Confirmation of Trypanosome Parasitaemia in Previously Serologically Positive Individuals in the Abraka Area of Delta State, Nigeria

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ABSTRACT

This study was conducted to follow-up trypanosome serologically positive individuals, with the aim of confirming the presence of trypanosome parasitaemia in them over the study period of 12 months. The study was carried out at the Eku Baptist medical centre Abraka, Delta State between June 2002 and May 2003.

Confirmation was by the detection of trypanosomes in blood, lymph node glands and cerebrospinal fluid microscopy. Of the booked population (n=128), 82 (64.1%) were studied, 46 (35.9%) lost to follow-up. Of the studied 82 cases, 14 (17.1%) were confirmed as having sleeping sickness, 40 (48.8%) remained unconfirmed cases while 28 (34.1%) seroconverted and were discharged from follow up. Of the 14 (17.1%) confirmed cases, 9 (64.3%) were confirmed in the last six months of the 12 months period of follow-up, compared to 5 (35.7%) in the first six months ($X^2 = 4.5$, df = 1, P = 0.04).Long-term monitoring of serosuspects is essential in this endemic area for arresting transmission of the disease and prevention of associated morbidities and mortalities.

INTRODUCTION

Sleeping sickness a lethal African disease caused by the parasite *Trypanosoma brucei* gambiense, remains a major public health concern in the Abraka area of Nigeria in view

Key Words: Gambiense sleeping sickness, serosuspects, reservoirs for infection, followup, Nigeria.

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Correspondence: Dr. (Mrs.) Airauhi L.U Department of Medical Microbiology, University of Benin Teaching Hospital, P.M.B. 1111 Benin - City, Nigeria. of the immense social impact of the disease on individuals, families and affected communities^{1,2}. The control of *gambiense* sleeping sickness includes detection and treatment of cases³. Subjects infected with gambiense sleeping sickness are often aparasitaemic1 when screened due to fluctuating levels of parasitaemia.² Seropositive individuals who remain undetected due to occult parasitaemia constitute an important population to consider. This is because they serve as reservoirs to maintain and ensure transmission in any focus since they remain apparently healthy with no specific symptoms and are thus not treated⁴. Some serosuspects who could not be confirmed during a previous study were booked and followed up at 6 and 12 months⁵.

This study reports the finding of a preliminary follow-up of serosuspects in the earlier described Abraka sleeping sickness focus (ASSF) in Nigeria⁶.

SUBJECTS AND METHODS

In the Abraka sleeping sickness focus (ASSF), during an active surveillance study, 128 seropositive subjects with no trypanosomes seen in their blood, lymph gland juice (IGJ) and cerebrospinal fluid (CSF) were detected. These individuals hereafter referred to as serosuspects were booked for follow up from the date of initial diagnosis between 8th April and 11th May 2002. The following information were obtained from the records of the subjects at the Baptist Medical Centre (BMC) Eku: age, sex, community of domicile, date of initial diagnosis, results of follow-up and date when confirmed.

During the follow-up of the booked population, a subject remained either a serosuspect, seroconverted or confirmed as a sleeping sickness. A confirmed sleeping sickness patient is defined as one having trypanosomes in blood, LGJ or CSF. A seroconverted subject is defined as a previously CATT positive subject who test negative subsequently.

Serological analysis

CATT was performed according to the procedures described by the manufacturers (Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium)⁷ Reagents were reconstituted and test carried out using blood collected by finger prick as described by Magnus *et al*⁷. One drop of well-homogenized CATT reagent was put on test card area and a drop of test blood added. The mixture was spread within 1mm from the edge of the test area using a stirring rod. Positive and negative controls were set up for all the screening. Test results were recorded as either positive or negative.

Parasite testing

Serosuspects were screened for confirmation of sleeping sickness using blood collected into 5 millitres Ethylene Diamin Tetraacetic Acid (EDIA) bottle for microscopic studies. Giemsa stained blood films were examined microscopically for trypanosomes. Haematocrit concentration technique (HCT) was performed by filling capillary tubes to about $^{2}/_{3}$ full, sealed with plasticine and spun in a microhaematocrit centrifuge to concentrate the trypanosomes. Buffy coat smears were made and examined for the presence of trypanosomes which were characterized by morphology⁸. Subjects with negative result were screened further by LGJ microscopy. CSF screening was done for all subjects; those who were positive were classified as being in late stage ONS stage II of infection. Other parasitic confirmation using blood and LGJ microscopy were classified as early haemolymphatic phase I. Those who were negative for trypanosomes were booked for further followup.

Medical Ethics

Approval by the Ethical Committee of the University of Benin Teaching Hospital was obtained before the commencement of the study. A consent form was signed by study partipants and in case of children by their parents/guardian.

Results

One hundred and twenty eight CATT positive cases (71 males and 57 females) were initially booked for follow-up after diagnosis of seropositivity. However, in this study only 82 CATT positive cases, made up of 47(57.3%)males and 35(42.7%) females were enrolled giving a male:female ratio of 1.3:1.0, no statistical significant difference existed in the follow-up population by sex ($X^2 = 0.32$, df = 1, P = 0.57), table 1.

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Of the 82 CATT positive serosuspects that were followed-up, 40(48.8%) cases remained unconfirmed at the end of the follow-up period. 14(17.1%) were confirmed to have sleeping sickness. Of the 14 confirmed cases, 9(64.3%) were confirmed in the last six months of the 12 months follow-up period compared to 5(35.7\%), in the first six months (P=0.04), table 2.

However, 28 cases (34.1%) seroconverted and were discharged from follow-up. All those that sero converted were aged 16 years and above with more males than females 17(60.7%) Vs 11(39.3%), but this was not significant (X2 = 0.02, df = 1, P = 0.81).

Of the 40(48.8%) cases that remained unconfirmed, 9(22.5%) were aged 16 years or below and 31(77.5%) were aged above 16 years, table 3. The 14(48.8%) cases that were confirmed during the period of study, 2(14.3%) were in stage I of the disease while 12(85.7%) were in stage II. Forty six subjects were however lost during the follow-up period, 12(26.1%) of them could not be traced

Table 1: Serosuspects by age and sex

using the addresses given in a previous survey while 34(63.9%) decided not to continue with the study.

Most studies on the control of sleeping sickness reported the difficulties in confirming sero suspects which include poor treatment seeking behavior due to low level of knowledge⁴ about the disease⁵.

Discussion

This paper documents the finding of the first follow up of serosuspects in the ASSF, Nigeria. Of the 128 serosuspects booked only 82 (64.1%) were followed-up while 46 (35.9%) were lost to follow up. Among the followedup population, there were 47 males and 35 females giving a male: female ratio of 1.3:1.0 while the lost population consists of 26 males and 20 females giving a male: female ratio of 1.3: 1.0. The sex distribution among the followed-up and lost population was similar. The age distribution in both populations was also similar. The confirmation of sleeping sickness revealed that a significantly higher

	Booked	Followed	Lost	P Value	
	n (%)	n (%)	n (%)		
Total	128 (100.0)	82 (64.1)	46 (35.9)		
Male	71 (55.5)	47 (57.3)	24 (52.2)	P > 0.05	
Female	57 (44.5)	35 (42.7)	22 (47.8)		
Age					
<u><</u> 16yrs	17 (13.3)	9 (11.0)	8 (17.4)	P > 0.05	
<u>></u> 16yrs	111 (86.7)	73 (89.0)	38 (82.6)		

 $X^2 = 4.53$, df = 1, P= 0.041

Table 2: Confirmation of sleeping sickness among serosuspects (n=54)

Follow up	Confirmed cases		Unconfirmed cases		
Interval	n	8	n	90	
0 - 6mths	5	(35.7)	4	(10)	
7 - 12mths	9	(64.3)	36	(90)	
Total	14	(100)	40	(100)	

	Serosuspects		Uncon	Unconfirmed		Seroconverted		Confirmed cases		P value
	n	(%)	n	(%)		n	(%)	n	(%)	
Total	82	(100)	40	(48.8)		28	(34.1)	14	(17.1)	
Male	47	(57.3)	22	(55)		17	(60.7)	8	(57.1)	P > 0.05
Female	35	(42.7)	18	(45)		11	(39.3)	6	(42.9)	
Age										
< 16 yrs	9	(11.0)	9	(22.5)		0	(0.0)	5	(35.7)	P < 0.05
> 16yrs	73	(89.0)	31	(77.5)		28	(100.0)	9	(64.3)	

Table 3: Outcome of follow-up of serosuspects by age and sex

n = number

% = Percentage

number of serosuspects were confirmed during the last six months of the follow up (P < 0.05) with both sexes and age groups infected. The distribution of serosuspects by age and sex is similar to earlier observed patterns in this focus. Seroconvertion was not recorded among subjects less than 16years, while 28/73 (38.41%) of those aged above 16years seroconverted. We are unable to explain this pattern. It could however be a chance occurrence and so should be interpreted with caution, as it may not necessarily be that those aged 16 and below do not seroconvert.

These observation suggests that both sexes and all age group are at equal risk of infection as earlier reported in this focus⁶ Our confirmation of sleeping sickness among previously aparasitaemic CATT positive subjects is an indication of the epidemiological importance of this population in the control of the disease.

We have demonstrated that a reasonable proportion of serosuspects were confirmed during this study thus ensuring that the population of reservoir hosts is reduced by the confirmation of cases and prompt treatment. Long-term follow-up of serosuspects in this endemic focus is required to interrupt transmission of sleeping sickness; we envisage the sustenance of follow-up of serosuspects as part of effort to control this lethal parasitic disease in Nigeria.

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