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Full Length Research Paper

Sero-epidemiological study of Human African Trypanosomiasis in non-endemic areas

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Human African Trypanosomiasis surveys were conducted using Card Agglutination Test for Trypanosomiasis (CATT) in displaced people's camps and prisoners in Khartoum state, who originated from endemic areas in southern Sudan, the result showed that 60 out of 211 cases and 38 out of 144 cases were positive (28.4% and 26.4%, respectively), however Parasitological examination did not show any parasite. A comparison between CATT and Card Indirect-Agglutination Antigen Test for Trypanosomiasis (CIATT) for the diagnosis of sleeping sickness was done on 250 serum samples the result indicated that 71 positive cases (28.4%) for CATT and only 15 positive cases (6%) for CIATT. CATT and CIATT were found similar in their cross reactivity which resulted in the detection of the false positive cases of sleeping sickness from patients with malaria and leishmaniasis from non-endemic areas of sleeping sickness (46% and 42.9% detected by CATT, respectively) and (28% and 34.3% detected by CIATT, respectively).

Key words: Sleeping sickness, CATT, CIATT, Non-endemic areas

INTRODUCTION

African trypanosomiasis (sleeping sickness) occurs in 36 sub-Saharan countries, within the area of distribution of the tsetse fly. There are two subspecies of Trypansoma brucei which are pathogenic for man, T. b. gambiense and T. b. rhodesiense. They are transmitted to human by the bites of various species of Glossina (tsetse) (WHO, 2000).

Diagnosis of sleeping sickness is based on parasitological and serological examinations. There are two field tests for diagnosis of the disease, namely Card Agglutination Test for Trypanosomiasis (CATT) for diagnosis of T. b. gambiense disease (Magnus et al., 1978), the other is the Card Indirect-Agglutination Antigen Test for Trypanosomiasis (CIATT) for the diagnosis of T. b. gambiense and T. b. rhodesiense disease (Nantulya et al., 1992; Komba et al., 1992).

Sleeping sickness is believed to be existed in Sudan and was recorded in different areas of the southern part of the country (Snow et al., 1991). This study was planned to evaluate Card Agglutination Test for Trypanosomiasis (CATT) and Card Indirect Agglutination Antigen for Trypanosomiasis (CIATT) for diagnosis of the disease in other parts of the country (non-endemic areas).

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MATERIALS AND METHODS

Study site

The study was conducted in Khartoum state. It's well outside the tsetse belt in Sudan.

Sampling

A total of 355 blood samples for serum separation were collected aseptically from displaced and prisoners who originality from endemic areas in Southern Sudan according to the willingness of the people for sampling (Convenience sampling methods

Table 1: Sero-positivity of sleeping sickness using CATT in Khartoum state

Source of sera	No. examined	No. of positive cases using CATT
Displaced people's camps		
Eid Hussien	30	6 (20%)
Mayo	164	45(27%)
Jebal Aulia	17	9(52.9%)
Sub-total	211	60(28.4%)
Prisoners		
Dabc men prison	30	14 (46.7%)
El Giref men prison	29	11(37.9%)
Omdurman women prison	85	13(15.3%)
Sub-total	**144**	**38(26.4%)**
Total	**355**	**98 (27.6%)**

(Thrusfield, 1995).

A serum from 35 patients with Leishmaniasis and Plasma from 50 patients with malaria from trypanosomiasis-free areas was obtained from the Faculty of Medicine, University of Khartoum and Tropical Medicine Research Institute.

Parasitological examination

Thin blood films

Thin blood films were prepared on slides, dried and fixed with alcohol and stained with 3% diluted Giemsa's stain solution for 30 minutes, washed in buffered distilled water (PH 6.8-7.2). They were left to dry and then examined microscopically.

Serological tests

Card Agglutination Test for Trypanosomiasis (CATT)

The test was carried out as described by Magnus et al. (1978). About 45 µl of well-homogenized CATT reagent was put on each test area of the card, then 20 µl of serum or plasma was added and mixed by rod. The card was shaken for 5 minutes on a rotator.

Card Indirect-Agglutination Antigen Test for Trypanosomiasis (CIATT)

CIATT was done by mixing 25 µl of serum or plasma with 25 µl of CIATT reagent and shaken on the rotator for 5 minutes (Nantulya et al., 1992; Komba et al., 1992).

Data management and analysis

Data analysis was performed using SPSS 10.0.7 for windows 98. Chi square was used to know significant level for sero-positivity of sleeping sickness with sex as well as comparison between CATT and CIATT. It is difficult to estimate the sensitivity and specificity of CATT and CIATT because parasitological examinations (Gold Standard Test) gave negative results. Then, the agreement between different tests (Kappa statistic) without assuming one test is the best was employed as described by Thrusfield (1995). Kappa statistic ranges from one (complete agreement) to zero (no agreement), negative values indicated that agreement was less than expected by chance, other point estimates are:

0.20 slight agreement
0.21-0.40 fair agreement
0.41-0.60 moderate agreement
0.61-0.80 substantial agreement
>0.81 almost perfect agreement

RESULTS

Serological examination using CATT on 211 and 144 serum samples from displaced people's camps and prisoners in Khartoum state, who originated from endemic areas in southern Sudan, showed 60 positive cases (28.4%) and 38 positive cases (26.4%) respectively. However, a microscopic examinations using thin blood film and thick blood film gave negative results in all samples (Table 1). Positive cases were found statistically to be higher in males than females ($P< 0.05$).

A comparison between CATT and CIATT for diagnosis of sleeping sickness was made on 250 serum samples from displaced people's camps and Omdurman's women prison, the result showed 71 positive cases for CATT (28.4%) but only 15 positive cases were detected by CIATT (6%) (The intensity of reaction is given in (Table 2 and 3). The difference between two tests statistically was significant ($P<0.05$). Agreement between the two tests (Kappa statistic) was indicated a slight agreement 0.18.

Plasma from 50 patients with malaria and serum from 35 patients with leishmaniasis from trypanosomiasis-free areas were examined serologically using CATT and CIATT. The result showed 23 (46%) and 15 (42.9%) positive cases for CATT, respectively. While, CIATT detected 14 (28%) and 12 (34.3%) positive cases, respectively. Statistically CATT and CIATT were found similar ($P>0.05$) in the detection of the false positive cases from patients with malaria and leishmaniasis from non-endemic areas.

DISCUSSION AND CONCLUSION

High sero-prevalence of sleeping sickness using CATT was recorded in Khartoum state. However, Khartoum

Table 2: The intensity of reaction using CATT in Khartoum state

Source of sera	No. examined	Positive Reactors											
		whole serum				diluted serum 1:4				diluted serum 1:8			
		+++	++	+	±	+++	++	+	±	+++	++	+	±
Displaced people's camps & Omdurman's women prison	250	1(0.4%)	18(7.2%)	17(6.8%)	35(14%)	0(0.0%)	0(0.0%)	11(4.4%)	0(0.0%)	0(0.0%)	0(0.0%)	2(0.8%)	0(0.0%)

(+++) very strong agglutination (++) strong agglutination (+) good agglutination (±) doubtful reaction (very fine agglutination)

Table 3: The intensity of reaction using CIATT in Khartoum state

Source of sera	No. examined	Positive Reactors					
		whole serum		diluted serum 1:4		diluted serum 1:8	
		++	+	++	+	++	+
Displaced people's camps & Omdurman's women prison	250	7(2.8%)	8(3.2%)	1(0.4%)	2(0.8%)	0(0.0%)	1(0.4%)

(++) strong reaction (+) weak reaction

state is known as a non-endemic area. This could be explained by the population movement from endemic areas. The sero-prevalence was found higher in males than in females (36.1% and21.8%, respectively). It is possible the males are in more frequent contact with the tsetse infested areas where they are come from for hunting, fishing, farming and herding (WHO. 2000). Table 3

As seen from the results there was a variation between positive cases detected by CATT and CIATT (28.4%and 6%, respectively). This could be attributed to the fact that CATT was developed to detect antibodies directed against a specific commonly occurring variable antigen of T. b. gambiense (Magnus et al, 1978). While, CIATT for diagnosis of both T. b. gambiense and T. b. rhodesiense is based on the detection of the specific circulating trypanosomal antigens in blood (Nantulya et al., 1992; Komba et al., 1992). Moreover, recent report has suggested that CATT is not effective in all T. b. gambiense endemic foci (Dukes et al., 1992).

Individuals who react positively in the CATT or CIATT and then gave a negative result for parasitological methods. This may be due to the variation between the regions as well as a number of positive tests are not confirmed by the presence of parasites in the blood, lymph node aspirates or cerebrospinal fluid (WHO, 2000). The finding was previously confirmed by Nantulya (1997) who explained that either some of the sero-positive patients were indeed infected but their parasitaemia is too low to be detected, or they were false positives. Furthermore, due to the lack of sensitivity and specificity of serological tests Becker, et al (2004) was developed real-time PCR assay and he indicated that it can be considered as a rapid and sensitive method suitable for the detection of T. brucei in human blood samples in routine clinical laboratory practice.

The false positive cases detected by CATT and CIATT in patients with malaria and leishmaniasis from non-endemic areas of sleeping sickness however, high sensitivity and specificity were recorded by different authors (Nantulya, 1997; Asonganyi et al, 1998; Chappuis, 2005). The disagreement is most probably due to the cross-reaction. More investigation is needed in order to establish possible interaction between both

malaria and leishmaniasis with human African trypanosomiasis.

In conclusion, sleeping sickness remains a major public health concern in south Sudan. Care must be taken in interpretation of serological tests. Based on that parasitological confirmation and clinical stage of the disease are needed before commencement of treatment.

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