Original article

Observation of blood microfilariae during human trypanosomiasis survey in Gambella, south west Ethiopia

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Abstract: While conducting a survey on sleeping sickness during 1989-92 in Gambella, South western Ethiopia, microfilariae of *Mansonella perstans* were detected in the blood of the indigenous population, the Anuaks (1%), and the refugees from Southern Sudan (4.1%). No blood microfilarial infection was detected in resettlers, who arrived in the area from drought affected regions of the country during the 1985/86 resettlement program. Among the diagnostic methods applied, nearly twice more microfilaraemic cases (4.1%) were detected by the Miniature Anion Exchange Centrifugation Technique (M-AECT) while only (1.9%) were detectable by Microhaematocrit Buffy Coat Technique (MHBCT) among the refugees. Using the conventional blood film methods (thin and thick smears) only fewer positive cases (1.0%) were detected compared to the above two techniques. Besides a known standard diagnostic methods for blood filariasis, however, the MHBCT seems preferable as field diagnostic technique. Because it is more rapid, simple to operate and does not necessitate as much advanced preparation and sterile condition as M-AECT, and could be a potential diagnostic tool for blood microfilariae. There is a significant difference ($P<0.01$) in age groups 15-30 years among Anuaks and refugees. There is no significant difference ($P>0.01$) in other age groups and sexes among Anuaks and refugees. However, there is a significant difference ($P<0.01$) in over all positivity among Anuaks and refugees. [*Ethiop. J. Health Dev.* 1997;11(1):1-5]

Introduction

Human trypanosomiasis is known to be endemic in Gambella, South Western Ethiopia (1,2). Meanwhile, different kinds of infectious agents that co-existed with sleeping sickness were also recorded. Among these lymphatic filariasis due to *Wuchereria bancrofti* (3) and a case of *Burgia* species (4) were reported. The area is also known to be endemic for Malaria, Onchocerciasis, Leishmaniasis and *Loa loa*. Some of the vectors of these parasites have also been incremented and described by previous investigators (5,6). The main objective of this study was to evaluate and apply a more sensitive, rapid, simple and reliable diagnostic technique for human trypanosomiasis in the field, therefore scoring for blood microfilarae along with trypanosomes in every Miniature Anion Exchange Centrifugation technique (M-AECT), Microhaematocrit Buffy Coat Centrifugation technique (MHBCT) and the classical methods such as stained blood films (thin and thick smears) and wet film mounting were conducted.

Methods

Study area and population

Gambella is located 800 km from Addis Ababa at latitude 8^0N and longitude 34 ^{o}c at an altitude of 500 metres. The Baro, Gilo, Aluwero, Akobo rivers and several other small to moderately sized

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rivers criss cross the Region. The mean annual rainfall is 980mm and mean relative humidity is

between 45% and 75% (7).

The indigenous population included in this study belongs to the Anuak tribe. Although the majority of the population were formally organized into farmers association, they do not produce cash crops. They live on fishing, hunting and on small scale subsistence farming, growing maize and sorghum. The adult males still travel away from their villages to gold mining fields or coffee growing areas to make money.

The refugees migrate from different areas of southern Sudan which are known to be endemic for human trypanosomiasis and filariasis. A large number of migrants have occupied the western part of the region, in Itang site along the Baro- Akobo rivers and the second group arrived lately in 1987/88 to the southern part of the Region, at Pingnudo site along the Gilo river.

The Ethiopian settlers arrived to Gambella during 1985/86 resettlement programmes from drought affected Regions of Wollo, Tigrai and Shoa.

The study subjects, the indigenous population and the settlers were selected using a simple random sampling method and the refugees using systematic sampling. The list of the head of the house hold including the total population was obtained from the farmers association office in case of the settlers and the indigenous population. The number of house holds required was estimated and then 10% was randomly selected using random table. All members of the selected house holds were included in the study. As of the refugees their way of settlement was not suitable for the above method of sampling. So we used systematic sampling method. The list of the individuals was obtained from the block leaders. All were informed to come to their usual ration dispensing place by the leaders for the examination. 10% of the population was selected using every other 10th individual among the population. For all selected individuals physical examination was done by a physician before blood sample for haemoparasites was taken. All positive cases were treated in the nearby clinics and when found necessary also referred to the Gambella hospital.

Blood sample collection. A total of 875, 1800 and 1400 blood samples were collected and analyzed for haemoparasites from resettlers, indigenous populations and refugees, respectively, using conventional blood films and MHBCT applied for the three study group and M-AECT only for the refugees as it is described here under.

Blood films. Thick and thin blood smears were prepared on the microscope slide following the standard procedure recommended by WHO (8). The smears were allowed to dry and stained with Giemsa and examined for the presence of haemoparasites (trypanosomes, malaria, blood microfilaria) under the microscope at the magnification of x100. In addition to the stained blood smears preparation, wet films were mounted and examined on the spot for all clinically suspected individuals for sleeping sickness infections.

Microhaematocrit Buffy Coat Technique (MHBCT). The MHBCT, also known as Woo's method (9) was performed as modified by WHO (8). Briefly, about 60 μl of blood was taken by a finger prick in heparinized haematocrit capillary tubes in a duplicate from each patient. One end of the capillary tube was sealed with critoseal and centrifuged for 5 minutes at 3000 rpm. Then the capillary tube was attached on a microscope slide with scotch tape. Both Trypanosomes and blood microfilarae were detected in the buffy fraction of the capillary tube under the microscope at the magnification of x10 and then by x40 for confirmation.

Miniature Anion Exchange Centrifugation Technique (M-AECT). This technique was originally developed as a laboratory research tool to detect low parasitaemia of trypanosomes (10). Later on it was designed and prepared in a form of sterile kit, Para- Sight Sterile Kit for field application. It is based on the capacity of cellulose fragments equilibrated by a buffer, Phosphate Saline Buffer with Glucose (PBSG), PH, 8.0 to have a surface static electrical charge. Fresh infected whole blood is

allowed to run through a column of equilibrated DE-52 or pre- swollen Diethyl Amino Ethylene Cellulose column (DEAE or DE-52, whatman, England). The blood cells are retained by cellulose while the parasite (trypanosomes and blood microfilarae) are eluted with the buffer as

both differ in their electrical charges. The eluate is collected in a special tube centrifuged and the parasites concentrated at the tip of the tube. Then mounted on a viewing chamber and examined under the microscope with the magnification of x10 and x40 for both trypanosomes and blood microfilariae.

Results

Blood microfilariae *Mansonella perstans* were detected in the blood of 75 individuals, that is 1% from indigenous the Anuaks and 4.1% from the Sudanese refugees. The microfilarae of *M. perstans* distinguished from those of *W.bancrofti, Loa loa* and *Burgia* species by the absence of sheath. In addition positive stained blood smears were sent to London School of Hygiene and Tropical Medicine for confirmation. Among the other blood microfilariae cases of *W. bancrofti* was also detected. But the detailed information and result is supposed to be prepared by one of our colleagues (S.A)

During the screening of indigenous population, with Microhaematocrit Buffy Coat Technique (MHBCT) many more microfilaraemic positive cases were detected (1.0%) than with the conventional thin and thick stained blood smears (0.33%). Amongst the refugees Miniature Anion Exchange Centrifugation Technique (M-AECT) detected more than twice (4.1%) as many microfilaraemic cases as MHBCT (1.9%) and more than four times as stained blood smears (1.0%) (Table 1). However, all cases detected by the stained blood smears were also detected by the other two methods. Using wet film mounting, only a single case of microfilaria was detected which also was detected by stained blood films.

The infection was higher in young age group, 15-30 years, (61.1%) and (84.2%) in Anuaks and refugees, respectively and have a significant difference ($P<0.01$) than children 5-14 years and adults above 30 years. No microfilaria was diagnosed in children below 5 years. Higher positivity was observed in males (66.7%) and (94.7%) in Anuaks and refugees, respectively. Regarding the over all positivity, there is a significant difference ($P<0.01$) among the two study groups (Table 2). No blood microfilaria was detected in the blood of resettlers.

Table 1. **Determination of *Mansonella perstans* infection rate by Blood films, MHBCT and M-AECT.**

Groups	Total Exam.	Blood Thin Pos	film thick% Pos	MHBCT		M- AECT			
				Pos	% Pos	Pos	% Pos	Total Pos	% Pos
Anuaks	1800	6*	0.33	18	1.0	-	-	18	1.0
Refugee	1400	14	1.0	26	1.9	57#	4.1	57	4.1
Settler	875	-	-	-	-	-	-	-	-

* is also detected by MHBCT

includes cases detected by the other two methods

Table 2: **Age and Sex distribution of *M. Perstans* infections**

Group	Total Pos	Sex	Age

			1-4	5-14	15-30	31+	(NO) Pos
		M	-	3	7	2	12
				16.7%	38.9%	11.1%	66.7%
Anuaks	18	F	-	1	4	1	6
				5.6%	22.2%	5.6%	33.3%
N=1800		Total	-	4	11	3	18
				22.2%	61.1%	16.7%	100%
		M	-	4	47	3	54
				7.0%	82.5%	5.3%	94.7%
Refugee	57	F	-	2	1	-	3
				3.5%	1.8%	-	5.3%
N=1400		Total	-	6	48	3	57
				10.5%	84.2%	5.3%	100 %

Discussion

During a sleeping sickness survey in 1989-92 in Gambella, South Western Ethiopia, microfilariae of *Mansonella Perstans* were detected in the blood of the indigenous population, the anuaks (1%) and the refugees from Southern Sudan (4.1%). No blood microfilarial infection was observed in the resttlers.

The infection rate is higher in young age groups, 15-30 years 84.2% in the refugees and 61.1% in Anuaks than the children below 15 years and older age groups. Eventhough it is not statistically significant there is some differences in positivity in males than females in both study subjects. This could be attributed to the occupational division and the biting nature of the vector (6). Males visit forests more frequently than females, and the culicoides bite mostly in dense patchy forests than open fields.

The pathology ascribed to *M. perstans* infection is not well recognized nor is the clinical symptomology well recorded (12). It was of interest to us therefore that many of the individuals who had high microfilariae count commonly complained of joint and back pains, headache, weakness, pruritus, etc. Did sleeping sickness whose symptoms are well known suggests them (8) or were they due solely to *M. perstans* infections regardless of the co-existence of malaria infection in the area. While we can not speculate, it is better to take into account that even an infection which normally might cause little or no illness could become serious in combination with other intercurrent diseases.

Previous studies indicated that there was a prevalence of 24% *Wuchereria bancrofti* infections among the Anuaks in the area (3). In the present study cases of *W. bancrofti* were detected among the indigenous population, the Anuaks (NRHI, unpublished report). That is the microfilariae of *W. bancrofti* was identified from stained blood smears by the presence of a sheath and subterminal arrangement of the nuclei.

The interesting observation made in the present study is that, M-AECT detect almost more than twice as many microfilaraemic cases as MHBCT and more than four times by stained blood smears among refugees which is quite in agreement with Lumsden et.al. and others (10,11). Due to the shortage of the kit we could not apply M-AECT, during the screening of the indigenous population the Anuaks and the resettlers.

Though M-AECT is the most sensitive test known so far, it needs further improvement in field conditions. However, the preparation of sterile kit of optimal size under difficult conditions avoids the need for refrigeration (8,11). Thus sterile kits are stable, and always the sterility is needed to prevent faulty diagnosis due to the presence of other motile micro-organisms in the column. As compared to M-AECT, MHBCT is relatively less sophisticated and could therefore easy to manipulate in the field.

Based on these facts, the complete epidemiological profile of lymphatic filariasis and other blood

filariasis in the area, including periodicity, density of the microfilaria and clinical picture of the disease should be studied. By large, as compared to M-AECT, MHBCT with its relative merits of low sophistication, could therefore, find application in some blood filariasis survey. In addition application of standard diagnostic techniques such as Millepore filtration methods, Knott's concentration technique (12,13), etc. for blood filariasis examination is recommendable.

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