These findings require further investigations.

Diagnosis

Diagnosis of leprosy is based on clinical evaluation and bacteriological confirmation, mainly using skin smears. Histopathological examinations of lesions can often be very useful. A recent study showed that the midfinger smear did not contribute useful information compared with the classical compulsory (both ear lobes) and optional (four active lesion) sites [23]. On the other hand fine needle cytology from skin lesions achieved reliable diagnosis with excellent correlation with bacteriological index, clinical and histopathological findings [24]. A retrospective study from India by Nadkarni and Rege showed a very high type-wise correlation between histopathological and clinical diagnosis and classification pointing to the fact that experienced physicians can clinically diagnose and classify with a reasonable accuracy for both therapeutic and research purposes [25].

Among the serological diagnostic methods for leprosy, the ELISA test using trehalose 6,6'-dimycolate as an antigen was found to be simple, fast and reproducible and useful especially in smear negative cases [26]. Anti PGL-I assay using *M. leprae* particle agglutination was also a sensitive and a specific diagnostic tool for the diagnosis of leprosy especially in multibacillary patients. It also provided an alternative tool in monitoring patients under MDT [27]. Seroprevalence of antibodies to PGL-I among school children could potentially be used as an indicator of the magnitude of the leprosy problem in a selected area [28]. Tomimori-Yamashita *et al.* have shown that there is a straight correlation between anti PGL-I determination using blood collection on a filter paper and the traditional sera collection method [29]. This blood collection method could be useful for investigation of new leprosy cases in the field especially in contacts [29]. A dipstick assay to detect antibodies to PGL-I of *M. leprae* using stable reagent was found to be suitable for use in tropical countries [30]. This test is applicable for use in close contacts but is not yet ready for mass screening [31]. On the other hand, antibodies to sulfatide were not of a diagnostic value because levels were elevated in both leprosy patients as well as healthy controls in the endemic areas [32].

The evaluation of nerve function is extremely important in the detection of silent neuritis and the nerve damage in reversal reactions. Although nerve conduction measurements proved to be more effective in detecting nerve function impairment comparable results were obtained with a combination of physical palpation for nerve thickening and the graded nylon test [33••]. Similar conclusions have been reached by other studies. A field trial in China recorded that early detection and treatment of nerve function impairments could be performed practically and effectively by well trained field staff using voluntary muscle test and touch sensitivity test [2]. The Semmes Weinstein monofilament and ballpoint sensory testing and voluntary muscle testing in trained and experienced hands are reliable and repeatable to a level allowing confident use of results in clinical monitoring and research [34].

Treatment

MDT remains the best chance of controlling leprosy and the disability associated with the disease [35]. However, since disability depends on early diagnosis MDT alone cannot solve the problem of disability. Incidences of disabilities in leprosy are affected by many factors. Delay in diagnosis and treatment and increasing age are the main risk factors for presentation with impairment [36].

Yearly skin slit follow-up examination in patients on the WHO/MDT programme did not show the expected 1 log unit per year decrement of bacteriological index despite adequate compliance. Viable bacilli could be isolated from some of these patients [37]. Gupte *et al.*

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found viable organisms in 10–13% of patients on MDT during a follow up period of six months to three years [38]. Similarly, when *M. leprae* were looked for in skin and nerve biopsies and lymph node aspirates after the completion of MDT in smear negative multibacillary patients, a pretreatment bacteriological index of 4+ or more was significantly associated with the presence of *M. leprae* at the end of treatment. This was particularly found in lymph node aspirates [39]. These studies demonstrate that close follow up of such patients should be mandatory after stoppage of MDT.

The WHO Expert Committee on Leprosy on the basis of field trials and clinical studies has concluded that a single dose of a combination of rifampicin, ofloxacin and minocycline is an acceptable and cost effective alternative for the treatment of single-lesion paucibacillary leprosy. They also suggest that the duration of MDT regimen for multibacillary leprosy could possibly be shortened to 12 months [40]. These recommendations have generated a great deal of controversy. In a commentary, Waters proposes a longer follow up before recommending the shortening of MDT in multibacillary cases, particularly those with a bacteriological index of more than 4.0 [41]. He also states his reservations on the hasty introduction by the WHO Expert Committee of the third one-day MDT regimen before the results of the WHO ofloxacin trial [41]. A similar concern has also been expressed by Lynch who disagrees with the shortened MDT regimen because of the increased rate of relapse among patients that had a bacteriological index of 4.0 before the start of MDT. He suggests that such a shortened course of MDT therapy should be considered only for those with low bacteriological index confirmed at referral centres, while the usual 24 months of MDT should be given for patients in the field where the bacteriological index cannot be measured [42]. Ramu suggests that the single rifampicin, ofloxacin and minocycline regimen should be instituted with care because all single lesions should not be considered paucibacillary. Relapse lesions of lepromatous and borderline lepromatous leprosy (following dapsone monotherapy) and with a high bacteriological index may present as single lesion [43]. Katoch has been critical of the use of the rifampicin, ofloxacin and minocycline regimen in single lesion leprosy. The spectrum of established leprosy cases treated with MDT can shift to fresh evolving and single lesion cases which may progress into lepromatous and tuberculoid cases. The one-day dose may not be able to eliminate all bacilli [44].

The shortened MDT and single dose therapy for single lesions require careful rethinking and further evaluation. The problem of relapse could pose serious obstacles to the efforts of eliminating leprosy.

The principles of management of reversal reactions are maintenance of antimycobacterial drugs, effective and prolonged anti-inflammatory therapy, and adequate analgesia and physical support during the phase of active neuritis [45]. Corticosteroids are still the cornerstone of drug therapy. It has been shown that treatment of acute nerve function impairment at field level by paramedical workers using a standardized regimen of prednisolone is feasible, practical and effective [46]. Bernink *et al.* in their study in Indonesia found that well-instructed field workers proved to be competent in detecting and treating leprosy reactions with prednisolone, which preserved or considerably improved nerve function [47]. Early diagnosis of reversal reactions will have to be encouraged through education of patients to self-report for treatment when they recognize the symptoms of reaction and neuritis. Health workers at peripheral levels should also be well trained to treat the condition promptly [48].

Thalidomide has been used successfully in the treatment of ENL. However, the dangers associated with the teratogenic properties of this drug has been emphasized [49,50]. This has prompted the search for other less toxic anti-inflammatories. There are data to suggest that pentoxifylline inhibits tumour necrosis factor-alpha production in ENL [51]. In one preliminary study involving four cases, severe Type 2 reactions showed a fast response to clofazimine and pentoxifylline [52] but this finding needs to be evaluated in large studies.

In the area of immunotherapy several vaccine trials have been undertaken in India. Gupte concluded that ICRC vaccine (vaccine containing ICRC bacilli, which are leprosy-derived cultivable mycobacteria) and the combination vaccine (BCG+ killed *M. leprae*) met the requirements of public health utility [53]. Using secondary data analysis of previous case-controlled studies Kulkarni *et al.* concluded that the protective effect of the BCG vaccination is differential across severity of leprosy as it brings about a shift in the immune response to a higher level of cell mediated immunity [54]. A population-based case controlled study performed in Central India revealed that BCG vaccination has a beneficial role in prevention of leprosy. It was more effective during the first decade of life, among females and in lower socioeconomic strata [55]. An immunotherapeutic vaccine which used *Mycobacterium w.* and had completed phase III therapeutic trial was found to be effective in inducing a fall of bacteriological index, histological upgrading and a reduction in the incidence and severity of Type 2 reaction in multibacillary patients who are either slow or nonresponders to standard MDT [56•]. A hospital based trial showed that the administration of *Mycobacterium w.* vaccine to standard MDT also

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resulted in histological upgrading, conversion to lepromin positivity and did not precipitate neuritis or deformity [57]. Inspite of these encouraging reports, in an editorial review Whitty convincingly argues that immunotherapy can not presently be recommended in routine clinical practice because of the apparent increase in Type 1 reactions and the absence of long-term follow up data on relapses and disability [58].

Rehabilitation

Rehabilitation is an essential component of the management of disabilities [illegible] multidrug therapy and integration of leprosy into primary health care Kazen warns of the possible neglect of care after cure. He points at the increasing need for empowerment of patients with the responsibility for their own ulcer prevention and also of health units for basic surgical treatment of ulcers [59]. Similar advocacy of self care is reflected in the Chinese literature [60,61]. Duerkson gives a very elaborate, systematic and easy to grasp review on the surgery of common paralytic conditions and post surgical rehabilitation in leprosy [62].

Conclusion

WHO/Multiple Drug Therapy has revolutionized the management of leprosy, and its success and impact on leprosy control has been tremendous. However, MDT alone can not solve the problem. The future of leprosy control programmes therefore depends on health education and the involvement of communities. The integration of leprosy into the primary health care as recommended by the WHO Expert Committee will ensure early detection and treatment of leprosy [40]. However, to get health professionals trained and motivated to carry on the leprosy programmes will be a great challenge.

The future challenges of leprosy control and research programmes in the 21st Century will require the concerted efforts of governments and nongovernmental institutions, communities and health professionals in ensuring early detection and treatment as well as follow up and rehabilitation of the already identified cases. The success of control programmes needs to be monitored through incidence data particularly in endemic areas.

As identified by Jacobson and Krahenbuhl future areas of research should include molecular biology of *M. leprae* and the host response, simple and specific methods of identifying *M. leprae* in biopsy specimens, and use of environmental samples and skin slits to find an individual's exposure to the bacillus. In addition the classification of Type 1 and Type 2 reactions, their differentiation from relapses, and the identification of immunoregulatory cytokines to predict reactions need to be studied further [63].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- * of special interest
- ** of outstanding interest

1 Weekly Epidemiological Record. Global leprosy situation. 1999; 38:313–320.
**
This record has the latest statistics on the global leprosy situation. It contains prevalence and detection of leprosy by regions and countries as well as numbers of patients cured with MDT.

2 Jiang J, Watson JM, Zhang GC, Wei XY. A field trial of detection and treatment of nerve function impairment in leprosy—report from National POD Pilot Project. Lepr Rev 1998; 69:367–375.

[illegible] 2004 new leprosy cases in Bangladesh (The Bangladesh Acute Nerve Damage Study). Lepr Rev 1999; 70:140–159.
A carefully monitored study assessing the frequency of nerve function impairments in relation to the leprosy classification of the patients and the peripheral nerves affected.

4 Abel L, Sanchez FO, Oberti J, Thuc NV, Hoa LV, Lap VD, et al. Susceptibility to leprosy is linked to the human NRAMP1 Gene. J Infect Dis 1998; 177:133–145.

5 Rambukkana A, Salzer JL, Yurchenco PD, Tuomanen EL. Neural targeting of Mycobacterium leprae mediated by the G domain of the laminin-alpha 2 chain. Cell 1997; 88:811–821.

6 Rambukkana A, Yamada H, Zanazzi G, Mathus T, Salzer JL, Yurchenco PD, et al. Role of alpha-dystroglycan as a schwann cell receptor for Mycobacterium leprae. Science 1998; 282:2076–2079.

7 Yamada H, Chiba A, Endo T, Kobata A, Anderson LV, Hori H, et al. Characterization of Dystroglycan-Laminin in Peripheral Nerve. J Neurochem 1996; 66:1518–1524.

8 Oldfors A. Macrophages in peripheral nerves: an ultrastructural and enzyme histochemical study on rats. Acta Neuropathol (Berl) 1980; 49:43–49.

9 Scollard DM. M. leprae: infection of vascular and lymphatic endothelial cells of the epineurium and perineurium in experimental lepromatous neuritis. Nihon Hansenbyo Gakkai Zasshi 1999; 68:147–155.

10 Griffin JW, George ER, Chaudhry V. Wallerian degeneration in peripheral nerve disease. Bailliere's Clin Neurol 1996; 5:65–75.

11 Turk JL, Curtis J, De Blaquiere G. Immunopathology of nerve involvement in leprosy. Indian J Lepr 1991; 63:483–491.

12 Bourrel P. Surgical rehabilitation. Lepr Rev 1991; 62:241–254.

13 Abulafia J, Vignale RA. Leprosy: pathogenesis updated. Int J Dermatol 1999; ** 38:221–224.
An extensive updated review of the pathogenesis of leprosy with emphasis on immunologic mechanisms.

14 Solomon S, Kurian N, Ramadas P, Rao PS. Incidence of nerve damage in leprosy patients treated with MDT. Int J Lepr Other Mycobact Dis 1998; 66:451–456.

15 Jacob M, Mathai R. Diagnostic efficacy of cutaneous nerve biopsy in primary leprosy. Int J Lepr 1998; 56:56–60.

16 Suneetha S, Arunthathi S, Job A, Date A, Kurian N, Chacko CJ. Histological studies in primary neuritic leprosy: changes in the nasal mucosa. Lepr Rev 1998; 69:358–366.

17 Suneetha S, Arunthathi S, Chandi S, Kurian N, Chacko CJ. Histological studies in primary neuritic leprosy: changes in the apparently normal skin. Lepr Rev 1998; 69:351–357.

18 Manandhar R, LeMaster JW, Roche PW. Risk factors for erythema nodosum leprosum. Int J Lepr Other Mycobact Dis 1999; 67:270–278.

19 Roche PW, Le Master J, Butlin CR. Risk factors for type 1 reactions in leprosy. Int J Lepr Other Mycobact Dis 1997; 65:450–455.

20 Lockwood DN, Sinha HH. Pregnancy and leprosy: a comprehensive literature review. Int J Lepr Other Mycobact Dis 1999; 67:6–12.

21 Hamerlinck FF, Klatser PR, Walsh DS, Bos JD, Walsh GP, Faber WR. Serum neopterin as a marker for reactional states in leprosy. FEMS Immunol Med Microbiol 1999; 24:405–409.

22 Schon T, Gebre N, Sundqvist T, Habtemariam HS, Engeda T, Britton S. Increased levels of nitric oxide metabolites in urine from leprosy patients in reversal reaction. Lepr Rev 1999; 70:53–55.

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23 Hari L, Suryanarayanan D, Thomas A, Joseph P. An assessment of the value of midfinger smears in multibacillary leprosy patients. Lepr Rev 1999; 70:47–51.

24 Singh N, Bhatia A, Arora VK, Bhattacharya SN. Fine-needle aspiration cytology of lepromatous leprosy. Lepr Rev 1998; 69:145–150.

25 Nadkarni NS, Rege VL. Significance of histopathological classification in leprosy. Indian J Lepr 1999; 71:325–332.

26 Wang L, Izumi S, He H, Fujiwara N, Saita N, Yano I, *et al.* Serodiagnosis of Hansen's disease/leprosy by enzyme-linked immunosorbent assay using cord factor (trehalose 6,6'-dimycolate) as an antigen. Nohon Hansenbyo Gakkai Zasshi 1999; 68:165–174.

27 Kampirapap K. Mycobacterium leprae particle agglutination in diagnosis and monitoring treatment of leprosy. J Med Assoc Thai 1999; 82:1020–1024.

28 van Beers S, Hatta M, Klatser PR. Seroprevalence rate of antibodies to phenolic glycolipid-I among school children as an indicator of leprosy endemicity. Int J Lepr Other Mcyobact Dis 1999; 67:243–249.

29 Tomimori-Yamashita J, Nguyen TH, Maeda SM, Flageul B, Rotta O, Cruaud P. Anti-phenolic glycolipid-I (PGL-I) determination using blood collection on filter paper in leprosy patients. Rev Inst Med Trop São Paulo 1999; 41:239–242.

30 Buhrer SS, Smits HL, Gussenhoven GC, Ingen CW Van, Klatser PR. A simple dipstick assay for the detection of antibodies to phenolic glycolipid-I of *Mycobacterium leprae*. Am J Trop Med Hyg 1998; 58:133–136.

31 Causative organism and host response [Workshop Report]. International Leprosy Congress, Beijing, 7–12 September 1998. Lepr Rev 1999; 70:95–102.

32 Spierings E, de Vlieger M, Brand A, Klatser PR, Ottenhoff TH. Antibodies to sulfatide in leprosy and leprosy reactions. Am J Trop Med Hyg 1999; 61:495–499.

33 ** Samant G, Shetty VP, Uplekar MW, Antia NH. Clinical and electrophysiological evaluation of nerve function impairment following cessation of multidrug therapy in leprosy. Lepr Rev 1999; 70:10–20.
This is a useful study that compared the results of electrophysiological studies with those of physical examinations in the evaluation of nerve function impairment.

34 Anderson AM, Croft RP. Reliability of Semmes Weinstein monofilament and ballpoint sensory testing, and voluntary muscle testing in Bangladesh. Lepr Rev 1999; 70:305–313.

35 Ladhani S. Leprosy disabilities: the impact of multidrug therapy (MDT). Int J Dermatol 1997; 36:561–572.

36 Meima A, Saunderson PR, Gebre S, Desta K, van Oortmarssen GJ, Habbema JD. Factors associated with impairments in new leprosy patients: The AMFES Cohort. Lepr Rev 1999; 70:189–203.

37 Habtemariam H. Viability and drug sensitivity of *M. leprae* isolated from long-term WHO/MDT treated multibacillary leprosy patients. Lepr Rev 1999; 70:43–46.

38 Gupta UD, Katoch K, Singh HB, Natrajan M, Sharma V, Katoch VM. Detection of viable organisms in leprosy patients treated with multidrug therapy. Acta Leprol 1999; 11:89–92.

39 Sharma A, Sharma VK, Rajwanshi A, Das A, Kaur I, Kumar B. Presence of *M. leprae* in tissues in slit skin smear negative multibacillary (MB) patients after WHO-MBR. Lepr Rev 1999; 70:281–286.

40 WHO Expert Committee on Leprosy. World Health Organ Tech Rep Ser 1998; 874:1–43.

41 Waters MFR. Is it Safe to Shorten Multidrug Therapy for Lepromatous (LL and BL) Leprosy to 12 months? Lepr Rev 1998; 69:110–111.

42 Lynch P. Multidrug therapy [letter]. Lepr Rev 1999; 70:70–71.

43 Ramu G. Single-dose rifampicin, ofloxicin and minocycline (ROM) therapy for single leprosyl [letter]. Lepr Rev 1998; 69:78–82.

44 Katoch VM. Is there a microbiological rationale for single-dose treatment of leprosy? [editorial]. Lepr Rev 1998; 69:2–5.

45 Britton WJ. The management of leprosy reversal reactions. Lepr Rev 1998; 69:225–234.

46 Croft RP, Richardus JH, Smith WC. Field treatment of acute nerve function impairment in leprosy using standardized corticosteroids regimen – first year's experience with 100 patients. Lepr Rev 1997; 68:316–325.

47 Bernink EH, Voskens JE. Study on the detection of leprosy reactions and the effect of prednisone on various nerves, Indonesia. Lepr Rev 1997; 68:225–232.

48 Disease and Disease Control. [Workshop Report] International Leprosy Congress, Beijing, 7–12 September 1998. Lepr Rev 1999; 70:78–84.

49 Annas GJ, Elias S. Thalidomide and the Titanic. Reconstructing the technology tragedies of the twentieth century. Am J Public Health 1999; 89:98–101.

50 Lary JM, Daniel KL, Erickson JD, Roberts HE, Moore CA. The return of thalidomide: can birth defects be prevented? Drug Saf 1999; 21:161–169.

51 Sampaio EP, Moraes MO, Nery JA, Santos AR, Matos HC, Sarno EN. Pentoxifylline decreases *in vivo* and *in vitro* tumour necrosis factor-alpha (TNF-alpha) production in lepromatous leprosy patients with erythema nodosum leprosum. Clin Exp Immunol 1998; 111:300–306.

52 Welsh O, Gomez M, Mancias C, Ibarra-Leal S, Millikan LE. Pharmacology and therapeutics: a new approach to type 2 leprosy reaction. Int J Dermatol 1999; 38:931–933.

53 Gupte MD, Vallishayee RS, Arantharaman DS, Nagaraju B, Sreevatsa , Balasubramanyam S, *et al.* Comparative leprosy vaccine trial in South India. Indian J Lepr 1998; 70:369–388.

54 Kukarni HR, Zodpey SP. Differential protective effect of Bacillus Calmette-Guerin vaccine against multibacillary leprosy in Nagpur, India [in process citation]. Public Health 1999; 113:311–313.

55 Zodpey SP, Bansod BS, Shrikhande SN, Maldhure BR, Kulkarni SW. Protective effect of Bacillus Calmette-Guerin (BCG) against leprosy: a population-based case-control study in Nagpur, India. Lepr Rev 1999; 70:287–294.

56 * Talwar GP. An immunotherapeutic vaccine for multibacillary leprosy. Int Rev Immunol 1999; 18:229–249.
The paper reports on the results of Mycobacterium w. vaccine used as an adjunct to chemotherapy in urban and rural leprosy control centres in India.

57 Sharma P, Kar HK, Misra RS, Mukherjee A, Kaur H, Mukherjee R, Rani R. Induction of lepromin positivity following imuno-chemotherapy with *Mycobacterium* w. vaccine and multidrug therapy and its impact on bacteriological clearance in multibacillary leprosy: report on a hospital-based clinical trial with candidate antileprosy vaccine. Int J Lepr Other Mycobact Dis 1999; 67:259–269.

58 Whitty C. *Mycobacterium* w. immunotherapy in leprosy [editorial]. Lepr Rev 1998; 69:222–224.

59 Kazen RO. Management of plantar ulcers in leprosy. Lepr Rev 1999; 70:63–69.

60 Zhen B. Effect of self-care on 56 cases of leprosy [in Chinese]. China Lepr J 1998; 14:159–160.

61 Zheng Z, *et al.* Effect of self-care in 856 persons affected with leprosy [in Chinese]. China Lepr J 1998; 14:154–156.

62 Duerkson F. Surgery of common paralytic conditions. Lepr Rev 1998; 69:390–396.

63 Jacobson RR, Krahenbuhl JL. Leprosy [seminar]. Lancet 1999; 353:655–660.

Revmatiske manifestasjoner ved HIV-infeksjon

Pasienter med HIV-infeksjon kan ha symptomer, kliniske funn og serologiske abnormaliteter som likner på det man finner ved revmatiske sykdommer.

Vi vil i denne artikkelen gi en oversikt over revmatiske symptomer og sykdommer som kan være manifestasjoner av HIV-infeksjon. Artikkelen bygger på nyere litteratur identifisert ved søk i Medline. Noen av assosiasjonene mellom HIV-infeksjon og revmatiske sykdommer kan være tilfeldige, mens andre synes å være direkte relatert til infeksjonen. De revmatiske manifestasjonene er en rekke leddsyndromer og autoimmune sykdommer, som Reiters syndrom, psoriasisartritt, HIV-assosiert artritt, septisk artritt, et Sjögren-liknende syndrom, myopatier og systemisk vaskulitt.

For klinikeren kan dette by på store diagnostiske og terapeutiske utfordringer, spesielt fordi de revmatiske manifestasjonene kan opptre før kliniske tegn på HIV-infeksjon, og fordi tradisjonell behandling av revmatisk sykdom med steroider og cytostatika kan forverre HIV-infeksjonen.

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Rheumatological manifestations of human immunodeficiency virus.
Tidsskr Nor Lægeforen 2000; 120: 1326–8.

Background. The recognised clinical spectrum of disease associated with HIV infection is rapidly expanding and now includes a variety of rheumatological manifestations.

Material and methods. In this review of the literature of the last 15 years, we present the most common rheumatic manifestations described in association with HIV infection.

Results. Manifestations include a wide array of articular syndromes and autoimmune manifestations such as Reiter's syndrome, psoriatric arthritis, HIV associated arthritis and septic arthritis. Autoimmune diseases associated with HIV infection include a Sjögren-like syndrome, myopathies and systemic vasculitis.

Interpretation. Rheumatological manifestations of HIV infection may present earlier than clinical signs of the infection itself. Steroid and cytostatic treatment of rheumatic diseases may worsen the HIV disease.

Siden det første tilfellet av immunsviktsyndromet AIDS ble beskrevet i 1981, er mange kliniske symptomer og funn blitt assosiert med HIV-infeksjon. Blant de kliniske funnene finnes revmatologiske manifestasjoner og autoimmune sykdommer. I 1987 ble det rapportert om 13 pasienter med HIV-infeksjon og samtidig Reiters syndrom (1), og dette markerte starten på en rekke studier hvor man undersøkte om det kunne være en sammenheng mellom HIV-infeksjon og revmatiske autoimmune sykdommer. Disse studiene har imidlertid vært vanskeliggjort ved at man har hatt problemer med å finne egnede kontrollpersoner til prospektive, kontrollerte studier. En del av rapportene har også vært anekdotiske og lite systematiske.

Epidemiologiske studier har vist høyst varierende resultater med tanke på prevalens av revmatiske manifestasjoner hos HIV-infiserte. Dette illustreres ved at det på den ene side er vist at risikoen for revmatiske manifestasjoner er betydelig økt ved HIV-infeksjon, mens det på den annen side kan være holdepunkter for at HIV-infeksjon kanskje beskyttet mot revmatologisk sykdom. I en av de få prospektive studiene som er utført, fant man at hos 300 HIV-infiserte som ble fulgt i tre år, utviklet 8 % forskjellige former for artritt og 5 % myositt, og autoimmun bindevevssykdom ble funnet hos 2 %. Dette er langt høyere enn forventet (2).

Man har gradvis dannet seg et bilde av hvilke revmatiske sykdommer som kan være assosiert med HIV-infeksjon, spesielt på bakgrunn av tidlige studier hvor man foreslo en sammenheng med psoriasisartritt (3) og Reiters syndrom (1). Det er fortsatt uklart om en del revmatiske manifestasjoner hos HIV-infiserte representerer en tilfeldig assosiasjon, men det synes klart at enkelte revmatiske manifestasjoner er knyttet til infeksjonen. Hvilke mekanismer som kan gi revmatiske manifestasjoner ved HIV-infeksjon, er fortsatt lite kjent. En mulig mekanisme er endrede immunreaksjoner som fører til økt HIV-ekspresjon, samtidig som vertens forsvarsmekanismer mot fremmede antigener endres. Denne spesielle formen for aktivering av immunapparatet kan også bidra til den kroniske inflammasjonen og tendensen til opptreden av autoimmunitet som ofte sees ved HIV-infeksjoner. I tillegg vil en sentral dysregulering i immunapparatet være assosiert med infiltrasjon av CD8-positive T-lymfocytter, som er typisk for flere av [illegible] revmatiske [illegible]sykdommene – slik som [illegible] infiltrerende lymfocytose-syndrom og visse former for myositt.

De revmatologiske manifestasjonene som er beskrevet ved HIV-infeksjoner kan inndeles i to hovedgrupper: leddsykdommene og de autoimmune bindevevssykdommene (tab 1).

Leddsykdommene

Leddsykdommene som er beskrevet ved HIV-infeksjon er artralgier, Reiters syndrom, psoriasisartritt, HIV-assosiert artritt og septisk artritt.

Artralgier

Artralgier og myalgier er de vanligste revmatologiske symptomene ved HIV-infeksjon (4). Artralgiene er ofte lette, fluktuerende og polyartikulære, og representerer et ukarakteristisk symptom som man ofte ser ved virusinfeksjoner i sin alminnelighet.

Reiters syndrom

Reaktiv artritt og Reiters syndrom som en følge av infeksiøse tarmsykdommer og veneriske sykdommer er blitt beskrevet hos HIV-infiserte både i tidlige og sene stadier av sykdommen (5), men det er fortsatt usikkert om patogenesen er knyttet til HIV-infeksjonen, eller om det er relatert til økt insidens av infeksjoner. Det sees spesielt affeksjon av knær, ankler og forføtter, affeksjon av ledd i øvre ekstremiteter er mer sjeldent. Entesopatier, tendinitter og andre ekstraartikulære manifestasjoner er blitt rapportert å være like alminnelig som hos personer uten HIV-infeksjon. Revmatisk sykdom i columna er ikke vanlig, og røntgenologisk verifisert sakroileitt er svært sjeldent. Den kjente assosiasjonen mellom reaktiv artritt og vevstypen HLA-B27 er blitt bekreftet hos HIV-infiserte individer av kaukasisk opprinnelse, mens en slik assosiasjon ikke er påvist hos svarte i Zimbabwe (6).

Det kliniske bildet ved Reiters syndrom er svært varierende, og i de fleste tilfeller er tilstanden mild og lar seg behandle med antiflogistika alene. Hos noen individer kan imidlertid tilstanden være alvorlig, med røntgenologisk påvisbar periostitt med erosjoner, og konvensjonell behandling med

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antiflogistika og spesifikke antireumatika kan ha liten effekt.

Det er beskrevet at Reiters syndrom kan opptre flere år før klinisk påvisbar HIV-infeksjon (7).

Psoriasisartritt

Et spektrum av psoriatriske hudmanifestasjoner kan finnes hos HIV-infiserte, og både hud- og leddmanifestasjoner kan være utbredt og til dels alvorlige (3, 8–11). Artritt og enthesopatier, slik som beskrevet for Reiters syndrom, kan også sees sammen med psoriatriske hudmanifestasjoner. Artritt assosiert med psoriasis er ofte vanligere hos HIV-infiserte enn det man ser på psoriasis hos ikke-HIV-infiserte. I en studie av 18 menn med HIV-infeksjon og psoriasis ble assymetrisk polyartritt påvist hos 32% (10), mens en prevalens mindre enn 10% er det som vanligvis observeres hos ikke-HIV-infiserte psoriasispasienter.

Siden keratoderma blenorrhagicum og pustuløs psoriasis ofte er vanskelig å skille fra hverandre, kan det være problematisk å avgjøre om det foreligger inkomplett Reiters syndrom eller psoriasisartritt. Dette har ført til at noen mener at Reiters syndrom og psoriasisartritt er to sider av samme tilstand hos HIV-infiserte individer (10). Det kliniske bildet varierer mye, og både hud- og leddmanifestasjonene varierer fra lette til alvorlige.

De fleste pasientene responderer godt på antiflogistikabehandling, mens de med artritt kan ha effekt av behandling med sulfasalazin. Den antivirale medisinen zidovudin er ofte effektiv til å kontrollere hudmanifestasjonene, mens den er mindre egnet til å behandle leddmanifestasjoner (12). Liknende studier med nyere antivirale midler er ikke publisert.

HIV-assosiert artritt

Det er seronegativ artritt som dominerer hos HIV-infiserte (2), og det er beskrevet to former. Den ene er karakterisert ved subakutt oligoartikulær artritt, som utvikler seg over 1–6 uker og har en varighet på opptil seks måneder. Denne formen involverer vanligvis store ledd, hovedsakelig knær og ankler, og den er ikke-erosiv, med lette inflammatoriske kjennetegn. Den andre formen for artritt kalles gjerne «the painful articular syndrome», og er ikke beskrevet hos andre enn HIV-infiserte (13). Denne formen kan finnes hos opptil 10% av AIDS-pasienter, og presenterer seg som akutt, svært smertefull og skarp smerte i ledd, hovedsakelig knær og albuer. Denne tilstanden kan være fra timer til flere dager og krever ofte behandling med sterke gruppe A-analgetika. Undersøkelse av leddvæske ved denne formen viser bare lett inflammatorisk tilstand, i form av få betennelsesceller.

I behandlingen av artritt er ofte antiflogistika godt nok, siden inflammasjonen ofte er beskjeden (14), gjerne kombinert med intraartikulære steroider. Av spesifikke medikamenter er i kasuistiske rapporter sulfasalazin og antimalariamidler også blitt rapportert å ha effekt og være sikre i bruk. Bruk av metotreksat og andre immunsuppressiver er imidlertid forbundet med risiko for opportunistiske infeksjoner og utvikling av Kaposis sarkom, og bør derfor unngås. Det er ikke dokumentert at antivirale medisiner har effekt på leddmanifestasjoner hos HIV-infiserte.

Tabell 1 Revmatologiske manifestasjoner rapportert hos HIV-infiserte

Leddmanifestasjoner	Autoimmune sykdommer
Artralgi	Diffus infiltrerende lymfocytose-syndrom
Septisk artritt	
Reiters syndrom	Myositt
Psoriasisartritt	Vaskulitter
HIV-assosiert artritt	[illegible] erythema-[illegible]sk tilstand
	Positive serologiske prøver

Sammenhengen mellom HIV-infeksjon og revmatoid artritt er omstridt. Tidligere observasjoner om at HIV-infeksjon hos pasienter med revmatoid artritt kunne gi remisjon av leddgiktssykdommen, førte til at man konkluderte med at HIV-infeksjon og revmatoid artritt ikke kunne være til stede samtidig hos samme person (15, 16). Imidlertid har senere studier vist at progredierende revmatoid artritt med ledderosjoner kan finnes hos HIV-infiserte, på tross av kraftig reduksjon av antallet CD4-positive lymfocytter (17).

Septisk artritt

Til tross for at HIV-infiserte har økt risiko for å pådra seg infeksjoner, er septisk artritt kun rapportert få ganger. Septisk artritt sees vanligvis ved avansert sykdom, og de vanligste patogene mikrobene er Staphylococcus aureus, Streptococcus pneumoniae, Sporotrix schenckii, Cryptococcus neoformans, Candida albicans, Histoplasma capsulatum, Mycobacterium avium-intracellulare, i tillegg til atypiske mykobakterier.

Klinisk atskiller septisk artritt seg ikke fra det som observeres hos ikke-HIV-infiserte. I tillegg til å undersøke leddvæsken på vanlige mikrober, som angitt, må det også undersøkes på sopp og mykobakterier. Behandlingen av septisk artritt må være bredspektret inntil svar på spesifikt bakterielt agens foreligger. Det må tas hensyn til pasientens dårlig fungerende immunapparat når behandlingsvarigheten skal bestemmes (18).

Autoimmune bindevevssykdommer

Et bredt spektrum av bindevevssykdommer er beskrevet hos HIV-infiserte.

Diffus infiltrativ lymfocytose-syndrom

En av de bindevevssykdommene som beskrives spesifikt hos HIV-infiserte, er en variant av primært Sjögrens syndrom. De kan utvikle et syndrom med bilateral parotishevelse, tørre øyne og tørr munn samt infiltrasjon av lymfocytter i spyttkjertler og viscerale organer. Selv om likheten med Sjögrens syndrom er slående, er dette en distinkt sykdomsenhet (19, 20). Benevnelsen diffus infiltrativ lymfocytose-syndrom brukes for å skille denne tilstanden fra primært Sjögrens syndrom. Ekstraglandulære komplikasjoner er svært vanlig ved denne tilstanden, og består av lymfocyttinterstitiell pneumonitt, lymfocyttmeningitt, nevropatier i kranialnervene, i tillegg til interstitiell nefritt og lymfocyttinfiltrasjon i tarmveggen. Dessuten har disse pasientene en klart økt risiko for å utvikle høygradig B-cellelymfom i spyttkjertlene. Autoantistoffer som man vanligvis assosierer med primært Sjögrens syndrom, slik som anti-SSA og anti-SSB, ser man sjelden ved denne tilstanden. I kontrast til primært Sjögrens syndrom er de infiltrerende cellene CD8-positive T-lymfocytter, mens det ved Sjögrens syndrom hovedsakelig er CD4-positive T-lymfocytter. I tillegg har pasienter med diffus infiltrativ lymfocytose-syndrom økt frekvens av vevstypen HLA-DR5 (svarte) og HLA-DR6 og -DR7 (kaukasere). Ved primært Sjögrens syndrom er det en overvekt av vevstypene HLA-DR2 og HLA-DR3.

Tørrhetsplagene og parotishevelsen hos pasienter med diffus infiltrativ lymfocytose-syndrom kan behandles effektivt med zidovudin. Hos pasienter med ekstraglandulære manifestasjoner og progredierende sykdom har høydose steroider vist seg å være egnet til å kontrollere tilstanden (20).

Myopati

Flere typer myopati er beskrevet hos HIV-infiserte. Muskelplager er vanligvis et av de første revmatologiske symptomene som opptrer, og de hyppigst registrerte muskelsykdommene er polymyositt/dermatomyositt, pyomyositt og myopatier som et resultat av opportunistiske infeksjoner som toksoplasmose eller ved antiviral zidovudinbehandling (21, 22).

Det anbefales å seponere den antivirale behandlingen med zidovudin dersom man mistenker HIV-assosiert myopati. Hvis dette ikke fører til bedring i løpet av to uker, anbefales muskelbiopsi. Dersom biopsien viser polymyositt, kan man forsiktig prøve behandling med steroider. Pasienter med HIV-infeksjon og myopati synes å respondere på lavere doser steroider enn andre med idiopatisk polymyositt. Så langt som mulig bør man unngå bruk av immunsuppressive medikamenter.

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Vaskulitt

Vaskulittiske syndromer rapportert i forbindelse med HIV-infeksjon spenner fra affeksjon av små kar, som man ser ved hypersensitivitetsvaskulitt, til affeksjon av middelstore arterier, slik som ved periarteritis nodosa, granulomatøse vaskulitter og primær angiitt i sentralnervesystemet (23, 24). Disse rapportene er hovedsakelig kasuistikker, noe som gjør det vanskelig å avgjøre om det er et tilfeldig sammentreff når vaskulittsykdommer og HIV-infeksjon opptrer samtidig. Sjeldne immunproliferative vaskulittiske tilstander, slik som angiosentrisk lymfom og lymfomatoid granulomatose, er dermot spesielle ved at [illegible] å opptre relativt hyppig ved HIV-infeksjoner, og manifesterer seg da oftest i lungene og i sentralnervesystemet. Det har ført til spekulasjon om HIV-indusert dysregulering av immunapparatet kan føre til proliferasjon av T-lymfocytter konsentrisk rundt kar, slik man ser ved disse sjeldne tilstandene.

Primær angiitt i sentralnervesystemet presenterer seg oftest som et progredierende tap av sentralnervøse funksjoner eller som fulminant hurtigutviklende encefalitt. Der finnes ikke prospektive studier vedrørende behandlingen av HIV-assosiert vaskulitt, men rapporter tyder på at pasientene responderer på tradisjonell vaskulittbehandling med steroider og cytostatika, selv om det kompromitterte immunsystemet gjør det vanskelig å gjennomføre en slik behandling. Enhver form for immunsuppresjonsbehandling bør kombineres med aggressiv antiviral og antibakteriell profylakse.

Systemisk lupus erythematosus-liknende syndrom

HIV-infeksjon og systemisk lupus erythematosus er systemsykdommer med variert organaffeksjon. Mange HIV-infiserte personer utvikler kliniske symptomer som kan være vanskelig å skille fra systemisk lupus erythematosus, slik som feber, dårlig allmenntilstand, vekttap, lymfadenopati, hudmanifestasjoner og nevrologiske manifestasjoner. Dette kan lett føre til diagnostiske og terapeutiske problemer, spesielt fordi det ved begge tilstander kan foreligge positive serologiske tester.

Serologiske og immunologiske prøver

Serologiske og immunologiske prøver er ofte patologiske ved HIV-infeksjon og likner det man ser ved autoimmune sykdommer (21, 25–27). Polyklonal hypergammaglobulinemi ser man nesten alltid hos HIV-infiserte, og høyt titer av IgG-antikardiolipin-antistoffer ser man hos opptil 50 % av disse pasientene, uten at det er forbundet med økt tromboserisiko. Antistoffer mot cytoplasmatiske antigener i nøytrofile granulocytter (ANCA) kan påvises hos opptil 42 % av HIV-infiserte pasienter, men i motsetning til hos ikke-HIV-infiserte er dette ikke assosiert med vaskulittsykdom. Både antinukleære antistoffer (ANA) og revmatoid faktor påvises noe hyppigere enn i normalbefolkningen. Økt mengde immunkomplekser påvises ofte, men er vanligvis ikke assosiert med immunkompleksykdom.

Et annet viktig poeng er at pasienter med autoimmune sykdommer har en tendens til å utvikle falskt positiv HIV-test på grunn av kryssreagerende autoantistoffer. Kryssreaksjon mellom HIV-antistoffer og autoantistoffer sees hos ca. 10 % av pasienter med systemisk lupus erythematosus når man bruker ELISA-teknikk for påvisning av antistoffer. Positiv HIV-test hos pasienter med systemisk lupus erythematosus bør derfor undersøkes med andre metoder, f.eks. Western blot (28).

Konklusjon

Revmatiske manifestasjoner er hyppig hos HIV-infiserte, og de vanligste manifestasjonene er uspesifikke og sees ved virusinfeksjoner i sin alminnelighet. Det synes likevel å være økt hyppighet av artritt, myositt og autoimmune sykdommer, og noen av disse tilstandene er spesifikke for HIV-infeksjon, de er ikke tilfeldig assosiert med infeksjonen. De revmatologiske manifestasjonene kan debutere før kliniske tegn på HIV-infeksjon, slik som beskrevet for Reiters syndrom. Tradisjonell behandling av de inflammatoriske revmatiske sykdommene med steroider og cytostatika fører ofte til økt risiko for opportunistiske infeksjoner og kanskje til økt risiko for malignitet hos HIV-infiserte.

Litteratur

1. Winchester R, Bernstein DH, Fischer HD, Enlow R, Solomon G. The occurrence of Reiter's syndrome and acquired immunodeficiency. Ann Intern Med 1987; 106: 19–26.
2. Calabrese LH. Rheumatic aspects of human immunodeficiency virus infection and other immunodeficient status. I: Klippel JH, Dieppe PA, red. Rheumatology. 2. utg. London: Mosby, 1998.
3. Duvic M, Johnson TM, Rapini RP, Freeze T, Brewton G, Rios A. Acquired immunodeficiency syndrome-associated psoriasis and Reiter's syndrome. Archives Dermat 1987; 123: 1622–32.
4. Buskila D, Gladman D. Musculoskeletal manifestations of infections with immunodeficiency virus. Rev Infec Dis 1990; 12: 223–5.
5. Keat A. HIV and overlap with Reiter's syndrome. Baillieres Clin Rheumatol 1994; 8: 363–77.
6. Davis P, Stein M, Latif A, Emmanuel J. Acute arthritis in Zimbabwean patients: possible relationship to human immunodeficiency virus infection. J Rheumatol 1989; 16: 346–8.
7. Espinoza LR, Aguilar JL, Berman A, Gutierrez F, Vasey FB, Germain BF. Rheumatic manifestations associated with human immunodeficiency virus infection. Arthritis Rheum 1989; 12: 1615–22.
8. Espinoza LR, Berman A, Vasey FB, Cahalin C, Nelson R, Germain BF. Psoriatic arthritis and acquired immunodeficiency syndrome. Arthritis Rheum 1988; 31: 1034–40.
9. Brancato L, Itescu S, Skovron ML, Solomon G, Winchester R. Aspects of the spectrum, prevalence and disease susceptibility determinants of Reiter's syndrome and related disorders associated with HIV infection. Rheumatol Int 1989; 9: 137–41.
10. Reveille JD, Conant MA, Duvic M. Human immunodeficiency virus-associated psoriasis, psoriatic arthritis and Reiter's syndrome: a disease continuum? Arthritis Rheum 1990; 33: 1574–8.
11. Kaplan MH, Sadik MS, Weider J, Farber BF, Neidt GW. Antipsoriatic effects of Zidovudine in human immunodeficiency virus associated psoriasis. J Am Acad Dermatol 1989; 20: 76–82.
12. Duvic M, Crane MN, Conant M, Mahoney SE, Reveille JD, Lehrman SN. Zidovudine improves psoriasis in human immunodeficiency virus positive males. Arch Dermatol 1994; 130: 447–51.
13. Rynes RI. Painful rheumatic syndromes associated with human immunodeficiency virus infection. Rheum Dis Clin North Am 1991; 17: 79–88.
14. Solomon G, Brancato L, Itescu S. Arthritis, psoriasis and related syndromes with HIV-infection. Rheum Dis Clin North Am 1993; 19: 59–78.
15. Bijlsma JWJ, Derksen RHWM, Huber-Bruning O, Boleffs JCC. Does AIDS «cure» rheumatoid arthritis? Ann Rheum Dis 1988; 47: 350–1.
16. Amor B. Rheumatoid arthritis and AIDS. J Rheumatol 1989; 16: 845.
17. Ornstein MH, Kerr LD, Spiera H. A re-examination of the relationship between active rheumatoid arthritis and the acquired immunodeficiency syndrome. Arthritis Rheum 1995; 38: 1701–6.
18. Itescu S. Adult immunodeficiency and rheumatic disease. Rheum Dis Clin North Am 1996; 22: 53–73.
19. Itescu S, Brancato LJ, Buxbaum J, Gregersen PK, Rizk JJ, Croxson TS et al. A diffuse infiltrative CD8 lymphocytosis syndrome in human immunodeficiency virus (HIV) infection: a host immune response associated with HLA-DR5. Ann Intern Med 1990; 112: 3–10.
20. Itescu S. Diffuse infiltrative lymphocytosis syndrome in human immunodeficiency virus infection – a Sjögren-like disease. Rheum Dis Clin North Am 1991; 17: 99–116.
21. Kaye BR. Rheumatologic manifestations of infection with human immunodeficiency virus (HIV). Ann Intern Med 1989; 111: 158–67.
22. Dalakis MC. Retrovirus and inflammatory myopathies in humans and primates. Ballieres Clin Neurol 1993; 2: 659–91.
23. Calabrese LH. Vasculitis and infection with the human immunodeficiency virus. Rheum Dis Clin North Am 1991; 17: 131–47.
24. Gherardi R, Belec L, Mhiri C, Gray F, Lescs MC, Sobel A. The spectrum of vasculitis in human immunodeficiency virus infected patients: a clinicopathologic evaluation. Arthritis Rheum 1993; 36: 1164–74.
25. Kopelman RG, Zolla-Pazner S. Association of human Immunodeficiency virus infection and autoimmune phenomena. Am J Med 1988; 84: 82–8.
26. Morrow WJW, Isenberg DA, Sobol RE, Stricher RB, Kieber-Emmons T. AIDS virus infection and autoimmunity: a perspective of the clinical, immunological, and mononuclear origins of the autoallergic pathologies associated with HIV disease. Clin Immunol Immunopathol 1991; 58: 163–80.
27. Muller S, Richalet P, Laurent-Crawford A, Barakat S, Riviere Y, Porrot F et al. Autoantibodies typical of non-organ-specific autoimmune disease in HIV-seropositive patients. AIDS 1992; 6: 933–42.
28. Calabrese LH, Proffitt MR, Segal AM. Clinical significance of serological reactivity to human T-cell lymphotropic virus type-III (HTLV-III) in patients with autoimmune disease. Arthritis Rheum 1986; 29 (suppl 2): 21.

Advances in immunosuppression for renal transplantation

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Renal transplantation is the treatment of choice for patients with end-stage renal disease.[1] Over the past 20 years, significant improvement in the success of renal transplantation has been noted. Most of this success is attributable to the introduction of cyclosporine A in the early 1980s, which improved renal allograft survival by approximately 15 % at one year post transplant.[2] However, long term renal allograft survival has not appreciably increased in the cyclosporine era and this, at least partly, is because of the nephrotoxic effects of cyclosporine.[2,3] The search for equally, or more effective, but less toxic, drugs is far from over. This review will summarise the various drugs and regimens used for immunosuppression following renal transplantation.

Standard immunosuppressive protocols in use at present consist of a combination of drugs that are given as initial and/or induction therapy in the immediate post transplant period, maintenance therapy given throughout the life of the graft, and therapy for acute rejection if and when rejection occurs. As the T cell is the central role player in the recognition of foreign antigens and the subsequent process of rejection, all current immunosuppressive drugs therefore target T cell activation and cytokine production, clonal expansion or both.[4]

The major immunosuppressive agents that are currently in use are the calcineurin inhibitors (cyclosporine and tacrolimus), azathioprine, mycophenolate mofetil, corticosteroids and antibody preparations (polyclonal or monoclonal). Each of these drugs will be reviewed briefly followed by an outline of standard protocols in use. Table I outlines the mechanism of action of the immunosuppressive drugs used.

cyclosporine

Cyclosporine is a cyclic polypeptide of fungal origin comprising 11 amino acids with a molecular weight of 1200. Its immunosuppressive effect depends on the formation of a complex with its cytoplasmic receptor protein, cyclophylin. The complex then binds to calcineurin which normally dephosphorylates nuclear regulatory proteins resulting in the expression of critical T cell activation genes including those of interleukin -2 (IL-2). Inhibition of calcineurin by the binding of the cyclosporine-cyclophylin complex therefore inhibits IL-2 gene transcription and production.[5,6] As the introduction of cyclosporine has been shown to increase graft survival, at least in the short term, most transplant recipients are currently maintained on a regimen that includes cyclosporine.

The standard formulation of cyclosporine with its poor and variable oral absorption has been replaced by the newer microemulsion formulation called Neoral which has better and more consistent bioavailability.[7] Therapy with cyclosporine is associated with several side effects including acute and chronic nephrotoxic effects, hypertension, hepatotoxicity, neurotoxicity, gingival hyperplasia and malignancy. The acute nephrotoxic effects occur secondary to renal vasoconstriction and are reversible while the

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chronic non-reversible nephrotoxic effects are probably due to a combination of persistent renal vasoconstriction and the induction of the potent fibrogenic growth factor, transforming growth factor β.[8]

Monitoring of trough concentrations and tailoring dosage to each patient is essential to minimise the toxic effects of cyclosporine while simultaneously maintaining adequate immunosuppression. Higher doses (eg. Neoral 8-10mg/kg/day) are used for initial therapy and the dose can be lowered (Neoral 3-5/kg/day) by six to12 months post transplantation. Whereas the majority of centres maintain cyclosporine as long as the graft is functioning, some have advocated the replacement of cyclosporine with another immunosuppressant after a few months post transplant.[8]

Table I: Mechanism of action of immunosuppressive agents*

Agent	Molecular target	Effect
Corticosteroids	Cytosolic receptors and nuclear proteins	Blocks transcription of cytokine genes (IL-1, IL-2, IL-6, TNF-α, IFN-γ)
Cyclosporine	Binds cyclophilins and inhibits calcineurin	Inhibits IL-2 production (IL-2)
Tacrolimus (FK506)	Binds FKBP-12, inhibits calcineurin	Inhibits IL-2 production
Azathioprine	Metabolites bind DNA	Inhibits purine synthesis, blocks DNA and RNA synthesis
Mycophenolate mofetil	Inhibits inosine monophosphate dehydrogenase	Blocks de-novo pathway of purine synthesis, blocks glycosylation
Sirolimus	Binds FKBP-12, blocks mTOR kinase	Blocks IL-2 induced cell cycle progression
ATG/ALG	Binds multiple antigens on lymphoid cells	Complement-mediated lysis, opsonisation and clearance, possibly ↓ IL-2 receptor
OKT3	Binds T cell CD3	Complement-mediated lysis, opsonisation and clearance, modification of CD3 receptor
Daclizumab, Basiliximab	Binds α-subunit of IL-2 receptor	Down-regulation of receptor, CD4 depletion

*Table modified from Kahan et al [2]

An important consideration in patients on cyclosporine therapy is that several drugs interfere with its metabolism causing either high or low blood levels. Calcium channel blockers, antifungal agents like ketoconazole and fluconazole and the macrolide antibiotics, erythromycin and clarithromycin, are among the group of drugs that slow cyclosporine metabolism by inhibiting hepatic cytochrome P450 activity, thus increasing cyclosporine levels. These drug interactions have been exploited for clinical use; examples are the addition of ketoconazole or diltiazem to lower the dose of cyclosporine, thus lowering drug costs.[10] Other medications such as the anticonvulsants (phenytoin, carbamazepine) and the antituberculosis agents (isoniazid and rifampicin) decrease cyclosporine blood levels by enhancing hepatic cytochrome P450 activity.

Tacrolimus (FK506)

Tacrolimus is a macrolide antibiotic compound that shares many of the properties of cyclosporine. Like cyclosporine it binds to a specific intracytoplasmic binding protein, FKBP12, and the resulting complex inactivates calcineurin with prevention of IL-2 gene transcription and IL-2 production.[11] Tacrolimus is more potent than cyclosporine on a milligram for milligram basis because of a greater affinity of its complex with FKBP12 to calcineurin. Tacrolimus-based immunosuppressive regimens have been shown to have a significantly reduced risk of acute rejection episodes.[12] Tacrolimus may also be beneficial as rescue therapy in patients with recurrent or resistant rejection episodes on cyclosporine where a reasonable response rate has been reported.[13] Tacrolimus is as toxic to the kidneys as cyclosporine. In addition, its use has been associated with a high incidence of diabetes, more prominent neurotoxic effects and less frequent hypertension, hirsutism and gingival hyperplasia

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compared to cyclosporine. As the controversy as to whether the benefits of [illegible], such as reduced rejection rate, outweigh its potential risks, most centres continue to adhere to a cyclosporine based regimen.

Corticosteroids

Steroids have been in use in clinical transplantation since the early 1960s and are used in maintenance immunosuppression and in the management of acute rejection episodes. Steroids are non-specific anti-inflammatory agents whose major immunosuppressive effect is the inhibition of almost all known cytokines. This effect is mediated via inhibition of nuclear factor KB activation and by binding to gluco-corticoid response elements in the promoter regions of cytokine genes.[2,14]

Generally, oral steroids are used in high doses in the immediate post transplant period and, over the subsequent several months, doses are tapered to a low maintenance dose. Slow tapering of steroids and ultimate withdrawal have been attempted to minimise toxicity but this is often accompanied by delayed acute rejection and a decrease in renal function.[15]

High dose intravenous methylprednisolone has an established role in the management of acute rejection with a response rate of 60% to 70%.[14,16]

The side effects of steroids are well known and include hypertension, glucose intolerance, dyslipidaemia, osteoporosis, aseptic necrosis of bone and susceptibility to infection.

Azathioprine

Azathioprine is an antimetabolite, a derivative of 6-mercaptopurine that inhibits DNA synthesis. It has been used as an immunosuppressive agent since the early 1960s and remains a part of the triple immunosuppressive protocol of many centres.

More recently, it is gradually losing ground to another antiproliferative agent, mycophenolate mofetil. Azathioprine is a purine analogue and inhibits purine nucleotide synthesis and interferes with the synthesis and metabolism of RNA.[17] The usual oral dose when used with cyclosporine is 1-2mg/kg/day. Higher doses of 2-3mg/kg may be administered when it is used as the primary immunosuppressant. Its most important side effects are bone marrow suppression, hepatotoxicity and susceptibility to infection and neoplasia. The blood count should be monitored regularly and the drug withheld if the white cell count falls below 3000/ml.

Mycophenolate mofetil (MMF)

Mycophenolic acid (MPA), the active compound in mycophenolate mofetil, is a reversible inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH). IMPDH is a critical enzyme in the 'de novo' synthesis of purines and, as lymphocytes rely on de novo purine synthesis more than other cell types, MPA has a relatively specific antiproliferative effect on lymphocytes.[18]

Mycophenolate has been used as a replacement for azathioprine in maintenance immunosuppression and as rescue therapy in patients with refractory rejection episodes. In clinical trials, where it was given as part of combination immunosuppressive therapy and compared with azathioprine containing regimens, mycophenolate resulted in a lower incidence of acute rejection (50% reduction) in the first year and decreased requirement

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for high dose steroids and antilymphocyte antibody therapy to treat rejection.[19]

Follow-up results at three years, though not statistically significant, showed a trend towards lower rejection rates and better graft survival.[20] MMF has also been successfully used in the treatment of acute rejection and in reversing refractory acute rejection.[21] Hence, MMF is currently being used for maintenance immunosuppression in many centres replacing azathioprine and is also finding a role in the treatment of rejection; doses employed are 2gm/day to 3gm/day. Its major side effect is gastro-intestinal toxicity manifesting as diarrhoea, oesophagitis or gastritis.

Sirolimus (Rapamycin)

Sirolimus is a macrolide antibiotic of actinomycetal origin that is structurally related to tacrolimus and binds to the same intracytoplasmic receptor as tacrolimus, FKBP12. However, its mechanism of action is different. It inhibits intracellular signalling distal to the IL-2 receptor and inhibits progression of the T cell into the S phase of the cell cycle.[22]

After having undergone studies on its safety and efficacy, sirolimus is currently being investigated for use in maintenance therapy with or without cyclosporine.[23] The major side effects are thrombocytopaenia and hyperlipidaemia. Results of ongoing trials need to be rigorously evaluated before sirolimus can become part of the 'routine' immunosuppressive armamentarium.

Polyclonal and monoclonal antibodies

Various polyclonal antibodies, antilymphocyte serum (ALS), antilymphocyte globulin (ALG) and antithymocyte globulin (ATG) have been available for use in renal transplantation for over two decades. Polyclonal antibodies are effective agents for rejection prophylaxis as part of induction therapy in the early post transplant period and for treatment of steroid resistant rejection.[24] They are given intravenously for a period of seven to 14 days. Presently, ATG is the most widely used polyclonal antibody. Their side effects include chills, fever and anaphylaxis, soon after administration and a serum sickness-like illness during prolonged course.

The monoclonal antibody OKT3 is a murine IgG antibody that binds to the CD3 complex, a part of the T cell antigen receptor, and its administration results in the depletion of CD3 positive T cells. It is highly effective for the treatment of acute rejection both as primary treatment or for steroid resistant rejection and it may also be used for induction therapy.[25] OKT3 is given intravenously for 10 to 14 days. OKT3 has, however, several serious adverse effects resulting from massive cytokine release including pulmonary oedema, encephalopathy and nephrotoxicity. Both OKT3 and polyclonal antibodies significantly increase the risk of infection and the development of post-transplant lymphoproliferative disease.

More recently, better understanding of the immune response and the biotechnological revolution have resulted in the production of genetically engineered chimeric (humanized) monoclonal antibodies. Two such antibodies are basilixiamab (Simulect) and daclizumab (Zenapax) which are anti IL-2 receptor monoclonal antibodies. They have shown promising results in reducing the incidence of acute rejection when used in induction therapy with a much better safety profile and ease of administration compared to earlier antibody preparations.[26,27]

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Immunosuppressive regimens/protocols

Immunosuppressive protocols may conveniently be divided into protocols for:

i) Initial immunosuppressive therapy (including induction therapy).

ii) Maintenance immunosuppressive therapy.

iii) Therapy of acute rejection.

Initial immunosuppressive therapy: This refers to therapy in the first three months after transplantation during which the risk of graft rejection is at its highest. Immunosuppression should therefore be at its maximum levels in this period. In most patients the basic triple drug protocol of cyclosporine, azathioprine and steroids (at doses higher than during maintenance therapy) is used.[14] In the last few years, protocols using tacrolimus (FK506) and mycophenolate mofetil in place of cyclosporine and azathioprine respectively have emerged as alternatives to the standard protocol.[12,18] Patients presumed to be at high risk of rejection, for example, presensitised patients or those undergoing a second or subsequent transplant are candidates for induction immunosuppressive therapy. This involves the use of monoclonal or polyclonal antibodies for several days (duration depending on the antibody preparation) in the period immediately after transplantation.[2-5] Induction protocols reduce early acute rejection in high risk patients and avoid the use of nephrotoxic immunosuppressive agents (cyclosporine and tacrolimus) in the first few days post transplantation. The potent immunosuppressive effect of the antibody preparations significantly increase the risk of serious infection and malignant disease. The recently introduced humanised antibody preparations may achieve the same goal with less side effects.

Maintenance immunosuppression: The major agents that are currently used in maintenance therapy are cyclosporine, corticosteroids and azathioprine or mycophenolate mofetil.[11] Using such a combination of drugs that are active at various steps in T cell activation allows one to use lower doses of each drug, thus reducing side effects to a minimum while at the same time maintaining adequate immunosuppression. As referred to earlier, some centres attempt to stop cyclosporine or steroids in the long term but triple therapy remains the standard against which all protocols for maintenance immunosuppression must be measured. The aim of research into new and better protocols for maintenance immunosuppression would be to find the right combination that reduces the risk of rejection while simultaneously avoiding serious side effects associated with long term immunosuppression.

Therapy for acute rejection: Intravenous pulse methylprednisolone is the first line therapy for acute uncomplicated renal allograft rejection in a 'low risk' patient. The drug is given intravenously (500-1000mg/day) for three to five days.[28] After completion of the steroid pulse, oral steroids are restarted at maintenance levels or are given in higher doses and rapidly tapered to maintenance levels. The expected reversal rate with pulse methylprednisolone therapy is 60% to 70% for the first episode of acute cellular rejection.[29]

In steroid resistant cases, i.e. those with a lack of improvement in urine output or plasma creatinine within five days, second line therapy with antibody preparations should be considered. Treatment with OKT3 or polyclonal antibodies results in a reversal rate of 80% to 90%. While it is very well known that the antibody preparation are more effective in reversing acute

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rejection episodes in general, they are reserved for cases of steroid resistant rejection, recurrent rejection and severe vascular rejection.[24,29]

Tacrolimus has been effectively used as rescue therapy for refractory rejection in these patients under primary cyclosporine immunosuppression and is thus an option for such patients.[13] Another valuable alternative for the treatment of refractory rejection is mycophenolate mofetil.[5]

Conclusion

Immunosuppressive treatment for renal transplantation has come a long way from its early beginning with 6 mercaptopurine (from which azathioprine was later derived). An increasing number of drugs have been made available over the years parallelling progress in the understanding of cellular and molecular mechanisms of allograft rejection. However, as is clear from the foregoing, presently used immunosuppressive drugs and protocols are far from optimal. The search for more selective and effective drugs and protocols will thus continue until the eventual goal of transplantation tolerance with long term graft function is achieved.

References

1. **Goodman WG and Nissenson AR.** Options for patients with end-stage renal disease. In Danovitch GM (Ed). *Handbook of Kidney Transplantation, New York, NY, Little, Brown 1996 p2.*
2. **Denton MD, Magee CC and Sayegh MH.** Immunosuppressive strategies in transplantation. *Lancet 1999, 353 : 1083-1091.*
3. **DeMattos A, Olyaai, AJ Bennett WM.** Nephrotoxicity of immunosuppressive drugs: Long term consequences and challenges for the future. *Am. J. Kid. Dis. 2000, 356: 333-346.*
4. **Suthianthiran M, Strom TB.** Renal Transplantation. *N. Engl. J. Med. 1994, 331:365-376.*
5. **Danovitch GM.** Immunosuppressive medications and protocols for kidney transplantation. In Danovitch G.M. (Ed). *Handbook of Kidney Transplantation, New York, NY, Little, Brown 1996. pp 55-94.*
6. **Kahan B.** Cyclosporine. *N. Engl. J. Med. 1989, 321:1725-1738.*
7. **Cole E, Keown P., Landsberg D.** et al. Safety and tolerability of Cyclosporine microemulsion during 18 months of follow-up in renal transplant recipients: a report of the Canadian Neoral Study Group. *Transplantation 1998, 65 : 505-510.*
8. **Bennett WM, De Mattos A, Meyers MM et al.** Chronic Cyclosporine nephropathy, the Achilles heel of immunosuppressive therapy. *Kidney Int. 1996, 50 : 1089-1100.*
9. **Hollander AA, van Saase JL, Koote AM. et al.** Beneficial effects of conversion from Cyclosporine to Azathioprine after kidney transplantation. *Lancet 1995, 345 : 610-614.*
10. **First MR, Schroeder TJ, Michael A.** et al. Cyclosporine – ketoconazole interaction : Long term follow-up and preliminary results of a randomized trial. *Transplantation 1993,55 : 1000 – 1004.*
11. **Velle JP, Sayegh MH.** Maintenance pharmacological immunosuppressive strategies in transplantation. *Postgrad. Med. J. 1997, 73 : 386-390.*
12. **Firsch JD, Miller J, Deierhoi MH.** et al for the FK 506 Kidney Transplant Group. A comparison of tacrolimus (FK506) and Cyclosporine for immunosuppression after cadaveric renal transplantation. FK 506 Kidney Transplant Study Group. *Transplantation 1998, 65: 142-145.*
13. **Jordan ML, Shapiro R, Vivas C. A.** et al. FK 506 'Rescue' for resistant rejection of renal allografts after primary cyclosporine immunosuppression. *Transplantation 1994, 57 : 860-865.*
14. **Strom TB, Tilney NL.** Immunobiology and Immunopharmacology of graft rejection. In Diseases of the Kidney. Schrier R W and Gottschalk C W (Ed) 5th edition 1993 Little, *Brown and Company pp 2879-2910.*
15. **Ratcliffe PJ, Dudley CRK, Higgins RM.** et al. Randomised controlled trial of steroid withdrawal in renal transplant recipients receiving triple immunosuppression. *Lancet 1996, 348 : 643-648.*
16. **Gray D, Daar A, Shepherd M.** et al. Oral versus intravenous high dose steroid treatment of renal allograft rejection. *Lancet 1978, 1: 117-120.*
17. **Walker RG, d'Apice AJF.** Nonspecific immunosuppression : Azathioprine and steroids. In Morris P. J. (Ed). *Kidney Transplantation, Principles and Practice (4th Ed) 1994, W.B. Saunders pp 202-204.*
18. **Halloran P, Mathew T, Tomlanovitch C.** et al for the International Mycophenolate Mofetil Renal Transplant Study Group. Mycophenolate Mofetil in Renal Allograft Recipients. *Transplantation 1997, 63 : 39-47.*
19. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. *Transplantation 1996, 61 : 1029-37.*
20. **Mathew T.** for the Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, long term, randomized multicentre study of mycophenolate mofetil in cadaveric renal transplantation. *Transplantation 1998, 65 : 1450-1454.*
21. The Mycophenolate Mofetil Acute Renal Rejection Study Group. Mycophenolate Mofetil for the treatment of a first acute renal allograft rejection. *Transplantation 1998, 65: 235-241.*
22. **Morris RE.** Mechanisms of action of new immunosuppressive drugs. *Kidney Int. 1996, 53: S27-38.*
23. **Groth CG, Bäckman L, Morales J M.** et al. for the Sirolimus European Renal Transplant Study Group. Sirolimus (Rapamycin) – based therapy in human renal transplantation. *Transplantation 1999, 67 : 1036-1042.*
24. **Powelson JA, Cosimi AB .** Antilymphocyte globulins and monoclonal antibodies. In Morris P J (Ed). *Kidney Transplantation: Principles and Practice (4th Ed) 1994. W B Saunders pp 215-232.*
25. **Schroeder TJ, First MR.** Monoclonal antibodies in organ transplantation. *Am J Kid Dis 1994, 23 : 138-147.*
26. **Nashan B, Moore R, Amlot P.** et al. for the CHIB 201 International Study Group. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. *Lancet 1997, 350 : 1193-1198.*
27. **Vincent F, Kirkman R, Light S.** et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. *N Eng J. Med 1998, 338 : 161-165.*
28. **Vineyard GC, Fadem SZ, Dmochowski J.** et al. Evaluation of corticosteroid therapy for acute renal allograft rejection. *Surg. Gynecol Obstet 1974, 138 : 225-229*
29. **McKay DB, Milford EL, Sayegh MH.** Clinical aspects of renal transplantation. In Brenner B. M. (Ed) *Brenner and Rector's The Kidney (5th Ed) 1996, WB Saunders pp 2606-2652.*

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Assessing the impact of an innovative curriculum on medical graduates: the Jimma experience, Ethiopia

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Abstract: Jimma Institute of Health Sciences (Jimma University) has adapted an innovative curriculum since its establishment. This study was carried out to assess the impact of its curriculum on its medical graduates. An attempt was made to involve graduates who were serving in the different parts of the country. To increase reliability and validity, multiple raters and multiple item scaling were used. The assessment of the raters on the different skills of the graduates was similar. The competence of the graduates on the majority of the skills was 4 on the five-point scale. JIHS medical graduates' Community-Oriented outlook and practice was high. The influence and impact of Community-Based Education on their commitment, character and career was also found to be high. Relevant recommendations are made. [*Ethiop. J. Health Dev.* 2000;14:253-267]

Introduction

Jimma Institute of Health Sciences came into existence as a result of several developments, globally and within the country, that necessitated a new strategy for the development of resources for health. Internationally; there was a movement by professional associations like the World Medical Association and agencies like WHO, UNESCO, which held series of conferences and symposia on strategies for developing trained human resources (1,2,3). At country level, in the 1960, and 1970s, it was a period when developing countries came to realize that the education models of the western nations were not working adequately for their situation (4,5). In the late 1970s, countries of the world declared 'Health For All by the year 2000' and adopted the Primary Health Care (PHC) approach as a means to attain it (3).

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Parallel to the international educational movement, there was a similar process in Ethiopia spearheaded by health professionals and the Ministry of Health (MOH) on models of health professional training. The general consensus was to train a new type of health worker based on the needs of communities, (6, 7, 8). To realize this, the Jimma Institute of Health Sciences (JIHS) was established in 1983, ie, to implement an innovative curriculum of health sciences (8). The aim of the new curriculum was to produce new types of health workers who could be responsive to the health needs of society at the individual, family, and community levels (9). In order to realise this aim, the philosophy and strategy adopted by the Institute was Community-Oriented Education (COE) and Community Based Education (CBE), respectively (9). In the strategy the three important educational approaches were Community Based Training Programme (CBTP), Team Training Programme (TTP) and Student

Research Project (SRP) (9, 10, 11).

Many institutions are showing growing concern to evaluate the process and outcome of their curriculum due to a sense of obligation and social accountability (12,13). As more innovative schools started to evaluate their curriculum, several questions came to the scene. The different settings of innovation raise questions of what should be evaluated, how and when? Methods of assessment are issues of debate (12,14). Though there is the question of standardized evaluation method, the advantage of any evaluation is beyond doubt. It is necessary to serve multiple purposes, including its use to modify and refine curricula, to address the concern of stakeholders, and to overcome the unfounded fears towards innovation (11). Some guidelines and frameworks for general approaches to evaluation of CBE have been reported based on experiences gained so far. Significant emphasis is put on social responsiveness (12,13,15,16,17).

JIHS was committed to provide relevant health services to the community that could help the learner to identify, prioritize, and solve health problems by involving the community and other development sectors. In order to implement such an innovative plan, appropriate strategies were developed (9, 10, 11). The strategies were means to train students: in actual community settings, in teamwork, and in undertaking applied research. JIHS is training different categories of health personnel that comprises medical doctors, nurses, pharmacy, environmental health, and laboratory technicians. JIHS has never evaluated its graduates, but it needs such information to review its curriculum. Considering resources and communication problems, this assessment study is limited to the medical training outcome. It is fair and logical to involve major stakeholders in the evaluation of this new approach to training (18). The stakeholders include the school, students (graduates), teachers, work-mates, supervisors and employers.

The purpose of this study was to assess JIHS medical graduates' responsiveness to society needs at individual and community levels by assessing their skills on individual care, communication, epidemiological, health management, community involvement, teamwork, multisectorality, learning and teaching, and research undertaking.

Methods

CBE has been introduced at JIHS in mid 1980s. There is a dearth of information on CBE evaluation since its introduction. JIHS was being upgraded to a university recently. Considering the above JIHS nominated a committee to assess CBE comprehensively.

In order to measure the outcome of CBE, it was agreed to study only the medical category of JIHS graduates in view of resources and communication problems. Locating the place of work of JIHS medical graduates was difficult of as this was not available at any administrative level. Since there was no sampling frame, the option was to move from place to place in person and identify their placement with the assistance of Regional and Zonal Health Offices and colleagues. Earlier, a letter was dispatched to all Regional Health Bureau (RHBs) and other possible establishments which could employ or train JIHS graduates, explaining the intended study's importance and

requesting their collaboration in the study endeavor. Medical graduates who were included in the study were eight cohorts who graduated from 1991 to 1998. For data collection, individuals were assigned either by JIHS or the RHBs who moved from place to place, try to identify as many as possible JIHS medical graduates, deliver the written questionnaire to the four groups (the graduate, the graduates' work-mates, graduates' supervisors/ employers, instructors) and then collect the completed questionnaire. This activity has been carried out in most Regions of the country using air and road transport.

During the construction of the study instrument (a written questionnaire), previous evaluation studies of innovative schools were considered (12, 14, 17, 19, 20) and attempt was made to develop multiple item scaling technique for each skill measured (18, 21, 22, 23). The structured questionnaire was prepared with two parts. Part one comprises sixty questions to be answered by JIHS medical graduates. Part two comprises forty-eight questions to be answered by each of JIHS medical graduates' work-mates (non-JIHS graduates), supervisors/ employers, instructors (for those who were attending graduate studies). Each item was mostly measured on a five point scale. A sample of the study questionnaire is included in Annex 1. To assess the graduates' competency the above questions were clustered into nine skills in line with the study objectives to measure their responsiveness to society's needs at individual and community, levels: Skills of individual care (clinical), problem identifying, prioritizing and intervention (epidemiological), running health services (health management), communication at various levels through different means (communication) working together with professionals (team work), working with other sectors (multi-sectoral), working with the community at the various stages of health care (community involvement), sharing knowledge, experience, and motivation to learn (learning and teaching), as well as motivation and attempt to undertake community-based research (research). The instruction of the questionnaire invites the different raters to participate in assessing the competence of JIHS medical graduates and rely on the raters conscientiousness in completing the questionnaire.

In order to increase reliability and validity, multiple raters and multiple item scaling were used (18, 21, 22, 23). The data collection was conducted in 1999/2000. The collected data were then entered into a computer and routine checking and cleaning of data was carried out. For data analysis, both descriptive and analytical approach were attempted using SPSS 7.5.1 for windows.

Results

The total number of the eight cohorts who graduated from 1991 to 1998 was 374 of which 33 (8.8%) were females (Table 1). The different groups who participated in the study were JIHS medical graduates (n=160)*, graduates' work-mate (n=183), graduates' supervisors/

Table 1: Graduates distribution, by year of graduation, and graduates included in the study with percent covered. JU, March 2000

Year of graduation	Number of Graduates			Graduates in the sample			Percent Covered
	Male	Female	Total	Male	Female	Total	
1991	56	10	66	23	0	23	34.8
1992	59	5	64	22	0	22	34.4
1993	39	1	40	16	0	16	40.0
1994	48	4	52	18	3	21	40.4
1995	39	3	42	13	0	13	31.0
1996	33	2	35	17	0	17	48.6
1997	36	4	40	22	1	23	57.5
1998	31	4	35	20	4	24	68.6
Total	341	33	374	151	8	159	42.5

The number of graduates who participated in the study were 160, but in this table it is 159, this is because one of the graduates didn't respond on year of graduation. A similar discrepancy could be observed in the other tables when a study participant or participants didn't respond for a certain question.

Table 2: Respondents distribution, by age and sex, March 2000

	Respondents' Category								Total	
	Graduates		Work-mates		Supervisors /Employer		Instructors			
	n	%	n	%	n	%	n	%	n	%
Age group										
< 30	92	57.5	69	38.1	15	23.8	0	0.0	176	43.6
30-39	68	42	98	54.1	33	52.4	9	60.0	199	49.3
40+	0	0.0	14	7.7	15	23.8	6	40.0	29	7.2
Total	160	100.0	181	100.0	63	100.0	15	100.0	404	100.0
Sex										
Male	152	95.0	168	91.0	57	90.5	15	100.0	377	92.9
Female	8	5.0	15	8.2	6	9.5	0	0.0	29	7.1
Total	160	100.0	183	100.0	63	100.0	15	100.0	406	100.0

employers (n=63), and instructors (for graduates who were following graduate studies) (n = 15). Even though the plan was to have one work-mate for each JIHS graduate, in some cases, two work mates of graduates had participated in the study. Again since one person was supervisor for more than one graduate the total number of supervisors is less than the total number of JIHS graduates in the sample. Instructors assessed as heads of departments or in their areas of specialties and assessed the students as a group (aggregate). The mean ages for the different groups were: JIHS medical graduates' 28.9, work-mates 31.4, supervisors 35.6, and instructors 42.2 years. In the study population, the proportions of females were 5%, 8.2%, and 9.5% among JIHS graduates, work-mates, supervisors, respectively and none among instructors (Table 2). The Regional distribution of JIHS graduates in the study sample was 54 in Addis Ababa, 29 in Oromia, 23 in Southern Nations and Nationalities Peoples' Region (SNNPR), 20 in Amhara, 17 in Tigray, seven in Benshangul, five in Gambella, and three in Harari; and two and did not indicate their work place address (Table 3).

Table 3: Graduates' distribution, by Region, March 2000

Region	Number	%
Addis Ababa	54	34.2
Oromia	29	18.4
SNNPR	23	14.6
Amhara	20	12.7
Tigray	17	10.8
Benshangul	7	4.4
Gambella	5	3.2
Harari	3	1.9
Total	158	100.0

The major categories of JIHS graduates' workmates were general practitioner/ medical doctor (GP/MD) (85.9%), nurses (10.0%), and environmental health experts (2.4%), with about 9% who did not indicate their profession. The qualifications of JIHS graduates supervisors were: GP/MD (89.1%), nurses (9.1%) and more than 10% did not indicate their qualifications.

Places of work of JIHS graduates during the study were: health centers and hospitals (47.6%),attending graduate studies at Addis Ababa University (27.8%), MOH, RHB, ZHB and other

Table 4: Graduates' distribution, by current place of work and position, March 2000

Current place of work	Number	%
HC, Hospitals	60	47.6
Graduate study, AAU	35	27.8
MOH, RHB, ZHB	12	9.5
JIHS staff	10	7.9
Different GHS	4	3.2
NGO	3	2.4
PHS	2	1.6
Total	126	100.0
Current Position		
GP	55	39.6
Attending graduate studies	37	26.6
Office Head*	39	26.6
Instructor	10	7.2
Total	139	100.0

* Mainly heads of sections or team leaders in RHB, ZHD, H.C./hospital head

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Table 5: work-mates', supervisors', and instructors' evaluation on the competency of JIHS Medical graduates, March 2000

Raters	Score	Skills								
		clncl	EPid	HM	Com	CI	TW	MS	LT	R
work-mate	Mean	4.31	3.91	3.99	3.96	3.85	4.16	3.76	4.28	3.56
	SD	0.68	0.97	0.85	0.85	0.97	0.89	0.97	0.68	1.18
	n	120	71	112	81	102	119	77	77	97
Supervisor/ Employer	Mean	4.13	4.33	4.14	4.26	4.09	4.44	4.20	4.15	3.91
	SD	0.57	0.55	0.70	0.58	0.64	0.71	0.62	0.57	1.01
	n	36	27	37	23	35	41	30	51	33
Instructors	Mean	3.78	3.92	3.67	3.80	4.20	4.19	3.78	4.07	4.50
	SD	0.73	0.59	0.54	0.28	0.84	0.70	0.19	0.45	0.58
	n	6	2	4	2	5	8	3	7	4
Total	Mean	4.25	4.02	4.02	4.3	3.92	4.23	3.88	4.24	3.67
	SD	0.67	0.89	0.81	0.76	0.89	0.84	0.89	0.65	1.14
	n	162	100	153	106	142	168	110	183	134

Key: Clncl = clinical Epid = Epidemiological HM = Health management Com = communication
CI = Community involvement TW = Team work MS = Multi-sectoral, LT = Learning and teaching R = Research

GHS (government health services) (12.7%), JIHS staff (7.9%), NGO (non governmental health organization) (2.4%), PHS (private health services) (1.6%), and 34 respondents did not indicate their current places of work.

The evaluations of the three raters (work-mate, supervisor, instructor) of JIHS graduates on the various skills, in general, were similar (Table 5). The opinions of the four raters and graduates' self-assessment, by year of graduation, are presented in Tables 6 and 7, respectively.

Among the graduates, 84.8% were willing to work in any community. These views were also confirmed by the other three groups of raters to a similar extent. According to three groups of rater opinions graduates willing to work in any community were 94.5%.

The graduates' self assessment of their overall clinical and public health competences, compared to other conventional medical schools' graduates put themselves on the top quarter and above 67.9% and 83.5%, respectively. The cumulative score by the three raters was also 57% and 71% (Table 8).

Graduates were asked specific questions on the influence and impact of CBE on their character and career. In general over 80% had rated it positively (Table 9).

On the question of whether they would advise others to join JIHS for medical training, the response was no by 8.5% (graduates), 2.2% (work-mates), 6.6% (supervisors) and none of the instructors.

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Table 6. Skills rating scores by graduates, by different raters, March 2000

Raters	Score	Skills: Clncl①	Epid①	CI①	HM①	Com①	MS①	TW①	LT①	R①
Graduates (Self)	Mean	4.09	3.96	3.67	3.99	3.82	4.08	3.59	4.09	3.45
	SD	0.68	0.75	0.83	0.69	0.90	0.84	1.01	0.75	1.27
	n	150	116	135	125	138	142	119	145	139
Work-mate	Mean	4.31	3.91	3.99	3.96	3.85	4.16	3.76	4.28	3.56
	SD	0.68	0.97	0.85	0.80	0.97	0.89	0.99	0.68	1.18
	n	120	71	112	81	102	119	77	125	97
Supervisor/ Employer	Mean	4.13	4.33	4.14	4.26	4.09	4.44	4.20	4.15	3.91
	SD	0.57	0.55	0.70	0.58	0.64	0.71	0.62	0.57	1.01
	n	36	27	37	23	35	41	30	51	33
Instructors	Mean	3.78	3.92	3.67	3.80	4.20	4.19	3.78	4.07	4.50
	SD	73	.59	.54	.28	.84	.70	.19	.45	.58
	n	6	2	4	2	5	8	3	7	4
Total	Mean	4.18	3.99	3.86	4.00	3.87	4.16	3.73	4.17	3.56
	SD	0.68	0.82	0.84	0.72	0.89	0.84	0.97	0.69	1.21
	n	312	216	288	231	278	310	229	328	273

① p=0.022 ② p=0.003 ③ p>>0.05

Key: Clncl = clinical; Com = communication; MS = Multi-sectoral; Epid = Epidemiological; CI = Community Involvement; LT = Learning and teaching; HM = Health management; TW = Team work; R = Research

Table 7: Graduates' self assessment, by year of graduation, on clinical and public health competences/ March 2000

Year of graduation	Score	Skills								
		clncl	EPid	HM	Com	CI	TW	MS	LT	R
1991	Mean	4.09	4.23	3.98	4.36	4.19	4.52	3.88	4.26	4.00
	SD	.81	.73	.84	.32	.85	43	.93	.78	1.13
	n	22	18	21	19	21	21	20	23	23
1992	Mean	3.88	3.94	3.77	3.93	3.82	4.25	3.85	3.98	3.21
	SD	.69	.74	1.05	.78	.95	.67	.94	.87	1.22
	n	20	15	19	17	19	18	16	20	19
1993	Mean	3.94	3.70	3.25	3.77	3.31	3.42	3.50	3.89	3.29
	SD	.55	.92	.87	.79	.79	1.17	1.32	.61	1.27
	n	16	9	12	12	13	13	10	16	14
1994	Mean	4.18	4.02	3.76	3.98	3.88	4.08	3.58	4.13	2.94
	SD	.62	.75	.82	.64	.70	.75	1.03	.72	1.35
	n	19	15	17	16	18	20	15	18	18
1995	Mean	4.18	3.83	3.53	3.76	3.70	4.08	3.33	4.23	3.36
	SD	.42	.71	.52	.74	.63	1.03	1.15	.59	1.43
	n	13	10	12	11	11	13	10	12	11
1996	Mean	4.19	3.92	3.64	4.07	3.90	4.14	3.86	4.09	3.50
	SD	.64	.68	.68	.64	.72	.63	.82	.59	1.34
	n	16	14	15	14	13	14	12	14	14
1997	Mean	4.16	3.90	3.70	3.94	4.06	4.25	3.48	4.00	3.38
	SD	.66	.86	.86	.76	.75	.75	.84	.88	1.28
	n	21	18	20	18	20	22	16	18	21
1998	Mean	4.11	3.85	3.43	3.86	3.72	3.63	3.07	4.09	3.68
	SD	.86	.69	.68	.70	.70	.87	1.03	.79	1.16
	n	22	16	18	17	18	20	19	23	19
Total	Mean	4.08	3.95	3.66	3.98	3.86	4.07	3.57	4.08	3.45
	SD	.68	.75	.83	.68	.80	.84	1.01	.75	1.27
	n	149	115	134	124	133	141	118	144	139

Key: Clncl = clinical
Epid = Epidemiological
HM = Health management
Com = communication
CI = Community Involvement
TW = Team work
MS = Multi-sectoral,
LT = Learning and teaching
R = Research

Table 8: Overall JIHS graduates' competence in clinical and public health compared to other conventional medical school graduates, by different raters,March 2000

JIHS Graduates Overall	Raters Category								Total	
	Graduate		Work-mate		supervisor /Employer		Instructors			
	n	%	n	%	n	%	n	%	n	%
Clinical										
Bottom quarter	0	.0	1	.6	0	.0	0	0.0	1	0.3
Competence										
Lower middle quarter	0	.0	3	1.9	0	.0	0	0.0	3	0.9
Upper middle quarter	44	32.1	72	46.5	23	48.9	7	70.0	139	41.0
Top quarter	89	65.0	76	49.0	22	46.8	3	30.0	187	55.2
Exceptional	4	2.9	3	1.9	2	4.3	0	0.0	9	2.7
Total	137	100.0	155	100.0	47	100.0	10	100.0	339	100.0
Public Health										
Bottom quarter	1	.7	4	3.2	0	.0	0	0.0	5	1.6
competence										
Lower middle quarter	1	.7	7	5.6	0	.0	0	0.0	8	2.6
Upper middle quarter	21	15.1	40	32.0	14	32.6	3	50.0	75	24.4
Top quarter	87	62.6	68	54.4	25	58.1	3	50.0	180	58.6
Exceptional	29	20.9	6	4.8	4	9.3	0	0.0	39	12.7
Total	139	100.0	125	100.0	43	100.0	6	100.0	107	100.0

Table 9: Influence or impact of CBE according to graduates' self-assessment, March 2000.

Characteristics		n	%
CBE is expendable to other disciplines as it is practiced each year during my under-graduate training	Very poorly	18	12.2
	Poorly	17	11.5
	Well	36	24.3
	Very well	54	36.5
	Extremely well	23	15.5
Total		148	100.0
CBE has imfluenced me in a way that characterizes me to this day	Very poorly	12	7.6
	Poorly	17	10.8
	Well	36	22.9
	Very well	56	35.7
	Extremely well	36	22.9
Total		157	100.0
CBE has impact on my career and its influence does not fade with time	Very poorly	16	10.3
	Poorly	11	7.1
	Well	36	23.1
	Ver well	63	40.4
	Extremely well	30	19.2
Total		156	100.0
CBE has helped me in developing a holistic approach in addressing a problem	Very poorly	10	6.5
	Poorly	10	6.5
	Well	30	19.5
	Very well	59	38.3
	Extremely well	45	20.2
Total		154	100.0

Clinical = Clinica
Com = communicati
Mult-sectoral

Epidemiological
Community involvement
Learning and teaching

Health
Team work
Research

continued (Table 9)

Characteristics		n	%
CBE taught me how to communicate better with the patient, family and community	Very poorly	9	5.9
	Poorly	16	10.5
	Well	20	13.1
	Vell Well	61	39.9
	Extremely well	47	30.7
Total		153	100.0
CBE has equipped me to work at a grassroot level	Very poorly	11	7.2
	Poorly	15	9.8
	Well	27	17.6
	Very well	45	29.4
	Extremely well	55	35.9
Total		153	100.0

Discussion

The attempt in the present study was to include all JIHS medical graduates who were serving in the country during the study period. But we managed to include only 42.5% of the eight cohorts (Table 1).

In general, Region size seemed to have influenced distribution (number) of graduates (Table 3). The relatively bigger ratios in Addis Ababa and Oromia were due to the fact that 35 of the graduates were attending further studies in Addis Ababa University and 10 were staff members in JIHS, respectively.

Graduates' self-assessment by year of graduation was similar (Table 7) except for team work skill which was different, the difference being statistically significant ($p = 0.002$). This statistically significant difference has appeared between the cohorts of 1991 and 1993, 1998 ($p=0.004$, $p=0.011$, respectively).

Graduates expressed their observation that the impact of CBE does not fade with time (Table 9).

The judgements of the three raters (which did not include the graduates self-evaluation) on the nine different skills were similar (Table 5). The minor differences observed were not statistically significant. Skills which were rated above 4 (on the five-point scale) by each of the three raters were team work and motivation to learn and teach. Considering the total mean score of each rater, only community involvement, multisectoral and research undertaking scored less than 4 and above 3.5. But graduates' skill which scored the highest point (4.5) by the instructors was research undertaking. In general, according to the three raters, graduates' performance on clinical, epidemiological, health management, communication, teamwork, and learning and teaching skills were very good. But their performance on

community involvement, multisectoral approach and research motivation and undertaking did not attain very good level (Table 5).

The evaluation of the four raters, which includes the graduates' self-assessment, showed variations on clinical, health management, and multisectoral competency which was statistically significant. From Tables 5 and 6, it could be discerned that graduates had given lower mean scores to themselves compared to the sum of mean scores of the three raters. But on the remaining six skills, the evaluation score was similar without significant variation (Table 6). According to the three raters, it was clearly shown that JIHS medical graduates were strong on their performance on most of the nine skills (Table 5). Other international studies on innovative schools had revealed similar findings (19, 25).

The study results showed that JIHS medical graduates who were assigned in various responsible positions in the health services were 26.6% and those attending graduate studies were 27.8% (Table 4). Assignment to responsible positions will help graduates to reinforce the community-oriented approach in the health services which is also supported by the Government policy (26). Graduates' motivation for further training in any field should also be appreciated, since graduates are expected to adapt community-oriented approach. For example, surgeons for cases of trauma or goiter, should ask ' where do they come from?' (27). Following a community-based problem-solving approach should initiate activities to address the problem at its source rather than waiting behind the desk. This view is further reinforced by graduates' response that CBE did not fade with time and it has influence and impact on their character and career (Table 9).

According to the aggregate evaluation of the three raters, the performance of the graduates on community involvement, multisectoral approach and research undertaking was less than 4 on the five-point scale (Table 5). There is a need for a concerted effort by all stakeholders to enhance the students' learning process on the three skills during the undergraduate training.

The graduates' self-assessment, compared to graduates of other conventional medical schools in the contry was 4 and above (on a 5 point scale) in 83.5% for public health and in 67.9% for a clinical competence. The aggregate score by the other four raters was 57% and 71% for clinical and public health competence, respectively (Table 8). These findings show the strength of JIHS medical graduates on their competency on community-oriented approach. International studies on innovative schools had reported similar findings (19, 25).

Among the graduates those who were willing to work in any community were 84.8% and according the four raters jedgement it was 94.5%.

On the question of would you advise others to join JIHS medical training, the response was yes by 91.5% graduates, 97.8% work-mates, 93.4% supervisors/ employers and 100% instructors. These findings clearly showed that it is not only the JIHS graduates who had a strong opinion of their community-oriented outlook but their performance has also convinced their work-mates, supervisors/

employers and instructors.

Eighty percent of the graduates who responded to this survey believed that CBE had positive impact on their charater and career. Other international studies on innovative education had also revealed similar findings (19, 25).

Conclusions

JIHS medical graduates' community-oriented outlook and practice was high. This was demonstrated by their very good performance on most of the skills on which they were assessed. Graduates' commitment, and the influence and impact of CBE on their character and career was again found to be high.

Recommendations

1. The relatively lower scores on community involvement, multisectoral approach, and research undertaking skills need a concerted effort by all stakeholders during the undergraduate training.
2. It is high time that the University should establish a regular monitoring and evaluation process for its innovative curriculum.
3. It would be helpful if the University encourages its graduates to form alumini association so that on inventory and full address of the graduates could be available.

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References

1. The World Medical Association. 1972. Educating tomorrow doctors. Fourth World Conference on medical education. Copenhagen.
2. WHO. 1977. Thirtieth world health assembly. Verbatim records of plenary meetings reports of committees. Geneva.
3. WHO. 1978. Primary Health Care. Alema-Ata 1978. Health for all series No. 1. Geneva.
4. WHO. 1987. Community-based education of health personnel. Technical report series 746. Geneva.
5. WHO. 1984. The role of universities in the strategies for health for all. Resolution WHA 37.31. Geneva.
6. Addis Ababa University. 1975. Educational philosophy of the university. Addis Ababa. Amharic version.
7. Addis Ababa University. Medical Faculty. 1976. To study and review the curriculum of the faculty of medicine. A workshop. Unpublished document.
8. Ministry of Health. 1983. Project of Jimma Institute of Health Sciences. Addis Ababa. Amharic version.
9. Department of Community Health Jimma Institute of Health Sciences. 1987. Community-based training programme manual, part I. Jimma.
10. Department of Community Health, Jimma Institute of Health Sciences. 1988. Team Training Manual, part II. Jimma.

11. Department of Community Health, Jimma Institute of Health Sciences. 1996. Manual for Student Research Project. Jimma.
12. Woodward CA. Some reflections of evaluation of outcomes of innovative medical education programmes during the practice period. Annals of Community Oriented Education, 1992;5:181-191.
13. Kamien M, Boelenc, Heck J. Measuring the social responsiveness of medical schools. Education for Health, 1999;12:9-19.
14. Moja EA, Ghetti V. Assessing performance of medical schools. Annual of Community-Oriented Education, 1995;8:247-253.
15. Seefeldf M. Evaluating community based health programs. In: Schmidt H, Magzoub M, Feletti G, Noomanz, Vluggen P, eds. Handbook of community-based education: theory and practices. Maastricht: Network Publications, 2000:345-360.
16. Kamien M. Responding to society needs: one criterion in evaluating the education of general practitioners. Education for Health, 1996;9:147-153.
17. Mennin S, Woodward CA. Evaluating innovative medical education programmes: common questions and problems. Annals of Community-Oriented Education, 1992;5:123-133.
18. Rossi PH, Freeman HE, Lipsey MW. Evaluation, a systematic approach. 6th ed. Thousand Oaks: Sage, 1999:500.
19. Kumpusalo E, Neittaanmaki, Virjo I, Mattilak, Kujalas, Luhtala R, et al. Relevance of medical education to professional Needs of Doctors in Community-Oriented and traditional medical schools. Education for Health, 1996;9:167-178.
20. Das M, Murdoch J, Mpofu D, Bener A. The role of peer and self-assessment during experiential learning in Community Settings. Education for Health, 1996;9:239-249.
21. Streiner D and Norman G. Health measurement scales, a practical guide to their development and use. Oxford: OUP, 1989: 175.
22. Judd C, Smith E, Kidder L. Research methods in social relations. 6th ed. Fort Worth: Saunders College, 1991: 573.
23. Shi L. Health services research methods. Albany: Delmar, 1997:410.
24. Magzoub M, Schmidt H. Impact of community-based educational programs on graduates: recent findings and common problems. In: Schmidt H, Magzoub M, Feletti G, Noomanz, Vluggen P, eds. Handbook of Community-based education: theory and practices. Maastricht: Network publications, 2000:407-415.
25. Schmidt H, Dauphinee D, Patel L. Comparing the effects of problem-based and conventional curricula in an international sample. Journal of medical education, 1987;62:305-315.
26. Transitional Government of Ethiopia. Health Policy. Addis Ababa: 1993.
27. Hamad B. Community-oriented medical education. What is it? Annals of Community-Oriented education, 1991;4:129-138.

Annex 1: A sample of the study instruments

Graduates' areas of assessment

1. Clinical skill: it was measured through the following specific questions
   - 1.1 ability in making correct diagnosis (History taking, physical examination, and laboratory investigation)
   - 1.2 ability to do procedures (e.g. lumbar puncture, IV line, vein puncture, pleural tap etc.....)
   - 1.3 ability to make complete, accurate and clear patient records
   - 1.4 ability on patient management and follow-up
   - 1.5 Relationship, devotion and concern to patients

2. Epidemiological skills
   - 2.1 identify health problems of the community, rank in order of importance, identify factors associated with these problems and develop intervention based on local resources
   - 2.2 identify life patterns and occupation of the community and relationship with the environment
   - 2.3 determine priority health problems
   - 2.4 identify groups at risk
   - 2.5 conduct surveys for community diagnosis
   - 2.6 ability to relate problems of a patient with that of the community (Ability to see problems of a single patient in the context of the whole community)

3. Health management skill
   - 3.1 identify and correct any inadequacies in the health care facilities and in the community in the context of national health care system
   - 3.2 understand civil service regulations and government policy in health problems
   - 3.3 utilize appropriate resources

4. Communication skills
   - 4.1 prepare a report on the above tasks and present orally/writing
   - 4.2 use health information system in planning and evaluating health programmes
   - 4.3 practice health education/IEC
   - 4.4 elationship with professional colleagues his/her senior physicians and administration staff

5. Community involvement skills
   - 5.1 identify and consult with important people in the community to plan and implement health promotion based on local resources
   - 5.2 Communicate with the community

6. Team work skills
   - 6.1 collaborate in group work
   - 6.2 work as a leader of a health team, trainer, supervisor, assessor

7. Multisectoral skills
   - 7.1 work with others in community development
   - 7.2 intersectoral collaboration
   - 7.3 solicit assistance from government and private agencies

8. Learning and teaching skills
   - 8.1 recognize personal educational needs
   - 8.2 formulate a plan for learning
   - 8.3 ability and willingness to teach
   - 8.4 ability and willingness to learn

9. Research
   - 9.1 design and implement a community based research

10. Over all clinical competence compared to that of other medical schools graduate

11. Over all public health competence compared to that of other medical schools graduate

Several specific questions were also prepared to measure the influence and impact of CBE on graduates character and career.

ijd085

Report

Pattern of skin diseases at the University Teaching Hospital, Addis Ababa, Ethiopia

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Abstract

Background and objective Skin diseases are among the leading causes of hospital visits in Ethiopia. An analysis was performed to describe the pattern of skin diseases at a teaching hospital in Addis Ababa, Ethiopia.

Patients and methods All consecutive new patients seen at the dermatologic referral clinic of Black Lion Teaching Hospital (BLH) from June 1995 to July 1997 were included in this prospective analysis. The clinical examinations were performed by the author, so that uniformity of diagnosis and classification is assured.

Results A total of 1505 patients were assessed; 1093 (72.6%) were in the age group 21–40 years, and the female to male ratio was 1.2 to 1. The most common skin diseases were allergic skin diseases (25.5%), infections (25.4%), the photodermatoses (22.9%), followed by papulosquamous diseases (11.4%) and pruritis of unknown origin (3.3%). Skin manifestations of collagen diseases were less common findings (0.7%).

Conclusions The pattern of skin diseases observed in this analysis shows that allergic and infectious causes account for three-quarters of skin problems. Emphasis should be given to this group of common skin diseases at all levels of health care so that proper diagnosis, treatment, and referral can be practiced.

Introduction

It is essential to determine the prevalence/incidence of skin diseases in a community because it provides, among other things, an index of community development. There may, however, be diversity in definitions, classification, and diagnosis as different dermatologists have variable opinions in these dimensions. This problem is especially pertinent in an Ethiopian setting as Ethiopian dermatologists have different schooling backgrounds, thereby necessitating a uniform approach to avoid ambiguity in their work or practice. In this study, uniformity of evaluation is assured as the clinical examinations were performed by the author. Although single spot surveys may be of limited value, they still provide a clue to future multicenter research plans. Several similar studies have been performed in Ethiopia: 6500 skin diseases were evaluated by Schaller[1] in 1963 in Ethiopia, 1087 skin diseases were evaluated by Manzur and Seboxa[2] in Addis Ababa in 1984, and a total of 1521 patients were evaluated in Gondar Administrative Region by Sochor *et al.*[3] in 1986. In general, these studies documented that skin diseases were prevalent in the country and that allergic and infectious skin diseases were the commonest problems identified.

Materials and methods

This work reports a prospective study of patients seen at Black Lion Teaching Hospital (BLH) dermatology clinic from June 1995 to July 1997. Only Ethiopian patients attending the clinic for the first time and subsequently were included in the study. Patients with venereal diseases and leprosy, and inpatients, were excluded from the study. The patients were mostly from Addis Ababa. Sex, age, and the results of diagnoses were recorded.

The percentages given are based on the number of diseases and not on the number of patients because some of the patients had more than one disease.

Laboratory investigations and biopsy were performed when necessary. Skin tests were not performed. Allergic skin diseases and contact dermatitis were diagnosed on clinical grounds only and, when necessary, provocative tests were carried out.

Results

The total number of patients was 1505 and the number of skin diseases was 1748. Around 75% of patients were in the age group 21–40 years, and the male to female ratio was 1:1.2. The pattern and frequency of skin diseases are shown in Tables 1–5. Allergic skin diseases were observed

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Table 1 Skin disease patterns and frequency

Disease	No.	%
Allergic skin diseases	445	25.5
Infections	444	25.4
Photodermatoses	400	22.9
Papulosquamous diseases	205	11.7
Acne	44	2.5
Psoriasis	16	0.9
Pruritus	181	10.4
Total	1745*	100

*Some patients have more than one diagnosis.

Table 2 Patterns of allergic skin diseases

Allergic disease	No.	%
Dermatitis (386, 66.5%)		
Neurodermatitis	98	25.4
Contact dermatitis	71	18.4
Atopic dermatitis	57	14.7
Nipple + nummular dermatitis	46	11.9
Leg dermatitis	6	1.6
Dyshidrosis (pompholyx)	3	0.8
Unclassified dermatitis	105	27.2
Urticaria (34, 7.6%)		
Drug reaction (25, 5.8%)		

in 25.5% (426) of cases. Neurodermatitis and contact dermatitis form the most prevalent groups (25.4% (98) and 18.9% (71), respectively), followed by atopic dermatitis (14.7%, 57) (Table 2).

Nipple and nummular dermatitis were diagnosed in 36.3% (46) of cases, while leg dermatitis and dyshidrosis were found in 3.8% and 0.7%, respectively. Unclassified dermatitis was seen in 23.5% (105) of cases. Drug reactions, consisting of a mild maculopapular eruption and fixed drug eruptions, were observed in 5.8%, and urticaria in 7.6% of cases.

Infections were the next most common presenting problems (Table 3). Fungal infections were the most common, occurring in 61.5% of cases. Dermatophytosis was the most common fungal infection (77%) in our outpatient dermatology clinic, followed by pityriasis versicolor (13.6%). Bacterial infection accounted for 18.7% of all infections: impetigo (27.7%) was commonly observed, followed by pyoderma, furunculosis, and tropical ulcer. Viral infection was seen in 15.8% of cases, consisting of herpes zoster (50.0%), verrucae (31.4%), molluscum contagiosum (14.3%), and herpes simplex (4.3%).

Table 3 Patterns of skin infections

Infection (444, 25.4%)	No.	%
Fungal (273, 61.5%)		
Tinea faciei	31	7.0
Tinea corporis	27	6.1
Tinea cruris	21	4.7
Tinea capitis	11	2.5
Tinea manuum	6	1.4
Tinea pedis (plantis)	3	0.7
Pityriasis versicolor	60	13.5
Candidiasis	29	6.5
Bacterial (83, 18.7%)		
Impetigo	23	27.7
Pyoderma	21	25.3
Furunculosis	16	19.3
Tropical ulcer	9	10.8
Erysipelas	7	8.4
Tuberculosis of the skin	6	7.2
Leprosy	1	1.2
Viral (70, 15.8%)		
Herpes zoster	35	50.0
Condyloma acuminata	12	17.1
Molluscum contagiosum	10	14.3
Verruca vulgaris	8	11.4
Herpes simplex	3	4.3
Verruca plana	2	2.9
Infestations (18, 4%)		
Scabies	14	77.8
Onchocerciasis + helminthiasis	4	22.2

Photodermatosis was a fairly common finding (Table 4). Of the total patients seen, it accounted for 22.9% of cases. Most patients reported late in the disease course with residual hyperpigmentation (14.2%). Of the photodermatoses, the most commonly observed cases were found to be polymorphic light eruption.

Of the papulosquamous diseases, seborrheic dermatitis was observed in 50% of cases, acne in 26%, rosacea in 25%, and psoriasis in 4% (Table 5). Pruritis of unknown origin and other dermatologic skin diseases were observed in 10.8% of cases.

Discussion

The BLH dermatology clinic is the University's referral clinic in Addis Ababa. Patients are seen by internists and other specialists and straightforward cases are treated by them. Those referred to the dematology clinic are either refractory to treatment, or those difficult to diagnose. The highest number of cases observed were allergic skin diseases. This finding correlates well with studies in Gondor[3] and Kenya.[4] Of the allergic skin diseases seen, dermatitis is predominant, in contrast to a study in Nigeria

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Table 4 Patterns of photodermatosis

Photodermatosis (400, 22.3%)	No.	%
Polymorphic light eruption	326	80.0
Hyperpigmentation	57	14.2
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

Table 5 Frequency of papulosquamous skin diseases

Papulosquamous disease (200, 11.1%)	No.	%
Seborrheic dermatitis	60	30.0
Acne	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
Pityriasis rosea	50	25.0
Lichen planus	30	15.0
Psoriasis	8	4.0

where dermatitis is rarely observed.[3,4] A breakdown of the dermatitis figures shows that lichen simplex (neurodermatitis) is the most common, followed by contact dermatitis (both allergic and irritant types) and atopic dermatitis. The reason for the high prevalence of neurodermatitis is not clear. The incidence of contact dermatitis is frequent as in previous studies.[3-4,6] Urticaria and drug reaction were fairly common. The most common drug reactions were maculopapular eruption, followed by fixed drug eruption and urticaria.[5,6]

Infectious skin diseases were the next most common finding in this study. Such a high occurrence is similar to studies in other African countries.[2-4,6] Of the infectious diseases, superficial fungal infection was predominant. There is no satisfactory explanation for the high preponderance of dermatomycosis in both this study and a Nigerian study,[4,5] but the hot, humid environment is considered to be a predisposing factor and the disease is therefore very common in tropical countries.[7] In adult patients, tinea corporis is predominant, whereas in children tinea capitis is most common.[7,9] Tinea pedis and onychomycosis are fairly common in Ethiopian patients, in contrast to the study in Nigeria and a previous report by Manzur.[2,6] Pityriasis versicolor is a common finding in Ethiopia (13.6% of the total cases of superficial fungal infection). This finding correlates with similar findings in Malawi: 8% of the study group had extensive pityriasis versicolor, while 9.9% had a mild form of pityriasis versicolor.[10] Pityriasis versicolor is caused by a normal flora of the human skin (*Malassezia furfur*). Why this normal flora becomes pathogenic is not well understood, but a hot, humid environment is considered to be a predisposing factor and the disease is very common in tropical countries.[11]

Bacterial and viral infections are more or less equally represented. One can speculate about the reasons for the large number of bacterial infections:[2,6] heat, humidity, poor personal hygiene, absence of drugs and antiseptics, and inappropriate traditional treatment. The high incidence of viral infection may be due to the spread of human immunodeficiency virus (HIV) infection. There is an unusually high prevalence of herpes zoster in young patients.

The low incidence of scabies in the referral clinic is due to the elimination of cases by internists and residents, and the early diagnosis and prompt treatment. Otherwise, the incidence of scabies in the population is high,[1,6] which correlates with studies elsewhere.[2-5]

Photodermatoses, especially polymorphic light eruption and acquired facial hyperpigmentation, were frequently observed.[5,6,12] The reason for such a high number of cases may be that the patients were seen in the tertiary referral clinic. The causes of hyperpigmentation are identified from circumstantial evidence of exposure to known agents: hydroquinone-induced exogenous ochronosis from skin-bleaching creams, mercury deposits from mercury-containing skin-lightening soaps and creams, sulfonamide-related drugs, antimalarials, fixed drug eruptions, clofazimine, and photosensitive herbal applications. There are a number of photosensitizers that cause photodermatoses when applied to areas exposed to sunlight or ultraviolet light, such as the face, the neck, V-surface of the chest, the extensor surfaces of the forearms, and dorsum of the hands.[13] Such substances include vaseline, Whitfield's ointment, sulfonamide-containing ointments or powder, neomycin, Phenergan cream, and other antihistamines. Detailed investigation was performed to determine systemic causes of porphyria cutanea tarda, systemic lupus erythematosus, etc., but no evidence was detected. Earlier studies have shown that hyperpigmentation of the face and dorsum of the hands, with or without blisters, as a result of porphyria cutanea tarda is common in Ethiopia;[2,14] however, such observations were found in only 1.8% of cases in this study. The reason for the decline in incidence could be due to the fact that these patients were screened out by the internists who had the change to see them first in the general referral outpatient clinic. Systemic lupus erythematosus and systemic scleroderma are not uncommon diseases in our referral clinic.

Of the papulosquamous diseases, seborrheic dermatitis, psoriasis, rosacea, and acne were frequently seen. While

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the high incidence of seborrheic dermatitis may be attributed to the HIV pandemic (as more patients are referred to the clinic because of the chronic nature of the disease), the reason for the high occurrence of rosacea is difficult to explain. Rosacea is more common in middle-aged women and above. The high incidence of acne and lichen planus agrees with the observations of other studies in Ethiopia [illegible] the Nigerian study. In contrast it usually subsides, and heals by leaving hyperpigmentation, which resolves to a normal color after many months or years. In contrast to the observations in Nigerian patients, mouth lesions specific for the disease (lichen planus) have been found in Ethiopian patients.

Psoriasis is fairly uncommon in Ethiopia in contrast to its rarity in the Nigerian study.[5] Previous studies in Ethiopia have also shown a high occurrence of psoriasis.[1–3,6] Such high ocurrences have been reported in sub-Saharan Africa[15] and even in Togo.[16] The reasons for such a high occurrence should be studied.

The complete absence of basal cell carcinoma in this study provides strong support for the protection of melanin against the mnelanin against the carcinogenic action of ultraviolet light in Africans.[2,5]

Conclusions

The prevalence of skin diseases inethiopian patients seen at the dermatologic clinic of the University College Hospital, Addis Ababa, Ethiopia, from June 1995 to July 1997, was presented. More than 60% of patients were in the age group 20–40 years. Allergic skin diseases were the most frequently observed (25.5%), followed by infections (25.4%). Photodermatoses of the face were frequent (12.9%) and papulosquamous diseases were seen in 14.4% of cases. Explanations for the occurrence of the disease patterns were provided.

Most of the skin diseases can be categorized into four groups: allergic skin diseases, infections, photodermatoses, and papulosquamous diseases. More than half of the diseases were allergic skin diseases and infections. Therefore, teaching of health personnel at all levels should emphasize these groups of common skin diseases, such that correct diagnoses and treatment can be achieved at the peripheral centers, and only a minimum of patients need to be referred to specialized centers where dermatologists are available.

References

1 Mariam J, Sebsibe T. Skin diseases in Addis Ababa. *East Afr Med J* 1986; 63: 75–80.

2 Sachdeva H, Sebsibe T, Mariu M. Pattern of skin diseases in Gondar Administrative Region. *Ethiop Med J* 1986; 24: 191–192.

3 Patel RI. Skin diseases in Nairobi: a preliminary review. *East Afr Med J* 1966; 43: 530–532.

4 Shrank AB, Harman RRM. The incidence of skin diseases in a Nigerian Teaching Hospital Dermatologic Clinic. *Br J Dermatol* 1966; 78: 235.

5 Manzur J. Skin diseases in Addis Ababa. A preliminary review. *Ethiop Med J* 1981; 19: 123–134.

6 Shrum JP, Milikan LE, Bataineh O. Superficial fungal infections in the tropics. *Dermatol Clin* 1994; 12: 683–693.

7 Schachner LA, Hansen RC. *Paediatric Dermatology*. Vol. 2. Edinburgh: Churchill Livingstone, 1988.

8 Fitzpatrick TB, Eisen AZ, Wolff K *et al. Dermatology in General Medicine*, 3rd ed.

9 Ponnighaus JM, Fine PE, Saul J. The epidemiology of pityriasis versicolor in Malawi, Africa. *Mycoses* 1956; 39: 11–12.

10 Aljabre SH, Sheikh YH. Penile involvement of pityriasis versicolor. *Trop Geogr Med* 1994; 46: 184–185.

11 Olumide YM, Odunowo BD, Odiase AO. Regional dermatoses in Africa. Part I. Facial hypermelanoses. *Int J Dermatol* 191; 30: 186–189.

12 Warndorf JA. *Ethiop Med J* 1979; 16: 159–162.

13 Tsega E. Hyperpigmentation of the face and hands without blisters, in porphyria cutanea tarda. *Br J Dermatol* 1980; 103: 187–190.

14 Leder RO, Farber EN. The variable incidence of psoriasis in sub-Saharan Africa. *Int J Dermatol* 1997 36: 911–919.

15 Napo-Krura G, Pitchie B, Tchangae-Wala K. Non-tumor dermatoses diagnosed in the pathologic anatomy laboratory of the Tokoin University Hospital Centre, Lome (Togo). *Santé* 1997; 7: 75–80.

Stein DH. Fungal, Protozoan, and Helminth Infections. In (Schachner LA, Hansen RC, 2nd ed. Paediatric Dermatology Vol 2 Churchil LiVingStone Inc, 1988, 1295–1345

Martini AG, Kobayashi GS. Superficial fungal infection: Dermatophytosis, Tinea Nigra, Piedra. In (Fitzpatrick TB, Eisen Wolf K et. al. 4th ed. Dermatology in General Medicine, Vol 1 McGraw-Hill Inc, 1993, 2421–2451.

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Pattern of Skin Disease at the Ethio-Swedish Pediatric Hospital, Addis Ababa, Ethiopia

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Abstract: Although skin diseases are common in children, only a very few prospective studies are available, in the literature, mainly from developed countries. This work reports the pattern of skin diseases in a tertiary referral hospital in Addis Ababa, Ethiopia, from June 1995 to July 1997. A total of 1000 consecutive new pediatric patients, ages 0–12 years, were studied. The male:female ratio was 1:1. Allergic skin diseases were most frequently found (55%), followed by infections (33%) and photodermatosis (6%). Of the allergic skin diseases, atopic dermatitis was the most prevalent (47%), followed by seborrheic dermatitis (17.4%).

Allergic and infectious diseases are the major skin problems among children, and emphasis should be focused on these conditions during the training of health care workers at all levels, especially primary care physicians. It is essential to know the incidence of skin disease in a community because it gives information helpful for the planning of manpower development and budget allocation. Although a single spot survey may be of limited value, it may still aid in the planning of further multicenter research. Very few similar studies of children have been performed in Ethiopia and elsewhere (1–5). In our study, 1346 skin diseases were evaluated in 1000 children, ages 0 to 12 years.

MATERIALS AND METHODS

This article reports a prospective study of patients seen at the Ethio-Swedish pediatric clinic, Addis Ababa, Ethiopia, from June 1995 to July 1997. All new Ethiopian patients attending the pediatric dermatology clinic were included in the study. The percentages given are based on the number of diseases, not the number of patients, because some of the patients had more than one disease. All patients were seen by the author.

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Laboratory investigations and biopsy specimens were taken when necessary. Skin tests were not performed and allergic skin diseases and contact dermatitis were diagnosed on clinical grounds only. When necessary, provocative tests were performed.

RESULTS

The total number of patients was 1000, while the number of skin diseases was 1346. Patients ranged in age from birth to 4 years (45%) and from 5 to 8 years (37%) (Table 1).

The patterns of skin disease are shown in Table 2. Allergic skin disease (Table 3) was observed in 55% of patients, followed by infections (33%) Table 4). The allergic skin diseases consisted of atopic dermatitis (47%), and contact dermatitis together with diaper rash (5% of cases). Seborrheic dermatitis was the second most observed disease in those patients less than 4 years of age (17%), followed by hand and foot dermatitis (8%) and pityriasis alba (6.3%). Other allergic diseases observed included nummular eczema, strophulus, insect bite hypersensitivity, and unclassified eczema.

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PDE1793

TABLE 1. *Age Distribution of Patients*

Age (years)	Number of patients	Percentage
0–4	451	45.1
5–8	369	36.9
9–12	180	18.0
	1000	

DISCUSSION

The Ethio-Swedish pediatric dermatologic clinic is the university referral clinic in Addis Ababa, Ethiopia. Patients are seen first by pediatric residents and uncomplicated cases are managed by them. Patients referred to our dermatology clinic include those refractory to treatment, those in whom there is difficulty in establishing the diagnosis or who have recurrent, chronic, or relapsing disease.

Allergic skin disease was the most common pediatric skin disorder (1,2). Eczema, fungal and bacterial infections, parasitic infestations, viral infections, and photodermatosis, mainly polymorphic light eruption with or without residual hyperpigmentation, were significantly observed (1–5). Tinea capitis, impetigo, and viral warts were the common infections in children. Acne vulgaris, psoriasis vulgaris, erythema nodosum, and keloid were uncommon presentations (1,4,6). Pityriasis alba was a common finding (2,4). Atopic dermatitis tended to begin in infancy over the face and extensor surfaces of the extremities. As the child grew toward adolescence, it involved the flexor surfaces of the extremities (7,8).

Endogenous eczema (including atopic dermatitis and seborrheic dermatitis) was the most common eczema, with a tendency to decrease with age, whereas the frequency of nummular eczema, lichen simplex, pityriasis alba, and unclassified eczema tended to increase with age (1,2). Pityriasis alba is observed more clearly in our patients because the hypopigmented macules are more evident on dark skin (1). The center of some of these lesions becomes hyperpigmented, with a hypopigmented halo at the margin. This occurs in patients with tinea capitis or in patients who have been treated with Vaseline or Whitfield ointment and exposed to sunshine (9). The former patients have good results with griseofulvin treatment

TABLE 2. *Pattern of Pediatric Skin Diseases*

	Number of patients	Percentage
Allergic skin diseases	741	55.1
Infections	441	32.8
Photodermatoses	108	8.0
Miscellaneous	56	4.2

TABLE 3. *Allergic Skin Diseases*

	Number of patients	Percentage
Eczema		
Atopic dermatitis	337	46.9
Contact dermatitis + diaper rash	33	4.6
Nummular eczema	12	1.7
Lichen simplex chronicus (neurodermatitis)	4	0.6
Strophulus	19	2.6
Seborrheic dermatitis	125	17.4
Insect bite hypersensitivity	28	3.9
Hand and foot dermatitis	56	7.8
Pityriasis alba	45	6.3
Unclassified eczema	59	8.2
Urticaria	15	2.0
Drug reaction	8	1.1
Maculopapular rash	5	
Fixed drug eruption	3	

per os and combined topical salicylic acid and hydrocortisone cream. The latter patients are treated as cases of phototoxic contact dermatitis. Contact dermatitis is rare in children and some of the cases identified were mostly irritant contact eczema. This could be explained by the fact that allergen exposure is less frequent and the immune system is not yet well developed.

Infections were the next most common diseases diagnosed. Fungal infections were commonly observed (44%), followed by bacterial infections and parasitic infestations (30% and 17%, respectively) (4,10). Viral infections were seen in 10% of cases (2,4). Tinea capitis was the most commonly isolated fungal infection, followed by tinea corporis and faciei (9,11). *Trichophyton violaceum* was isolated in most cases (9,11). Of the bac-

TABLE 4. *Infections*

	Number of patients	Percentage
Fungal	193 (of 441)	43.8
T. capitis	106	54.9
T. faciei	20	10.4
T. corporis	36	18.7
T. unguium	14	7.3
T. pedis	8	4.1
Candidiasis	9	4.7
Bacterial	131 (of 441)	29.7
Impetigo	78	59.5
Pyoderma (abscess)	33	25.2
Folliculitis	13	9.9
Ecthyma	7	5.3
Infestations	74 (of 441)	16.8
Scabies	54	72.9
Pediculosis	15	20.3
Cutaneous leishmaniasis	5	6.8
Viral	43 (of 441)	9.8
Verrucae	28	65.1
Molluscum contagiosum	13	30.2
Herpes zoster	2	4.7

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Amir sultan (MD), 2014

terial infections, impetigo and other pyodermas were the predominant skin diseases. Infantile infections are increased due to the immaturity of the immune system, frequent skin-to-skin plus skin-to-fomite contact, and poor hygiene. As the child matures these factors become less significant (1,2,4,10).

Of the infestations, the numbers for scabies and pediculosis are low (4,10,12,13) compared to those of studies elsewhere. The reason for this is that most cases would have been treated by general practitioners, residents, or other specialists.

Verrucae and molluscum contagiosum are the next most common viral infections of children (1–5). These cases are also underreported, as most patients don't come to the hospital for treatment and some are advised by different specialists not to seek treatment, as these lesions resolve spontaneously.

Photodermatosis was a common finding in children (Table 5). Polymorphic light eruption was frequently observed in children 4–12 years of age. This is because the children go to school (kindergarten or primary school) and often walk long distances in daylight (14,15). Phototoxic contact dermatitis was seen most frequently in those about 8 years of age. These older children frequently apply Vaseline or Whitfield ointment for treatment of pityriasis alba or for any scaling lesions on the face, which they consider as fungal infections and therefore develop phototoxicity. Miscellaneous skin diseases seen uncommonly among our population of pediatric patients are indicated in Table 6.

CONCLUSION

The main skin diseases are of few categories —allergic skin diseases, skin infections, and infestations including viral infection and photodermatosis. It seems necessary therefore to ensure that the dermatologic education of residents in this university pediatric hospital focus on accurate recognition, diagnosis, and management of these common skin diseases.

TABLE 5. *Photodermatosis*

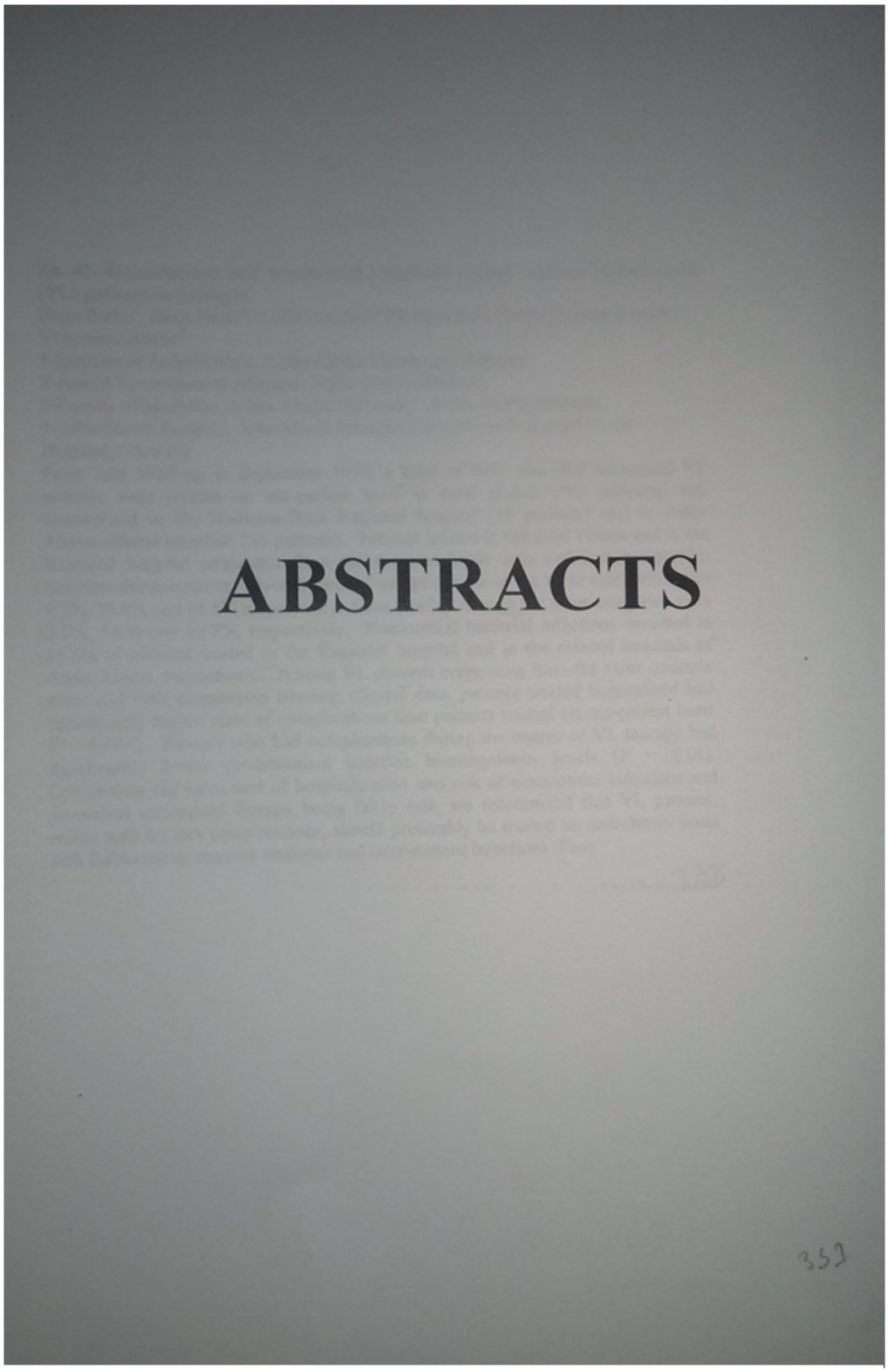
Polymorphic light eruption	98	90.7
Actinic prurigo	2	1.9
Phototoxic/allergic contact dermatitis	8	7.4

TABLE 6. *Miscellaneous*

Vitiligo	10	17.9
Ichthyosis vulgaris	8	14.3
Acne	5	8.9
Psoriasis vulgaris	4	7.1
Erythema nodosum	3	5.4
Keloid	3	5.4
Pityriasis rosea	2	3.4
Pruritus	21	37.5

REFERENCES

1. Goh CL, Akarapanth R. Epidemiology of skin disease among children in a referral skin clinic in Singapore. Pediatr Dermatol 1994;11:125–128.
2. Nanda A, Al-Hasawi F, Alsaleh QA. A prospective survey of pediatric dermatology clinic patients in Kuwait: an analysis of 10,000 cases. Pediatr Dermatol 1996;16:6–11.
3. Bowker NC, Cross HW, Fairburn EA, Wall M. Sociological implications of an epidemiological study of eczema in the city of Birmingham. Br J Dermatol 1976;95:137–144.
4. Figueroa JI, Fuller LC, Abraha A, Hay RJ. The prevalence of skin disease among school children in rural Ethiopia. A preliminary assessment of dermatologic needs. Pediatr Dermatol 1996;13:378–381.
5. Krowchuk DP, Bradham DD, Fleischer AB. Dermatologic services provided to children and adolescents by primary care and other physicians in the United States. Pediatr Dermatol 1994;11:199–203.
6. Al-Fouzan AS, Nanda A. A survey of childhood psoriasis in Kuwait. Pediatr Dermatol 1994;11:116–119.
7. Hanifin JM. Atopic dermatitis. J Allergy Clin Immunol 1984;73:215–222.
8. Rothe MJ, Grant-Kels JM. Atopic dermatitis. An update. J Am Acad Dermatol 1996;35:1–13.
9. Shibeshi D. Tinea capitis in an NGO established for care of children in the Ethiopian capital city Addis Ababa (unpublished).
10. Kottenhahn RK, Heck JE. The prevalence of pediatric skin diseases in rural Honduras. Trop Doct 1994;24:87–88.
11. Figuera JA, Hawranek T, Abraha A. Tinea capitis in southwestern Ethiopia: a study of risk factors for infection and carriage. Int J Dermatol 1997;36:661–666.
12. Mahe A, Cisse IA, Ousmane F, N'Diaye HT, Niamba P. Skin diseases in Bamako (Mali). Int J Dermatol 1998;37:673–676.
13. Sunder KO, Haimanot AT. Epidemic of louse-borne relapsing fever in Ethiopia. Lancet 1993;342:1213–1215.
14. Shrank AB, Harman RRM. The incidence of skin diseases in a Nigerian teaching hospital dermatologic clinic. Br J Dermatol 1966;88:235–241.
15. Manzur J. Skin diseases in Addis Ababa. A preliminary review. Ethiop Med J 1981;19:123–134.


ABSTRACTS

Ab 41- Inter-current and nosocomial infections among visceral leishmaniasis (VL) patients in Ethiopia

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4-Arba-Minch Hospital, Arbr-Minch Ethiopia (currently at Rogaland Center Hospital, Norway)

From July 1989 up to September 1997, a total of 247 non-HIV associated VL patients were treated on out-patient basis in rural clinics (195 patients), and hospitalised in the Northern-Omo Regional hospital (18 patients) and in Addis Ababa referral hospitals (34 patients). Patients treated in the rural clinics and in the Regional hospital originated from the same endemic area and had comparable baseline characteristics. Overall rates of complications in the three categories were 9.7%, 38.9% and 61.6% respectively. Case fatality rates in the three categories were 2.5%, 5.6% and 11.7%, respectively. Nosocomial bacterial infections occurred in 16.6% of patients treated in the Regional hospital and in the referral hospitals of Addis Ababa, respectively. Among VL patients originating from the same endemic place and with comparable baseline clinical data, patients treated hospitalised had significantly higher rates of complications than patients treated on out-patient basis ($P < 0.001$). Patients who had complications during the course of VL therapy had significantly lower pre-treatment baseline haemogolobin levels ($P = 0.01$). Considering the extra cost of hospitalisation and risk of nosocomial infections and petavelent antimonial therapy being fairly safe, we recommend that VL patients, unless with serious complications, should preferably be treated on ambulatory basis with follow-up to monitor response and inter-current infections if any.

Ab 39- Periodontal Disease and Dental Caries in Ethiopian Diabetic Patients, Tikur Anbessa Hospital (TAH), Addis Ababa, Ethiopia

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2-Mikias Dental Clinic, Addis Ababa, Ethiopia

This was a cross sectional study conducted to asses the prevalence of dental and periodontal problems in diabetic patients, and to asses the relation between dental and periodontal problems to the duration of diabetes, degree of control, type of diabetes, and oral health practices. Every third patient was selected by systematic random sampling technique from the registration book among patients coming for the regular follow up during the study period at the diabetic referral clinic of TAH. A physician recorded details of information in the questionnaire and examination of the oral cavity was conducted by a dentist. General hygienic condition, plaque score, periodontal indices, gingivitis score, degree of mobility, gingival recession. Probing depth, severity of bone loss and dental caries were defined according to the standard definition. A total of 649 diabetic patients were involved in the study. Among these 309 (47.6%) were males, 340 (52.4%) were females, 324 (49.9%) had Type 1 and 325 (50.1%) had Type 2 diabetes mellitus. The mean ± SD age was 42.0 ± 15.6 yrs. The mean duration of diabetes was 10.2 ± 6.6 years. 518 (79.6%) patients were on insulin, 121 (18.6%) were on oral agent and 12 were on diet. The prevalence of dental caries was 23% and gingivitis 96.1%, plaque 94.1%, gingival recession in 93.3%, abnormal mobility in 19%, bone loss in 47%. 20.5% of patients lost incisors. 5.9% canine, 17.0% premolar and 56.6% molar teeth. The mean duration of diabetes was significantly associated with general hygienic condition of the oral cavity, gingival recession, probing depth, severity of bone loss (p=0.01, p=0.0009, p=0.0002, p=0.02 respectively). There was no significant association between the mean fasting blood sugar and periodontal disease and dental caries (P>0.05). There was significant association between the type of diabetes and periodontal disease (P<0.05). There was significant association between the use of tooth brush and general hygienic condition of the teeth, plaque formation (p=0.01, p=0.28 respectively), but no significant association between gingivitis, probing depth, severity of bone loss and dental caries (p>0.05). In conclusion periodontal diseases are severe and more common in diabetes. We recommend strengthening health education about the oral health care practice and organizing an effective preventive and management dental service for diabetes.

ANALYSIS OF THYROID "COLD" NODULES AT TIKUR ANBESSA HOSPITAL A68

Solomon Damena

One hundred and forty-eight patients with solitary "cold" nodules which were investigated in our Nuclear Medicine Unit from October 1984 to October 1991 were reviewed. Only sixty-four patients have undergone surgical biopsy and/or fine needle aspiration cytology. The female to male ratio is 2.2:1 and the median age is 35 years. Histopathological findings revealed forty-two percent of the "cold" nodules were thyroid adenoma while thirteen percent were thyroid carcinoma. Of the eight patients with thyroid carcinoma, four had the papillary type. Histologically twenty-two and twenty-three percent of the solitary "cold" nodules revealed nodular colloid goitre and cystic degeneration respectively. Our study showed that there are fewer "cold" nodules which are histologically malignant than in the areas non-endemic for goitre. In addition, there are greater numbers of nodular colloid goitres and cystic degenerations.

RHEUMATIC HEART DISEASE: PREVALENCE AMONG SCHOOL-CHILDREN OF AN ETHIOPIAN RURAL TOWN

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**Department of Neurology, Umea University, Umea, Sweden

Rheumatic heart disease (RHD) is the most important cardiovascular disease in hospital practice in Ethiopia. However, the prevalence rate of RHD at the community level remains unknown. The current survey was undertaken to estimate its prevalence among school-children of a central Ethiopian rural town (Butajira). Accordingly, 92.6% of all the school children (n=3,235) in the town were screened. Their mean age was 13.4±3.5 years. Fifteen children, accounting for a rate of 4.6/1000, were noted to have findings consistent with RHD. Females and older children were more frequently affected. Only 2 of the 15 students were aware of their disease, and neither of them were on regular secondary prophylaxis. Our finding is consistent with the hospital-based reports that showed RHD to be a common disorder in clinical practice. At the same time, the lack of awareness about their disease noted among the cases is worrisome and deserves serious attention.

Abstract No 3

NEUROLOGICAL ASSESSMENT OF A COHORT OF CHILDREN BORN TO MOTHERS WITH LEPROSY AND HEALTHY CONTROLS (A9 STUDY) - 1. CLINICAL AND CONVENTIONAL TESTS

M. E. Duncan (1, 2), M. Hungnaw (3), H. S. H/Mariam (3), L. Selassie (2), Z. Melaku (4), R. Kazen (3), A. Challis (5)

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In a prospective (1975-1997) study (A9) of mothers with leprosy and healthy controls, and their children, 15/99 of children were found to have very early leprosy at puberty (1990). Despite MDT 13/15 showed new nerve damage (1993). In 1993, 40% of the A9 cohort who had easily palpable/slightly enlarged nerves without suspicious skin lesion showed early neurodysfunction. The aim of this study was to investigate if early nerve enlargement was a prodromal sign of leprosy.

Subjects: A9 cohort children: 85 (51 females and 34 males); distribution according to leprosy status of the mother multibacillary leprosy (MBK:47), paucibacillary leprosy (PBK:22) and non-leprosy (NLK:16). [K=kids!]. Control group: no known family or household leprosy contact (NKLCK:18).

Nerve enlargement: The A9 cohort had 45% with early/definite nerve enlargement (MBK 55%, PBK 36%, NLK 54%) compared with 25% NKLCK. Male:female ratio for nerve enlargement was 25:17, except in MBK (11:12). The ulnar nerve was the most frequently enlarged.

Sensory and motor nerve impairment (graded sensory testing (STG) and voluntary muscle testing (VMT)): A9 cohort had i) 32% sensory nerve impairment (MBK 40%, PBK 29%, NLK 14%) compared with NKLCK 13%, and ii) 12% motor nerve impairment (MBK 12%, PBK 14%, NLK 7%) compared with NKLCK 7%.

Nerve Conduction Velocity: measurements in 30 children who had evidence of neurodysfunction were all within limits of normal for the reference laboratory.

Am. Rev. Resp. Dis 143: A719
1991 (suppl 4 pt 2)

COMMUNITY ACQUIRED PNEUMONIA IN ADULTS IN ADDIS ABABA - ETIOLOGIC AGENTS AND THE IMPACT OF HIV INFECTION. A. Getachew, Black Lion Hospital, Addis Ababa University, Ethiopia.

Community acquired pneumonia (CAP) is the second most common cause of adult medical admissions in Addis Ababa and accounts for 17% of deaths. The etiologic agents of CAP in this setting have not been defined, but Strep. pneumoniae is believed to be the major pathogen. To determine the etiologic agents, and the impact of HIV infection, on CAP, we studied 110 consecutive patients between August 1987 and July 1989. All patients had a detailed clinical examination, chest X-ray, sputum gram and acid fast stain, serology for HIV and pneumococcal serotyping of sputa, sera and urine. 39 patients underwent transthoracic needle aspiration (TTNA). Acute and convalescent sera were studied for mycoplasma and common respiratory viruses in 42 patients. Cultures of blood and TTNA were performed but the results were not interpretable because of technical difficulties. Of the 110 patients, 62 were men and mean age was 35 years. A radiologic pattern of lobar or segmental consolidation was seen in 97%. Gram positive diplococci were the most important cause of CAP. Of 28 patients with gram positive diplococci on TTNA, 26 (93%) had the same organisms in their sputa.

RESULTS OF GRAM STAIN: SPUTA AND LUNG ASPIRATE IN 110 PATIENTS

	Sputum		Lung Aspirate	
	No.	%	No.	%
Gram positive diplococci	72*	65	28	72
Mixed organism	12	11	-	-
Gram negatives	3	3	1	2
Gram positive clusters	3	3	-	-
No bacteria but pus cells	-	-	10	26
No sputum	17	15	-	-
AFB	1	3	-	-

(* All or predominant gram positive diplococci)

The results of serologic assays for pneumococcal typing, CFT and HIV are pending. From these initial results we conclude that Strep penumoniae is the most important cause of CAP and that sputum gram stain is a rapid, inexpensive and reliable method of the diagnosis of CAP in this setting. (Supported by the Addis Ababa University Research and Publication Office and the Welcome Tropical Institute).

Abstract No 4

NEUROLOGICAL ASSESSMENT OF A COHORT OF CHILDREN BORN TO MOTHERS WITH LEPROSY AND HEALTHY CONTROLS (A9 STUDY) - 2. TESTS OF SMALL NERVE DYSFUNCTION

M. E. Duncan (1, 2), M. Hungnaw (3), H. S. H/Mariam (3), L. Selassie (2), Z. Melaku (4), R. Kazen (3), S. Hansen (5), G. A. Jamal (5), P. O. O. Julu (5), G. Ward (6), A. Challis (7)

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The aim of this study was to see whether neurodysfunction could be detected before irreversible nerve damage had occurred in the A9 cohort and healthy controls. The Thermal Threshold Tester (Triple-T: Medelec Ltd (see poster)) detects small nerve dysfunction in thinly myelinated Aδ (cold) and unmyelinated C (heat) fibres. The Laser Doppler blood flow meter (MicroFlo DSP: Oxford Optronix (see poster)) assesses function in autonomic (vasomotor) unmyelinated C fibres by recording skin blood flow response to inspiratory gasp (LD-IG) and cold challenge (LD-CC) in tips of index and fifth fingers.

Triple-T: The A9 group had significantly increased hot and cold thermal thresholds (MBK 70%, PBK 70%, NLK 75%) indicating small fibre damage compared with NKLCK (18%).

LD-IG and LD-CC: LD-IG showed abnormal traces with established and subclinical nerve damage. The fall from resting blood flow (baseline) to the lowest point of the curve in response to LD-IG and LD-CC is expressed as % of the baseline. The mean LD-IG and LD-CC % fall in index and fifth fingers showed no differences between A9 and control groups. The percentage of children having i) abnormal LD-IG were MBK 22%, PBK 19%, NLK 17%, NKLCK 37%; ii) abnormal LD-CC were MBK 38%, PBK 33%, NLK 36%, NKLCK 33%.

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4- Institute of Psychiatry, Umea University, Sweden

In an ongoing epidemiological study of course and outcome of schizophrenia and affective disorders a neurological study was done on 111 schizophrenic patients and 50 healthy controls using a Neurological Evaluation Scale (NES). Correlation of the neurological abnormalities with gender, age, history of treatment and duration of illness was done. It was found out that about 70% of the schizophrenic patients scored one or more abnormal neurological signs as compared to 25% in the healthy control group. There was no significant difference in the results in gender, age, history of treatment and duration of illness. Our result is consistent with the result of most other comparative studies.

AB5- Neurological Signs in Major Affective Disorders in Butajira-Ethiopia

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4- Institute of Psychiatry, Umea University, Sweden

In an ongoing epidemiological study of course and outcome of schizophrenia and affective disorders a neurological study was done on 51 cases of bipolar disorders, 30 cases of major depression and 50 healthy controls using a Neurological Evaluation Scale (NES). Correlation of the neurological abnormalities with gender, age, history of treatment and duration of illness was done. It was found out that about 73% of the bipolar disorder patients and 87% of cases of major depression scored one or more abnormal neurological signs as compared to 25% in the control group. There was no significant difference in the results in gender, age, history of treatment and duration of illness.

PREVALENCE OF LOW BACK PAIN AT AN AGRO-INDUSTRIAL COMMUNITY IN THE RIFT VALLEY

Z Meklau,* R T Haimanot,* G Shifera**
Addis Ababa and Nazareth, Ethiopia

ABSTRACT: A descriptive cross-sectional study is carried out at the Wonji Shoa Sugar Estate in the Rift Valley region of Ethiopia. A total of 1939 adults were surved with respect to Low Back Pain, LBP. The overall prevalence of LBP is 21.7 %. The prevalence is only slightly higher in males than in females. The highest frequency of low back pain (28.7 %) was found in the age group 50 years and above. The frequency of LBP is associated with duration of residence in the fluorotic Estate. 25.4 % of the Estate population seem to be completely ignorant the fluoride problems and the on going defluoridation program.

Keywords: Low back pain; Fluoride; Defluoridation program; Rift Valley; Ethiopia 347

PAN AFRICAN ASSOCIATION OF NEUROLOGICAL SCIENCES

ABSTRACT FORM

Title: LIMOGES EPILEPSY PROTOCOL RESEARCH TOOL IN TROPICAL COUNTRIES.

Author/s: Preux P.M., Druet-Cabanac M., Melaku Z., Bernet-Bernady P., Tapie Ph., Dumas M. and members of epilepsy research group

Centre: The institute of tropical Neurology (ITN), School of medicine, Under the auspices of Pan-African Association of Neurological Sciences (PAANS)

City/Country: Limoges, France.

Many studies have been carried out in Africa, but the most likely age-adjusted prevalence for active epilepsy in tropical countries as a whole, is between 10 and 15 in 1000 inhabitants. This is about twice the prevalence in western countries. Most of these studies have methodological biases due to lack of appropriate epidemiologic methodology and standardized diagnostic criterias.

Epilepsy was chosen as a major axis of research during the last meeting of PAANS in Addis Ababa (May 1994). Since then two important meetings have taken place during the Second International Congress of Tropical Neurology in Limoges (September 1994): a round table conference on epilepsy in the tropics and the annual meeting of the ILAE Commision for tropical diseases. All the participants of these meetings have proposed that the ITN take the responsibility of preparing a standardized protocol for epilepsy.

The protocol has four specific objectives:

- to develop a screening instrument for epilepsy in tropical countries,
- to describe the different types of epilepsy,
- to try to identify the etiologies of epilepsy,
- to assess the different aspects of treatment.

This Questionary is already applied in African countries. If it is supported by ILAE, it can be used worldwide. This protocol could be an important tool for reserch on epilepsy in tropical countries.

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Abstract No 5

ASSESSMENT OF THE A9 STUDY NERVE FUNCTION TESTS, HYPOTHESIS AND APPLICATIONS FOR LEPROSY ERADICATION

M. E. Duncan (1, 2), M. Hungnaw (3), H. S. H/Mariam (3), L. Selassie (2), Z. Melaku (4), R. Kazen (3), S. Hansen (5), G. A. Jamal (5), P. O. O. Julu (5), A. Challis (6)

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The prospective (1975-1997) study (A9) of mothers with leprosy and healthy controls, and their children, showed 15/99 of children with very early leprosy at puberty (1990). Despite MDT 13/15 showed new nerve damage (1993), while 40% of the A9 cohort with easily palpable/slightly enlarged nerves without suspicious skin lesion showed early neurodysfunction. In 1997 70% of the cohort had abnormal thermal thresholds, 32% had sensory impairment and 12% motor impairment. These findings from a well documented cohort are indicative of a significant level of as yet undiagnosed subclinical leprosy among teenagers and young adults in the "leprosy villages", potentially explosive and crippling in the event of widespread immunosuppression.

Hypothesis: Abnormal thermal thresholds, an effective measure of early small nerve fibre damage, are the first evidence of subclinical leprosy (which at present is not treated).

Application: Thermal Threshold Tester is simple to use, with high specificity and sensitivity. There is a strong case for using the A9 cohort with numbers augmented from surrounding villages, to carry out a controlled drug trial with appropriate MDT against placebos in both affected and unaffected populations to see whether treatment i) restores thermal thresholds to normal, and ii) prevents development of new cases and especially the nerve damage which causes disfiguration.

PAN AFRICAN ASSOCIATION OF NEUROLOGICAL SCIENCES

ABSTRACT FORM

Title: EPIDEMIOLOGY OF EPILEPSY IN AFRICA

Author/s: D. Houinato, P.M. Preux, Z. Melaku and M. Dumas

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Epilepsy is one of the commonest neurological disorders in Africa. The difficulties encountered in evaluating the prevalence of epilepsy are not only methodological problems (i.e definition of cases, collection of datas) but also the perception of epilepsy as a supernatural problem by the society. The differences in prevalence rate observed between African countries and Industrialized nations are due to methodological problems, inadequacy of medical infrastructures and specifities of certain etiologies (eg. cysticercosis). Globally, the prevalence of epilepsy in developing countries is twice that of developed countries.

In the majority of the published series from Africa.

- The incidence rate was around 73.3 per 100.000 inhabitants per year.
- The prevalence rate varies between 0.9 - 49 per 1000 in habitants.
- An overall male predominance has been observed.
- In almost all cases the seizures start in the first two decades of life.
- All studies have demonstrated the predominance of grand-mal seizures.

Epilepsy in Africa poses a real public health problem which merits particular attention of the authorities concerned.

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PE2

ABSTRACT FORM

Title: CYSTICERCOSIS AND NEUROCYSTICERCOSIS IN AFRICA

Author/s: P.M. Preux, Z. Melaku, M. Druet-Cabanac, G. Avodé, E.K. Grunitzky, B. Bouteille, M. Dumas

Centre: Institute Of Tropical Neurology, School of Medicine

City/Country: Limoges, France

Cysticercosis exists in Africa but its importance is not well recognized due to lack of diagnostic facilities. Absence of developed medical and sanitary infrastructures, and lack of trained medical personnel explains the relative scarcity of reports from this continent. For religious reasons, cysticercosis is almost nonexistent in Muslim parts of Africa where pork is not consumed. But, it affects almost all sub-saharan African countries where the necessary conditions for its development are reunited; which includes poor sanitary conditions, presence of free roaming pigs around residential areas, and their slaughtering without veterinary control. Cases of human cysticercosis have been reported in Benin, Burkino Faso, Burundi, Cameron, Ghana, Ivory Coast, Kenya, Madagascar, Rwanda, Senegal, South Africa, Togo, Zaïre, Zambia and Zimbabwe. Most of the works carried out in Africa had methodological biases due to appropriate epidemiologic methodology. There are very few population-based epidemiologic studies concerning human cysticercosis (Benin, South Africa, Togo). The overall cysticercosis prevalence rate varies depending on the population group studied methodologies and the diagnostic criterias used.

In Africa, the most common presenting clinical symptomatology is seizure, which have been reported in 30-60% of cases. Confirmation of diagnosis of neurocysticercosis requires use of modern medical imaging techniques which are expensive and not available in most African countries. In the absence of these examinations the diagnosis depends on clinical examination, serology, and circumstantial evidences, notably the presence of cysticerci in other systems.

Neurocysticercosis has a considerable impact on human health in Africa and its economic impac is certainly high, even if sufficient data is not available. Further epidemiological, socio-culteral and clinical studies must be carried out in different communities in order to determine the exact magnitude of the problem and propose control measures well-adapted to local conditions.

PI9

Ab 12. **PREVALENCE OF GESTATIONAL DIABETES MELLITUS IN RURAL PREGNANT MOTHERS IN NORTHERN ETHIOPIA.** Berhane Seyoum[1], Kassahun Kiros[2], Tesfay Haileselassie[2] and Abadi Leole[2].

[1]Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine
[2]Adigrat Hospital, Adigrat, Tigray. In a community based survey of gestational diabetes in 18 rural villages of the eastern zone of Tigray administrative region, northern Ethiopia, a total of 890 pregnant women with gestational age of 24 weeks and above were examined for gestational diabetes mellitus (GDM) based on WHO criteria. A 75gm oral glucose tolerance test was performed on each subject with measurement of glucose at 0 and 2 hours. Blood glucose was determined by glucose oxidase method using capillary blood (Accutrend alpha, Boehringer Mannheim). The mean age of the mothers was 27.4±7.1 years. Forty four percent of the subjects were multiparas. The prevalence rate of GDM was found to be 3.7%. The mean blood glucose 2 hours after the glucose load in those pregnant diagnosed to have GDM was 154.6±14.44mg%. The prevalence of GDM in this region of the country is high as compared to other parts of Africa. The possible role and contribution of exposure of the general population in this area to chronic malnutrition as a result of prolonged famine, drought and war, to the high prevalence of GDM warrants further study.

Ab 34. **A PILOT STUDY OF THE CLINICAL PROFILE OF MEDICAL EMERGENCIES AND GEOGRAPHIC ADDRESS OF PATIENTS SEEN AT THE TIKUR ANBESSA HOSPITAL OVER A TWO WEEK PERIOD.** Nebiyou Asnake, Mesfin Taye, Teshome Mengesha, Jemal Abdulkadir, Solomon Damena (Department of Internal Medicine, Addis Abeba University, Addis Abeba). A pilot study of the disease pattern and address of 244 patients seen during the two-week period November 9-23, 1992, in the Medical Emergency Unit (MEU) of Tikur Anbessa Hospital (TAH) was conducted. The age range was 15 to 75 years (median 30); male to female ratio was 1.3:1. Of 222 patients who came from a known location, 189 were from Addis Abeba and 13 were from elsewhere. Only 27 patients were from Woreda III where TAH and Zewditu Hospital are located. Eighty-two percent of the patients came to MEU on their own or were brought by relatives. The most common complaints were fever and headache (40%) and the ten commonest diagnoses were acute febrile illness (22.3%), bronchial asthma (6.6%), relapsing fever (6.3%), pneumonia (5.4%), clinically suspected AIDS (4.7%), hypertension (4.3%), peptic ulcer disease (3.9%), pulmonary tuberculosis (3.5%), upper GI bleeding (3.5%), and typhoid fever (2.7%). The duration of stay in MEU ranged from 5 minutes to 24 hours (median 4 hours). On the basis of an arbitrary formula apportioning emergency cases originating from Woreda III and each adjoining woreda between TAH and the other equally or more accessible hospitals or health centers according to the physical location and accepting all referrals to TAH as legitimate referrals, the total number of cases which could have been seen at the MEU would have been 98.40.2%) with commensurate overall saving of resources and better care for those served. The findings are discussed in the light of the urgent need to improve emergency health services in Addis Abeba.

SKELETAL FLUOROSIS AMONG RETIRING EMPLOYEES OF WONJI SHOA SUGAR FACTORY

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Nazareth and Addis Ababa, Ethiopia

SUMMARY: A retrospective study was done involving 263 retiring employees of Wonji Shoa Sugar Factory, an estate located in the Ethiopian Rift Valley and which is known for the high fluoride levels in its domestic water supplies. The radiological prevalence of skeletal fluorosis in the study sample was found to be 70.3 %. Skeletal fluorosis was associated with male gender ($p<0.05$) and also the prevalence was higher among factory and agricultural workers than among administrative workers ($p<0.05$). The association between male gender and fluorosis persisted within strata of occupation, indicating that the association was not mediated by occupation. The prevalence of clinical signs suggestive of skeletal fluorosis was markedly lower than the radiological prevalence and raged between 20.6 % and 40.2 % for the whole sample, indicating that many cases were asymptomatic. Further clinical epidemiological studies are suggested and strengthening of the existing defluoridation program is recommended.

Key words: Skeletal fluorosis; Occupational factors; Gender factors; Rift Valley; Ethiopia.

A review of lung cancer cases diagnosed between 1968 and 1984. Solomon Demena, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

A review was made of 51 cases of lung cancers diagnosed by the biopsy services of the pathological unit of the Black Lion Hospital, Addis Ababa, from 1968 to 1984 inclusive. The male-to-female ratio was 3.25 : 1. From 1968 to 1976 there were only 14 cases of lung carcinoma (27.5%), as compared to 37 cases (72.5%) from 1977 to 1984, showing a significant increase in diagnosis of lung carcinoma over the past 8 years. Histological typing showed that squamous-cell carcinoma cases numbered 22 (43%); the second most common was adenocarcinoma, 11 (21%) cases. From 1981, there were 28 cases of lung carcinoma from the Black Lion Hospital; 18 of these were revealed by bronchoscopy biopsy, and 2 cases by trephine lung biopsy. The question of whether this increase is due to improved diagnostic methods or to increased incidence requires further study.

The clinical characteristics of endoscopically proven duodenal ulcer cases in Ethiopian patients. Edemariam Tsega and Mulugetta Mengistu, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

During the 3 years April 1979 to April 1982, in a study of 144 cases (66% from Addis Ababa) of endoscopically proven duodenal ulcer, the mean age was 33.8 ± 12.9 years (range 14 – 75 years); the age group chiefly affected was 20 to 44 years; male-to-female ratio was 3.6 : 1.0. Local spices, alcohol, soft drinks and tension aggravated pain. Four patients were asymptomatic bleeders; the remaining 140 had pain, mainly epigastric burning; most had epigastric tenderness; 35 had exaggerated nasolabial fold. Endoscopic examination revealed that 63% had healed ulcers, and 37% fresh; over 50% of ulcers were located on the posterior and right side of the duodenal bulb. Of 66 patients who had blood group and rhesus factor tests, 33 were "O" positive and 19 "A" positive. Complications occurred in 107 (74%): 62 had bleeding (24 required blood transfusion), 21 gastric outflow obstruction, 20 intractable symptoms, 2 perforation and 2 posterior penetration. The positive correlation of endoscopic and barium meal findings was 86.6%. Duodenal ulcer cases may well be missed on diagnosis by symptomatology (only 45% were so diagnosed in this study) and barium meal examination.

High-speed air-drill biopsy of the lung in Addis Ababa. Bayu Teklu, Department of Medicine, Faculty of Medicine, Addis Ababa University.

A three-year (1982-1985) experience of high-speed air-drill biopsy of the lung at Tikur Anbessa (Black Lion) Hospital is reported. Thirty-six biopsies were made in 31 patients; a further 7 were postmortem (total: 43 biopsies). There were 17 males and 21 females, ages ranging from 14 to 74 years. Histology diagnosed fibrosis in 6; acute inflammation in 2; chronic inflammation in 2; 3 primary lung neoplasms, 3 secondary neoplasms and 1 tuberculosis. In 2 patients, pus was obtained; 9 biopsies were inadequate for interpretation, and 11 were normal. Three liver biopsies were inadvertently performed. Only one patient had significant haemoptysis, which quickly resolved without transfusion. A few developed pneumothoraces, but none required intercostal tubes. Routine post-biopsy chest X-rays were not necessary. The high-speed air-drill biopsy is a simple and safe procedure; even though the positive yield is not very high, it is recommended for use before thoracotomies are entertained.

Ab 8. TUBERCULOUS AND DIABETES MELLITUS. Yeweyenhareg Feleke[1], Getachew Aderaye[2], Jemal Abdulkadir[1] and Judith Bruchfeld[3]
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The present study was conducted to test the value of routine chest x-ray in diabetic patients for screening of pulmonary tuberculosis. This was a prospective study which was performed in 570 consecutive patients attending the diabetic clinic at Tikur Anbessa Hospital. The inclusion criteria were all diabetes who fulfill the WHO criteria of diagnosis of diabetes mellitus. Patients were interviewed and examined, the data were filled in the prepared questionnaire. Postero anterior chest routine annual CXR for early detection of TB and to determination serology for HIV, so that prophylactic chemotherapy can be given for seropositive diabetic patients.

Low-dose insulin in the management of diabetic ketoacidosis. Mulugetta Mengistu, Jemal Abdulkadir, Elehu Feleke, Seifu Daniel and Teshale Seboxa, Department of Medicine, Faculty of Medicine, Addis Ababa University.

The low-dose insulin regimen, continuous intravenous (IV) or hourly intramuscular (IM), has been demonstrated in special centres as safe, simple and effective in managing diabetic ketoacidosis (DKA). We tried the hourly IM regimen, 10 units IV and 10 units IM initially, then 5 units IM hourly, in 14 episodes of DKA in 12 patients (4 males and 8 females), under suboptimal conditions with limited biochemical monitoring. We used Dextrostix strips (Ames) for the hourly blood glucose determinations, augmented by periodic spectrophotometric determinations. The mean initial blood glucose was 513.7 ± 135.3mg%; the urine was strongly positive for ketones; and the level of consciousness varied from drowsy to comatose. The mean insulin requirement before changing to subcutaneous injections at a blood glucose level of 250mg% was 37.9 ± 13.1 units, fluid requirement was 2.5 to 6 litres, and the duration of treatment was 4.6 ± 2.4 hours. There was 1 death, not due to insulin resistance; all the other patients recovered uneventfully. This study reveals that, where laboratory facilities are limited, the small-dose regimen is applicable in DKA treatment, and possibly safer than the big-dose regimen, because of the lesser likelihood of severe hypoglycaemia and hypokalaemia.

Limited joint mobility in insulin-dependent and non-insulin-dependent Ethiopian diabetics: a controlled study. Mulugetta Mengistu and Jemal Abdulkadir, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

Limited joint mobility (LJM) was studied in 110 insulin-dependent (IDDM) and 190 non-insulin-dependent diabetics (NIDDM) and 300 controls. The age of the IDDM patients ranged from 14 to 62 years (mean ± SD = 35.5 ± 9.9 years), and that of the NIDDM patients ranged from 28 to 79 years (mean ± SD = 49.4 ± 12.0 years). The age of the controls ranged from 13 to 85 years (mean ± SD = 43.3 ± 14.0 years). Male-to-female ratio was 0.96 : 1.00. The prevalence of LJM was 44.5%, 25.3% and 6.7% in the IDDM, NIDDM and controls, respectively. The difference between the controls and each of the IDDM and NIDDM patients was significant ($P < 0.001$). Most of the cases with LJM showed minor changes, involving the proximal interphalangeal joints of the fifth fingers. In both the IDDM and the NIDDM group with LJM, the prevalence of retinopathy and nephropathy was higher than in patients without LJM; hence LJM may be taken as a valuable predictive sign for future complications.

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Ab 14. INSULIN STORAGE BY DIABETIC PATIENTS. BerhaneSeyoum[1], Jemal Abdulkadir[2], Y-Yeweyenhareg Feleke[1] and Yeshak Worku[2]
[1]Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University
[2]Department of Biochemistry, Faculty of Medicine, Addis Ababa University

A total of 140 Type I diabetic patients attending the diabetic clinic of the Tikur Anbessa Hospital were interviewed for information on how they store their insulin. The mean age of the patients was 31.0±10.2 years. The majority 84(60%) patients were males and the rest 56(45%) patients were females. The mean duration of diabetes mellitus was 8.3±5.4 years. Twenty percent of the patients had stopped their insulin for a few days to some weeks since their diagnosis. The most common reason was lack of insulin (55%). Only 26.4% of the patients store their insulin in a refrigerator, the rest they use clay pots filled with water and sand 17.8%, empty tins 17.1%, cupboards 17.1%, floors 15.7% and glasses 3.6%. The mean glycosylated hemoglobin was 11.1±2.3% (range 6.5-17.4%). The educational status, the income and the glycosylated haemoglobin were not statistically different among the different groups (P>0.05), whereas hyperglycaemic symptoms are significantly less in patients who store their insulin in the refrigerator (P<0.01). In the majority of our patients because of the poor socioeconomic status, the site of storage of insulin is likely to be hot, which might be associated with significant problems of tempreture related deterioration and thereby poor metabolic control.

Ab 30. HAEMOGLOBIN CONCENTRATION OF ETHIOPIANS RESIDING AT 3530 METERS ON THE SEMIEN PLATEAU Cynthia M. Beall[1], Amha Gebremedhin[2], Michael J. Decker[1], Gary M. Brittenham[1] and Milkias Shamebo ([1]Case Western Reserve University, Cleveland, OH, USA) and ([2]Addis Ababa University, Addis Ababa, Ethiopia). The paper reports the result of a study designed to obtain information on the haematological adaptation of high altitude Ethiopian natives and to compare the findings with those of other high altitude native population. Data were collected from 314 males and females 14 years and older resident around 3530m in the Amhara region of the Semien Mountains National Park, North Gondar, Ethiopia. Two hundred sixty two were free of self-reported illnesses, symptoms or conditions that might influence erythropoiesis. The average male haemoglobin concentration is 15.8gm/dl ± 1.1 (SD), range 12.8 - 19.1, n=142 compared with the female average of 15.0 ± 1.1 (SD), range 12.0 - 18.5, n=120. To control for different age distributions and a decline in haemoglobin concentration with age, the analysis was limited to young adults 20-39 years of age: males average 16.2gm/dl ± 1.3, range 13.4 -19.1, n=66 and females average 14.8 ± 1.0, range 12.0 - 16.8, n=48. The mean haemoglobin concentrations of the young adult males and females are just 5% and 9% higher than the sea level reference values of 15.4 and 13.6mg/dl, respectively, reported by Viteri and colleagues. This Ethiopian sample, in common with samples of Tibetan highlanders, exhibits a slight haematological response to high altitude hypoxia and contrasts with Andean highlanders marked (25-30% elevation of haemoglobin concentration) response.

Ab 10. SERUM 25-HYDROXYVITAMIN D (25-OHD) STATUS IN PREGNANT ETHIOPIAN AND NORWEGIAN WOMEN. Yeweyenhareg Feleke[*1], Jemal Abdulkadir[1], J Falch[2], JP Berg[2], OR Odegard[2], Tekle-Ab Mekbebe[3] and Robert Mshana[4]
[1]Endocrinology and Metabolism unit, Department of Internal Medicine, Faculty of Medicine, AAU, Addis Ababa, Ethiopia
[2]Hormone and Central laboratory, Aker University Hospital, Oslo, Norway
[3]Yekatit 12 Hospital, Addis Ababa, Ethiopia
[4]AHRI, Addis Ababa, Ethiopia

The main objective of this study was to determine the status of 25-Hydroxyvitamin D in term pregnant (38-42 weeks of gestation) Ethiopian women in comparison to age-matched term pregnant Norwegian women. 31 Ethiopians, 23 Norwegians were included in the study. Blood was drawn between August and September 1995 (end of the heavy rains) from the Ethiopians and in March 1996 (end of the winter) from the Norwegians. The separated serum was preserved at -20°C. 25-OHD, $1,25(OH)_2D$, and PTH were analysed by high pressure liquid chromatography, radio receptor assay and two-site immunoradiometric assay respectively. The serum levels of 25, hydroxyvitamin D was significantly lower in Ethiopian term pregnant women compared with the Norwegian term pregnant women (30.2±17.7nmol/lvs 43.1±21.3 nmol/l). The Ethiopians has higher levels of serum parathyroid hormone as compared to Norwegians (5.31±2.8pmol/l vs 3.1±1.3)pmol/l). This preliminary study shows that Ethiopian term pregnant women are at an increased risk of developing vitamin D deficiency with its adverse metabolic consequences to mother and infant. A more extensive survey is warranted to verify these preliminary findings.

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Ab 12. **25-HYDROXYVITAMIN D (25-OHD) IN NURSING STUDENTS FROM ADDIS ABABA IN COMPARISON WITH A SIMILAR AGE GROUP FROM OSLO, NORWAY.**

Yeweyenhareg Feleke[*1], Jemal Abdulkadir[1], J Falch[2], JP Berg[2], Robert Mshana[3] and OR Odegard[2]
[1]Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, AAU, Addis Ababa, Ethiopia
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[3]AHRI, Addis Ababa, Ethiopia

The main objective of the study was to obtain information on the level of 25-OHD at the end of the rainy season in young adult nursing school students from Addis Ababa (AA) in comparison with a similar group from Oslo, Norway, in the first week of September 1995 in Addis Ababa and March 1996 in Oslo which were considered comparable times after exposure to low intensity of sunshine. There were 30 subjects from Addis Ababa and 24 from Oslo aged 18-35 years. Subjects with a history of liver disease, renal disease, recent bone fracture or drug intake were excluded from the study. Blood was collected by venepuncture, serum was separated and kept at -20°C until shipment to Oslo for determination of 25-OHD by high pressure liquid chromatography, 1,25,$(OH)_2$D by radio receptor assay, and parathyroid hormone (PTH) by two site immunoradiometric assay. The serum level of 25-OHD was significantly lower in Ethiopians than Norwegians (24.7±10.1 nmol/l vs 85.1±30.3nmol/l), parathyroid hormone was higher in Ethiopians (6.6±4.1 pmol/l vs 2.6±1.1 pmol/l). Such a difference could indicate a tendency to secondary hyperparathyroidism in our population. This could further have a negative influence on the bone structure by reducing peak bone mass or increasing age-related bone loss. Therefore we recommend ultrasound study of bone to evaluate the bone mineral Density (BMD) in our population.

Ab 13. **VITAMIN D-RECEPTOR GENE POLYMORPHISM IN HEALTHY ETHIOPIANS.**

Yeweyenhareg Feleke[*1], Jemal Abdulkadir[1], J Falch[2], JP Berg[2], Robert Mshana[3], OR Odegard[2] and Tekle-Ab Mekbebe[4]
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The purpose of the study was to determine the type of vitamin D receptor Alleles (VDRA) in healthy Ethiopians. Determination of VDRA is important because the VDRA genotype can predict predisposition to osteoporosis or osteomalacia. Great interest has been generated by a recent report in which the authors claimed that as much as 75% of the total genetic effect on bone density may be related to a natural allelic variation or polymorphism within the VDR gene. The study showed that the higher bone density is associated with "b" allele of the VDR gene. The BB genotype is associated with a lower mean bone density and there is a 2.2 fold increase in prevalence of the BB VDR genotype in patients with osteoporosis. 102 healthy volunteers from Addis Ababa, age 19-40 years were included in the study. 37 males, 65 females, mean age was 22.9±4.6 years. 5 cc of EDTA blood was obtained by venepuncture and preserved at -20°C at AHRI laboratory. Analysis was made in Aker hormone and central laboratory, Oslo. DNA was extracted from whole blood, polymerise chain reaction (PCR) was used to amplify DNA sequence. VDRA distributions were Bb in 51 (50.0%), bb in 37 (36.3%) and BB in 14 (13.7%). So this study provided as the pattern of VDRA in Ethiopians which requires further study to determine the bone mineral density and VDR gene polymorphism simultaneously in Ethiopian population.

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Ab 8. TUBERCULOUS AND DIABETES MELLITUS. Yeweyenhareg Feleke[1], Getachew Aderaye[2], Jemal Abdulkadir[3] and Judith Bruchfeld[4]

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The present study was conducted to test the value of routine chest x-ray in diabetic patients for screening of pulmonary tuberculosis. This was a prospective study which was performed in 570 consecutive patients attending the diabetic clinic at Tikur Anbessa Hospital. The inclusion criteria were all diabetes who fulfill the WHO criteria of diagnosis of diabetes mellitus. Patients were interviewed and examined, the data were filled in the prepared questionnaire. Postero anterior chest x-ray was done for each patient. An average of 3 consecutive fasting blood sugar and symptoms related to diabetes were taken to assess the degree of diabetes control. A Radiologist and a Chest specialist read all chest x-ray. Out of 570 consecutive diabetic patients 297(52.1%) were males, 273(47.9%) were females, 306(53.8%) of them were IDDM, 264(38.3%) were NIDDM. The mean fasting blood sugar was 191.56±69.41mg/dl. Out of 570 patients 508 had chest x-rays taken. The results showed 25(4.9%) cases had abnormal chest x-rays, of these 11(2.2%) cases had active pulmonary tuberculosis, 12(2.4%) had old tuberculosis and 2(0.3%) had pneumonia. All of the active pulmonary tuberculosis cases were asymptomatic. 10(90.9%) of the 11 active pulmonary tuberculosis patients were IDDM. 6 of 11 patients were smear positive for acid fast bacilli (AFB), 5 were smear negative. 4 of 11 patients had interstitial involvement, 4 patients cavitary lesions, 2 had combined interstitial, alveolar and cavitary involvement, 1 had calcification and cavitary lesion. The disease burden in the chest x-ray showed minimal pattern in 6, moderate in 4 and sever in 1 patient. The chest x-ray side involved was unilateral in 9, bilateral in 2 patients. A routine chest x-ray detects an evidence of active tuberculosis in 2.2% of 508 diabetic patients. From this prospective study we have concluded that chest x-ray is useful for the early detection of pulmonary tuberculosis in asymptomatic diabetic patients. Therefore, we have recommended to do annual chest x-ray for IDDM patients.

A retrospective analysis of TB patients among students of Jimma Insitute of health Sciences

Lissane Seifu, MD and Sileshi T/Mariam, MD, MPH

ABSTRACT

A three year retrospective analysis of Tuberculosis (TB) patients who attended the student's clinic of Jimma Institute of Health Sciences (JIHS) was conducted. It is aimed to determine the prevalence of TB in the students and describe the clinical presentations and factors associated with the acquisition of TB.

Data were retrieved from the individual medical records and patients were interviewed to supplement missed major variables.

The overall prevalence of TB in the student population was 2.2% (49/2212). Out of the total 49 cases, 42(87.2%) were analyzed. Majority had Pulmonary TB, 83.3%. Group specific prevalence was highest in the Environmental Health students (4.3%) followed by medical students (2.9%) and Pharmacy students (2.8%). Nursing students and medical Laboratory students each had prevalence of 1.9% and 1.7% and 1.7% respectively. Prevalence of TB in medical students who had patient contact for training was 1.9% (8/428), and in those with no contact it has nearly doubled 3.7% (19/510). Information on variables necessary to determine factors related to TB acquisition, like contact history, past history of TB and duration of illness was incomplete, in 40.5%, 31% and 23.8% of the cases respectively.

In conclusion, TB has affected students of all categories of studies irrespective of contact to TB patients in the hospital for professional training. Information on determinant factors to TB acquisition is incomplete. Further cross-sectional study to know the determinant factors for TB acquisition is recommended.

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Ab 48. PREVALENCE OF RETINOPATHY IN DIABETIC PATIENTS ATTENDING THE TIKUR ANBESSA HOSPITAL (TAH) DIABETIC CLINIC. Berhane Seyoum, Jemal Abdulkadir, Yeweinghareg Feleke (Endocrinolgy and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, AAU, Addis Abeba), Zerae Mengistu (Department of Ophthalmology, Minilik II Hospital, Addis Abeba), Paulos Berhanu (Department of Medicine, University of Colorado, Health Sciences Center, USA), Yishak Worku, (Department of Biochemistry, Faculty of Medicine, AAU, Addis Abeba).
Gonfa Ayana (National Research Institute of Health, Addis Abeba). A total of 302 patients was selected by taking alternate patients from regular attendants of the TAH diabetic clinic to determine the prevalence of retinopathy. The mean age was 41.4 years (range 14-85). There were 160 males (53%) and 142 females (47%). One hundred forty (46.5) were type I and 162 (53.6%) were type II. The mean duration of diabetes was 9.4 years; mean haemoglobin A1c (HgA1c) was 10.4%. On the day of the examination the mean fasting blood glucose (FBG) and random blood glucose (RBG) were 195.5mg% and 273.1mg% respectively. The mean serum total cholesterol, triglycerides, LDL, VLDL and HDL were 166.5mg%, 129.9mg%, 94.5mg%, 24.4mg%, and 44.3mg%, respectively. The overall prevalence of retinopathy was 37.8% out of which 113 patients (36.1%) had background retinopathy and 5 patients (1.7%) had proliferative retinopathy. The retina could not be visualized in three patients because of dense cataract. Retinopathy correlated positively with age, duration of diabetes and blood pressure, r=0.19 (95%CI 0.14 to 0.30), r=0.43 (95%CI 0.33 to 0.52) and r=0.25 (95%CI 0.14 to 0.35), respectively. However retinopathy has no significant correlation with HgA1c and serum lipids. Prevalence of retinopathy was comparable in type I & type II ($P>0.05$). The prevalence of retinopathy in our patients relative to the duration of diabetes mellitus was high. Therefore, improvement of facilities for the diagnosis and treatment of retinopathy is recommended.

Ab 22. A SERO PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN ETHIOPIAN DIABETIC PATIENTS. Yeweyenhareg Feleke[1], Getachew Aderaye[2], Jemal Abdulkadir[1] and Judith Bruchfeld[3]
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[3]Department of Infectious Disease, Hudding University Hospital, Hudding, Sweden
A sero prevalence rates of HIV infection reach upto 20% of the adult population in some cities of central and East Africa. In general there is scanty information on the prevalence of HIV infection in diabetic patients. This prospective study was conducted to assess the sero prevalence of HIV infection in diabetic patients from the diabetic clinic of Tikur Anbessa Hospital from January 1997 to June 1997. 322 consecutive patients were interviewed and underwent through physical examination, blood was drawn by venepuncture and sent to the Armauer Hanssen Research Institute (AHRI) for screening by enzygnost ELISA method. The positive samples were reanalyzed by the same method, those repeatedly positive samples were analyzed by Wellcozyme HIV, VK57 ELISA. Those positives with latter test were considered as true positive. Those negative tests in the second Enzygnost ELISa test were considered as true negative. Those samples which tested positive in one of the two consecutive Enzygnost ELISA testes were subjected to westernblot test for confirmation in Hudding University Hospital, Sweden. Out of 322 diabetic patients there were 164(50.9%) males, 158(49.1%) females, 179(55.8%) were IDDM and 143(44.5%) of them were NIDDM. The mean age was 36.2 ± 15.5 years. 18(5.6%) patients had a history of sexually transmitted disease, 1(0.3%) patient had history of blood transfusion, 2(0.6%) patients have history of sharing needles. The sero prevalence of HIV in our diabetic patients was found to be 7%. For comparison 582 age and sex matched healthy blood donors from Ethiopian Red Cross between January 1997 to June 1997 were screened for HIV by Wellcozyme ELISA method showed sero prevalence of 6.2%. Therefore, the sero prevalence of HIV in our diabetic population is almost similar to the non-diabetic populations. In general it is advisable to strengthen the health education for the diabetic and non-diabetic populations.

Ab 13. IMPROVING METABOLIC CONTROL BY CORRECTING FAULTY INJECTION TECHNIQUES IN DIABETIC PATIENTS. Berhane Seyoum (Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, AAU, Addis Ababa, Ethiopia).

We had previously reported on the prevalence of wrong injection techniques in diabetic patients. In this study of 27 insulin requiring diabetic patients with faulty injection technique, instructions were given to correct their technique and thereby improve their metabolic control. The mean fasting blood glucose was 287.3±78.4mg% and the mean dose of insulin was 53.9±15.8 units before intervention. After correcting the injection technique the mean fasting blood glucose and the mean insulin dose were lowered to 120.3±23.1mg% and 46.8±12.5 units respectively ($P<0.001$). By correcting the injection technique, the metabolic control can be improved and the dose of insulin can be reduced significantly. These results demonstrate the importance of regular inspection of insulin injection areas in order to detect and correct faulty injection technique as an integral component of the means to achieve good diabetic control.

Ab 14. SURVEY OF THE HIGH RISK DIABETIC FOOT. Berhane Seyoum[1], Tezera Chaka[2]
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[2]Orthopaedic Department, Faculty of Medicine, Addis Ababa University, Addis Ababa

A cross sectional survey of the high risk diabetic foot was done on 223 diabetic patients attending the diabetic clinic of Tikur Anbessa Hospital. One hundred fifty one (67.7%) were NIDDM and 72(32.3%) were IDDM patients. The mean age and the mean duration of diabetes were 47.1±14.1 years and 11.1±6.4 years respectively. About 4.5% of the patients either walked barefoot or wore shoes only occasionally and in 43.5% of the patients, their shoes were ill-fitting. Only 57.8% of the patients washed their feet daily. On 40.4% of the diabetics, the shoes had ragged and uncomfortable insoles. Thirty one percent of the patients cut their nails using inappropriate and dangerous instruments such as razorblades and penknives. Inspection of the feet revealed that 137(61.4%) patients had skin abnormalities including skin cracks, cornes, mycosis and blisters, 172(77.1%) patients had nail abnormalities mainly thickened and ingrown nails and 62(27.8%) patients had foot structure abnormalities. More than half of the patients (68.6%) had neuropathy and 24.7% have signs of macroangiopathy. Fifty three (23.8%) patients did not know that foot care was important in diabetic patients. Those patients who knew about the importance of foot care were found to have significantly lower prevalence of foot and nail abnormalities ($P<0.001$). This survey showed a high prevalence of foot abnormalities in our diabetic patients, which emphasis the importance of health education on proper diabetic foot care.

Ab 42. OUTCOME OF DIABETIC PATIENTS WITH FOOT PROBLEMS IN TIKUR ANBESSA HOSPITAL: PRELIMINARY REPORT. Berhane Seyoum, Tezera Chaka, Wondimu Wolede. Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University
Orthopedic Department, Faculty of Medicine, Addis Ababa University

From Meskerem 1990 till Tir 1991, a total of 33 diabetic patients with foot problems were admitted to the medical wards of Tikur Anbessa Hospital, of whom 22(66.7%) were male and 11(33.3%) were females. The mean age was 54.7±15.1 years (range 26-87). The majority, 25 patients (75.8%) were Type 2, 8 patients (24.3%) were Type 1. The mean duration of diabetes mellitus was 13.8±8.1 years. Two patients presented with the foot problem and diabetes was diagnosed during evaluation in the Hospital. The main risk factors identified were neuropathy and macroangiopathy, which were seen in 84.8% and 63.6% of the patients respectively. Of the total, 15(45%) patients had foot gangrene, 12 (36.4%) patients had foot infection, 4(12.1%) patients had toe gangrene and 2(6.1%) patients had toe infection. In 21 (63.6%) patients X-ray of the foot showed some abnormalities and out of the 32 cultures taken from the foot, 28(87.5%) patients there was a positive culture. Major amputations were done in 18(54.5%) patients and 84.8% of the patients were discharged improved. However five patients (15.2%) died because of sepsis. The mean hospital stay was 48.9±28.5 days. In conclusion, it was noted that the bed occupancy rate to be high and efforts should be made to prevent amputations in diabetic patients.

Experiences in acute renal failure in the Renal Unit of Black Lion Hospital, Addis Ababa. Berhanu Habte, M. Almaguer, J.P. Alfonzo, and S. Arce, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

Case records were analysed of 38 patients (9 males, mean age 44 years, and 29 females, mean age 30 years) with a diagnosis of acute renal failure admitted to the Black Lion Hospital between March 1980 and March 1983. Of these, 34 patients had renal parenchymal causes for acute renal failure, and the remaining 4 patients had obstructive uropathy. Among the renal parenchymal causes, acute tubular necrosis (ATN) was the commonest cause of acute renal failure, followed by severe glomerulonephritis. Septic abortion was the most important pathogenetic factor leading to ATN, occurring in 20 patients; hypotension and septicaemia, next in importance, accounted together for 10. Oliguria occurred in 21 patients with ATN, but 4 had non-oliguric acute renal failure. In those with oliguria the initial phase had an average duration of 5.4 days, and the oliguric phase lasted 18 days. Haemodialysis was employed in 8 patients, peritoneal dialysis in 21, and 2 patients were treated with both. Eleven patients died, from renal failure in 4 cases, septic complications in 3, and haemorrhagic complications in 3. Septic abortion, a very common cause of ATN, requires that in such patients renal function must from admission be closely monitored.

Acute tubular necrosis in a developing tropical country. Berhanu Habte, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

Sixty-four patients with acute tubular necrosis (ATN), admitted to Tikur Anbessa Hospital, Addis Ababa, over a 5½-year period, are described. Their ages ranged from 13 to 67 years, with a median of 29 years. Forty-five (70.3%) were females. The major diseases leading to ATN were obstetric complications in 29 cases (45%); haemoparasites in 11 cases (17%); nephrotoxins in 7 cases; and severe infections and acute dehydration in 6 cases each. Oliguria occurred in 47 patients; non-oliguric ATN occurred in 15. The mean duration of the oliguric phase was 14.7 days, with a range of 7 to 28 days. Gastrointestinal upset, mental alterations and fluid overload were common features. Dialysis was indicated in 45 (70.3%) patients; of these, 9 could not be dialysed, as they had a rapidly fatal course. A total of 16 patients (25%) died. The major causes of death were the underlying illness in 8; other complications in 5; and uraemia *per se* in only 3 patients. It is concluded that obstetric complication and severe parasitic and bacterial infections are the leading causes of ATN in an underdeveloped tropical setting, and that dialysis treatment is effective in reducing mortality.

Pattern of glomerulardiseases in adult Ethiopians. Genene Bekele and Berhanu Habte, Department of Internal Medicine, Faculty of Medicine, Addis Ababa University.

Of 110 patients with a clinical diagnosis of primary or secondary glomerulonephritis admitted over a 2-year period to Tikur Anbessa (Black Lion) Hospital, Addis Ababa, 54.5% had nephrotic syndrome, 27.3% had chronic renal failure, 13.6% had acute nephritic syndrome, and 3.6% had asymptomatic proteinuria and/or haematuria. The commonest renal histological finding was diffuse proliferative glomerulonephritis of various types (42.5%). "Reactive amyloidosis" was found in 10%. No aetiological association could be made in the majority (60%) of the cases. Post-streptococcal infection and diabetes mellitus each accounted for 15.4% of all the glomerular diseases. Schistosomiasis, hepatitis B virus, syphilis, and quartan malaria apparently played an insignificant role, or none, in the causation of glomerulonephritis in this group of patients.

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Pattern of skin diseases in Gondar Administrative Region. Heinz Sochor, Teshale Schoxa and Mallede Mam, Gondar College of Medical Sciences.

A total of 1521 patients (approximately 80% older than 16 years; male-female ratio 2:1) was examined in the Department of Dermatology, Gondar College of Medical Sciences, between October 1985 and March 1986, in correlation with reports from elsewhere in Ethiopia. The most common skin diseases were infectious diseases (541: 36%), diseases with immunological disorders (401: 26%), diseases of epidermal appendages (81: 5.3%) and congenital disorders (51: 3.4%). The symptom pruritus as a common sensation was only found by itself in 23 cases (1.5%) without clear cause; it was found mainly in scabies (78: 5.1%) and in tuberculosis (27: 1.8%). The eczema diseases with irritant, allergic contact and microbial eczema (9.9%), together with seborrhoeic and atopic dermatitis (15.6%), formed the biggest group of all skin diseases. The different forms of exanthema (5.5%), the lichen group (5.1%) and the other diseases with itching made up a group indicating use of local traditional medicine, with negative and complicating effects on the skin. In Ethiopia, as in other parts of the world, the most common skin diseases were caused by the same micro-organisms, drugs, food-particles and other pathogenetic mechanisms.

The epidemiology of bronchial asthma in a healthy Ethiopian population. Teshale Schoxa, Arthur F. Fast and D. Goldblatt, Gondar College of Medical Sciences, Gondar.

One hundred apparently healthy Ethiopians (79 males and 21 females; between the ages of 19 and 36 years) were studied at the Gondar College of Medical Sciences. They included 25 interns, 25 medical students, 7 nurses, and 32 ancillary personnel. There were 25 males who stated they had smoked for at least 5-10 years; 18% gave a history of chronic cough, bronchitis or asthma. One-second forced expiratory volume (FEV1) of less than 75% of the predicated value was shown in 43% of the study group; 17% had greater than 15% improvement of FEV1 after use of a bronchodilator; 13% with a history of asthma had FEV1 less than 75% of the predicated value.

So-called "rare" skin diseases of the tropics observed in Gondar Administrative Region: analysis of case reports. Heinz Sochor, Teshale Schoxa and Zein Ahmed Zein, Department of Dermatology, Gondar College of Medical Sciences.

From a total of 1521 cases, 294 (19%) occurrences of so-called "rare" skin diseases of the tropics were observed in the Gondar College of Medical Sciences from October 1985 to March 1986. These included 57 genetic or hereditary diseases (atopic dermatitis, psoriasis, ichthyosis, follicular keratosis *et al.*); 152 immunological diseases (erythematodes, lichen group, erythema exudativum multiforme, erythema nodosum, dermatitis herpetiformis, pemphigus vulgaris, circumscribed scleroderma, Reiter's syndrome, *et al.*); 12 epidermal appendage diseases (alopecia totalis, rosacea, cheiro-podopompholyx, *et al.*); 57 microbial disorders (herpes simplex and zoster, condylomata acuminata, erysipelas, anthrax, gingivostomatitis, *et al.*); 7 metabolic disorders (lipomatosis, porphyria cutanea tarda, Fox-Fordyce's disease); 2 of unknown cause (ainhum); and 5 malignant diseases (T-non-Hodgkin's lymphoma); 2 anomalous diseases (naevus flammeus, haemangioma). The diagnosis was made from clinical, histological, immunological and microbiological findings, and by X-ray, as well as genetic heredity and the patients' history and progress under treatment. All skin diseases in all regions of Ethiopia should be investigated and registered. The skin/race differentiation and climate conditions involve speciality, but are not a fundamental cause of "rarity" of tropical skin diseases.

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Ab 44. OUTCOME OF THYROTOXIC PREGNANT PATIENTS: PRELIMINARY REPORT. Berhane Seyoum (Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine. Addis Ababa University). A total of 20 thyrotoxic pregnant patients were prospectively followed until delivery. All were confirmed to have thyrotoxicosis clinically and biochemically. Of whom 15(75%) had Graves' disease and 5(25%) had toxic goiter. The mean age was 27.4 ± 5.1 years and the mean gravidity and mean parity were 2.5±1.6 and 1.4±1.5 respectively. Out of the patients with Graves disease, 6 (40%) had classic eye signs. Fifteen patients (75%) received propylthiouracil and 5 patients (25%) received combination of propranolol and propylthiouracil. The mean duration of treatment was 31.6± 9.5 weeks (range 12-40 weeks). Seventeen patients (85%) delivered vaginally and 3 patients (15%) delivered by cesarean section, the indication being purely obstetrical. Three neonates (15%) had congenital goiter. All neonates were alive during delivery. The mean weight of the neonates was 2916.2±526.8 grams. No complications occurred to the mother during delivery. The rate of congenital goiter is high, however, the outcome is gratifying.

Ab 22. A SERO PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN ETHIOPIAN DIABETIC PATIENTS. Yeweyenhareg Feleke[1], Getachew Aderaye[2], Jemal Abdulkadir[1] and Judith Bruchfeld[3]
[1]Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, AAU
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[3]Department of Infectious Disease, Hudding University Hospital, Hudding, Sweden
A sero prevalence rates of HIV infection reach upto 20% of the adult population in some cities of central and East Africa. In general there is scanty information on the prevalence of HIV infection in diabetic patients. This prospective study was conducted to assess the sero prevalence of HIV infection in diabetic patients from the diabetic clinic of Tikur Anbessa Hospital from January 1997 to June 1997. 322 consecutive patients were interviewed and underwent through physical examination, blood was drawn by venepuncture and sent to the Armauer Hanssen Research Institute (AHRI) for screening by enzygnost ELISA method. The positive samples were reanalyzed by the same method, those repeatedly positive samples were analyzed by Wellcozyme HIV, VK57 ELISA. Those positives with latter test were considered as true positive. Those negative tests in the second Enzygnost ELISa test were considered as true negative. Those samples which tested positive in one of the two consecutive Enzygnost ELISA testes were subjected to westernblot test for confirmation in Hudding University Hospital, Sweden. Out of 322 diabetic patients there were 164(50.9%) males, 158(49.1%) females, 179(55.8%) were IDDM and 143(44.5%) of them were NIDDM. The mean age was 36.2±15.5 years. 18(5.6%) patients had a history of sexually transmitted disease, 1(0.3%) patient had history of blood transfusion, 2(0.6%) patients have history of sharing needles. The sero prevalence of HIV in our diabetic patients was found to be 7%. For comparisop 582 age and sex matched healthy blood donors from Ethiopian Red Cross between January 1997 to June 1997 were screened for HIV by Wellcozyme ELISA method showed sero prevalence of 6.2%. Therefore, the sero prevalence of HIV in our diabetic population is almost similar to the non-diabetic populations. In general it is advisable to strengthen the health education for the diabetic and non-diabetic populations.

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Ab 44. **LIPID PROFILE IN TYPE I AND TYPE II DIABETIC PATIENTS ATTENDING THE TIKUR ANBESSA HOSPITAL DIABETIC CLINIC.** Berhane Seyoum, Jemal Abdulkadir, Yeweinhareg Feleke (Endocrinology and Metabolism Unit, Department ofInternal Medicine, Faculty of Medicine, AAU, Addis Abeba), Paulos Berhanu (Department of Medicine, University of Colorado, Health Sciences Centre, USA), Zerai Mengistu (Department of Ophthalmology, Minilik II Hospital, Addis Abeba), Yishak Worku (Department of Biochemistry, Faculty of Medicine, AAU, Addis Abeba), Gonfa Ayana (National Research Institute of Health, Addis Abeba). A total of 302 patients attending the TAH diabetic clinic were selected for lipid determination in four months time. Every other patient was selected for the study. The mean age was 41.4 years (range 14-85 years). One hundred sixty (53%) were males and 142 (47%) were females. There were 140 (46.4%) type I and 162 (53.6%) type II patients. The mean duration of diabetes mellitus, haemoglobin A1C, fasting blood sugar and random blood sugar were 9.4 years, 10.4%, 195.5mg% and 273.1mg%, respectively. The mean cholesterol, triglycerides, LDL, VLDL and HDL were 166.5 mg/129.9 mg/dl, 94.5 mg/dl, 24.4 mg/dl and 44.3 mg/dl, respectively. Cholesterol levels > 200 mg/dl were found in 56 (18.5%) and 43 (14.2%) of type I and type II patients, respectively. Total cholesterol & triglycerides correlated positively with blood pressure, r=0.28 (95% CI. 0.17 to 0.38) and r=0.25 (95% CI 0.14 to 0.35), respectively. Total cholesterol was significantly higher in females than in males (179.5 mg/dl veraus 154.9 mg/dl, P<0.001) and in type II than in type I patients (183.1 mg/dl versus 147.2 mg/dl, P<0.001), respectively. Triglycerides and LDL cholesterol were significantly higher in type II diabetic patients (162.7 mg/dl versus 91.5 mg.dl, P<0.001 and 105.6 mg/dl versus 81.9 mg% /dl, P<0.001), respectively. HDL cholesterol was the same in both types of diabetic patients. Total cholesterol and triglycerides correlated with body mass index, r=0.40 (95% CI 0.30 to 0.49) and r=0.28 (??to 0.48), respectively. Smoking has no effect in cholesterol and Triglyceride levels (P>0.05). The study confirms that lipid values are high in type II diabetic patients. Hence, we recommend periodic check up of diabetic patients.

Ab 46. **PROFILE OF METABOLIC CONTROL IN DIABETIC PATIENTS ATTENDING THE TIKUR ANBESSA HOSPITAL (TAH) DIABETIC CLINIC.** Berhane Seyoum, Jemal Abdulkadir, Yeweinhareg Feleke (Endocrinology and Metabolism Unit, Department ofInternal Medicine, Faculty of Medicine, AAU, Addis Abeba), Yishak Worku (Department of Biochemistry, Faculty of Medicine, AAU, Addis Abeba), Paulos Berhanu (Department of Medicine, University of Colorado Health Sciences Center, USA), Zerae Mengistu, Gonfa Ayana (National Research Institute of Health, Addis Abeba). A total of 302 patients were selected by taking alternate patients from regular attendants of the TAH diabetic clinic for haemoglobin A1c (HgA1c) determination. The mean age was 41.4 years (range 14-85). There were 160 males (53%) and 142 females (47%). One hundred forty patients (46.4%) had type I and 162 (53.6%) had type II diabetes. The mean duration of diabetes was 9.4 years (range 1-34 years). The mean HgA1c was 10.4% ,.ange 5.9-17.4%); forty two (13.8%) patients had excellent (<7.9%), 37 (12.3%) good (8.0-8.9%), 49 (16.2%) fair (9.0_9.9%) and 174 (57.6%) patients had poor (≥ 10%) control. On the day of the examination, the mean fasting blood glucose (FBG) and random blood glucose (RBG) were 195.5mg% and 273.1mg%, respectively. HgA1c correlated negatively with age, r= -0.27 (95% CI -0.39 to -0.18) and positively with FBS and RBS, r=0.45(95% CI 0.36 to 0.54) and r= 0.49 (95%CI 0.40 to 0.57), respectively, but HgA1c had no correlation with sex, duration of diabetes, serum lipid level or retinopathy. HgA1c was significantly higher in Type I as compared to Type II patients (11.1% versus 9.8%, P<0.001). In conclusion, the overall metabolic control of our diabetic patients is not satisfactory. This is related possibly to inadequate care as well as adverse socioeconomic factors precluding home blood glucose monitoring, periodic measurements of HgA1c and strict dietary control, all of which would be difficult to improve in the short term.

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Ab 9. **A PRACTICAL APPROACH TO THE MANAGEMENT OF DIABETES DURING PREGNANCY: A PROGRESS REPORT.** Jemal Abdulkadir[1], Berhane Seyoum[1], Yeweyenhareg Feleke[1], Seyoum Yoseph[2], Taddesse Kitila[2], Fekade Deneke[2] and Bogale Worku[3]
[1]Endocrinology-Metabolism Unit, Dept. of Internal Medicine, Addis Ababa University, Addis Ababa
[2]Dept. of Obstetrics and Gynaecology, Addis Ababa University, Addis Ababa
[3]Naenatology Unit, Dept. of Paediatrics and Child Health, Faculty of Medicine, Addis Ababa

One hundred and twenty-one patients, 38 IDDM, 34 NIDDM and 49 gestational diabetics (GDM), were seen over an eight year period from November 1989 to November 1997 and followed for at least eight weeks to the end of pregnancy. The diagnosis of diabetes was according to the WHO criteria. Only one patient attempted planned pregnancy. Fifty-two patients (43.0%) were first seen during the first trimester while 67 (55.3%) were first seen during the second or third trimester. Dietrary advice, based on the habitual diet of the patient, emphasised adherence to the recommended quantity of food, avoidance of simple carbohydrates and, in relevant cases, reduction of fat intake. Insulin dose was adjusted on the basis of twice weekly preprandial and once weekly 2h postprandial blood glucose values. Patients were seen at least every two weeks during the first and second trimesters and every week during the third trimester. Good diabetic control (mean preprandial blood glucose of $=<100$mg/dl and/or postprandial $=<140$mg/dl) was achieved in 47.8% of the patients. Toxaemia or preeclampsia developed in 17 (14.0%) %), polyhydramnios in 4 (3.3%), infections in 7 (5.8%) and miscellaneous other complications in 4 (3.3%) patients. Mode of delivery was based on obstetric indications. Of 114 patients who had live deliveries 44 (38.6%) patients had spontaneous labour, 26 (22.8%) were induced and 44 (38.6%) had cesarean section. Twenty-eight (73.7%) IDDM, 34 (100%) NIDDM and 49 (100%) GDM had live deliveries. Twenty-five (22:5%) newborns had macrosomia, 24 (21.6%) had hypoglycaemia. Overall achievement of live deliveries in comparison to outcome in antecedent pregnancies was 28/38 (73.7%) vs11/31 (35.5%) in IDDM, $X^2$17.2, $x^2$8.6, $P<0.005$, 34/34 (100) vs 9/18 (50%) in NIDDM, $X^2$17.2, $P<0.0005$. It is concluded that a practical management of diabetes during pregnancy, based on the approach described above, can significantly improve the prospects for a live delivery in a situation where unfavourable socioeconomic conditions preclude implementation of the stricter measures adopted in developed countries aimed to achieve normoglycaemia and thereby pregnancy outcome comparable to that of nondiabetics.

Ab 28. **EFFECTIVE PERITONEAL DIALYSIS USING RINGER'S LACTATE AND 5% DEXTROSE IN 0.9% NaCl SOLUTIONS.** Yewondwossen Tadesse (Department of Internal Medicine, Faculty of Medicine, Addis Ababa University). Intermittent peritoneal dialysis was done in 18 patients with renal failure (13 acute and 5 chronic) using 2 litre cycles (40-50ml/kg in children) of Ringer's lactate solution alternating with a combination of Ringer's lactate and 5% dextrose in 0.9% NaCl solutions. Indications for dialysis included fluid overload, severe metabolic acidosis, uremic encephalopathy, pericarditis and electrolyte imbalances particularly hyperkalemia. All 14 of the 18 patients who had adequate dialysis session length (>/ 48 cycles) had reversal of the clinical and biochemical derangements of uremia resulting in at least a temporary improvement in their condition, 8 patients died in three (3) of whom only a few exchanges (10-18) were done and thus died before PD made any significant impact on their metabolic derangements. Of the remaining 5 deaths, 2 deaths occurred from progressive uremia in patients with end stage renal disease several weeks after PD and 3 deaths were related to infectious complications (pneumonia and sepsis). There were 3 procedure related complications, 2 cases of peritonitis and 1 patient with a skin infection at the site of catheter insertion. No side effects attributable to the dialysis solution were observed. These findings indicate that in places where the supply of proper peritoneal dialysis fluid is erratic and unreliable easily available intravenous fluids can be used for intermittent peritoneal dialysis with equally good results. The results of this study may be taken up for further research on a larger group of patients who require dialysis in a suboptimal environment.

Trend of Acute Myocardial Infraction Admissions over a Decade: Tikur Anbessa Teaching Hospital

Yoseph Mamo, MD* Kebede Oli. MMED, MD**

Abstract:

This is a case series analysis undertaken to evaluate the importance of acute myocardial infraction (AMI) as a cause of admission at the Tikur Anbessa Medical Intensive Care Unit (MICU) and the trend of that importance over a decade (1988-1997). Clinical presentations and the frequencies of major coronary risk factors in the individual patient were also assessed for the later half of the decade (1993-1997). In the decade under study a total of 2313 patients were admitted to MICU according to its register. Overall AMI was the third commonest cause of admission and accounted for 8.8% (N=203) of all MICU cases. AMI annual admissions increased consistently over the years. Of the 122 AMI admissions during the second half of the decade 92 charts were available for detailed analysis. 86% (79/92) fulfilled the stated criteria for the diagnosis. The mean age of these patients was 55.1 ± 13.0 years. Males constituted 82% of all AMI cases. Eighty seven per cent (69/79) of the cases were first admissions with the diagnosis of AMI. Ninety-four per cent (74/79) of them were brought to the emergency room due to chest discomfort and 20% were in frank pulmonary oedema. Nineteen per cent died in hospital. 81% and 34% of the patients and one or more and two or more major coronary risk factors excluding age and gender respectively. Raised total cholesterol and hypertension were the commonest risk factors being reported in 69% and 47% of AMI patients respectively. In conclusion this study has demonstrated that AMI is indeed on the rise at least at Tikur Anbassa Teaching Hospital. The conventional coronary risk factors seem to operate in the Ethiopian series as well. The study highlights the need for coronary risk factors surveys at least in the susceptible population group to assess the gravity of the problem.

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