ASSESSING RELIABILITY OF CARD AGGLUTINATION TEST FOR TRYPANOSOMIASIS IN SCREENING GAMBIAN SLEEPING SICKNESS AT ABRAKA ENDEMIC FOCUS

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ABSTRACT

Introduction: Human African trypanosomiasis (HAT) is one of the neglected tropical diseases. It is endemic in Abraka, south south , Nigeria . Clinical features of this disease are not sufficiently specific for diagnosis of HAT; a definition diagnosis relies on laboratory examination. The card agglutination test for trypanosomiasis (CATT) is a useful fast practical serological test for HAT screening in endemic areas. There are indications that CATT results may be misleading since it is not 100% sensitive. The objective of the study was to evaluate the reliability of CATT test in Abraka sleeping sickness endemic focus.

Method: This study was carried out in Oria-Abraka for about two years from march 2013. A total of 254 consenting individuals were screened with CATT reagent. All sero-positive individuals were further subdivided into mild, moderate and severely positive. Detailed clinical examination and parasitological evaluation of blood, csf and aspirates of lymph node, of all seropositive individuals were done to confirm the diagnosis of HAT. 26 seropositive were followed-up for 24months and one for 3months.

Results: 27 of the 254 consenting individual tested positive to CATT test, giving a seropositivity of 10.6%. All the seropositive individuals were parasitologically negative after series of parasitological examinations over 24months period. They were all also positive to malaria parasite test.

Conclusion: Card Agglutination Test for Trypanosomiasis is not specific enough for screening for trypanosome brucei gambiense infestation within the Abraka sleeping sickness endemic focus

Keywords:

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INTRODUCTION

Human African Trypanosomiasis (HAT) also called sleeping sickness is one of the neglected tropical diseases[1] caused by *Trypanosoma brucei gambiense*, in west Africa [1,2,3] and *T. b. rhodesiense* subspecies is found in central and southern Africa.[4] In Abraka, Delta State Nigerian, it is called '*Oga-Ovwere*' which means 'illness of sleep'. The early stage of the disease is difficult to differentiate from other common mild febrile illness even in the endemic areas. The late stage is characterized by excessive sleep which progresses gradually to seizure.[4] Without appropriate treatment intervention, the illness leads to death. Clinical features of the disease are not sufficiently specific for diagnosis. Definitive diagnosis of *T . b. gambiense* relies on laboratory examinations for parasite identification in blood, lymph node and or cerebrospinal fluid (CSF) [2]. A three step approach is utilized during control programmes while evaluation of individual patient involves screening, diagnostic confirmation and clinical staging [5]. The card agglutination test for trypanosomiasis (CATT) was developed in the late 1970s [6]. It can be performed on serum, capillary blood from finger prick or blood eluted from impregnated filter paper [7].It is a fast and practical serological test that allows hundreds of individuals to be screened daily.[8,9,10] The reported sensitivity is

within a range of 87 to 98% and the specificity has a range of 76 to 80% [8]. It is widely used for extensive population screening in endermic areas.[2,11]. There are indications that CATT result might be misleading since it is not 100% sensitive and the negative individual, may turn out to be parasitological positive. The objective of this study was to evaluate the reliability of CATT test in Abraka sleeping sickness endemic focus.

Methods and Materials

This investigation was carried out at Oria-Abraka in March 2013 during the one week medical out-reach to mark the 1st coronation anniversary celebration of the Ovie of Umeghuva Abraka kingdom. The kingdom is located between latitude 5º47'-6º15' N and longitude 5º42'-6ºE with a population of about 150,000 people.[12] The study communities were situated within a mixed rain forest and derived grassland vegetation with river Ethiope and its tributaries running through the kingdom.

The predominant economic activity is agrarian peasant farming with some artisans, transporters, traders and civil servants. All communities in the kingdom were duly sensitized for the outreach activities. Investigations commenced with daily enlightenment lectures on the nature, objectives and benefits of the investigations. Informed consent was sought and obtained from individuals (n=254) that volunteered to be screened. Questionnaire was administered to obtain participants' information on bio-data and their awareness of HAT.

Finger pricked blood was drawned into heparinized capillary tube. A drop of freshly CATT regent was added to a drop of blood, on a plasticized surface and mixed. All seropositive individuals were further sub-divided into three; using CATT reagent by serial double dilution. The reaction was graded as negative with no visible agglutination and slight, moderate or strong reactivity for those with visible agglutination as positive with 1:2-1:4, 1:8-1:16 and ≥1:32 titres respectively.

The seropositive individuals were given one week appointment for detailed clinical examination and parasitological investigations to confirm presence or absence of parasite in blood smears and aspirate from cervical lymph nodes and, CSF. FBC, blood smear for trypanosome, filarial and malaria parasite microscopy and urinalysis test. Only strongly seropositive volunteers had lumber puncture under standard procedure and the CSF analyzed for trypanosome, cell count, and protein estimation. 26 of the 27 seropositive individual were followed up monthly for 24 months and one for three months. The later relocated after three months. During the follow up visits, clinical examination and laboratory tests were performed monthly and half-yearly, respectively. Data obtained were subject to statistical analysis.

Results

A total of 254 individuals comprised of males (n=86) and females (n=168) were screened for HAT with CATT reagent. The age of the sample population ranged between 16 to 72 years with a mean of 42±2 years. 82.3% were farmers while 17.7% were artisans, traders, transporters, civil

Table 1: Seroreactivity of sera to CATT reagents.

No of individual	Negative		Positive		Total	
	n	(%)	n	(%)	n	(%)
Male	78	90.7	08	09.3	86	100
Female	149	88.7	19	11.3	168	100
Total	227	89.4	27	10.6	254	100

Table 2: Degree of Seropositive among Catt Positive individuals

Degree of CATT positivity	Male		Female		Total	
	n	%	n	(%)	n	(%)
Mild	05	62.5	05	26.3	10	37.0
Moderate	03	37.5	11	57.9	14	51.9
Severe	00	00.0	03	15.8	03	11.1
Total	08	100	19	100	27	100

servants and retired individual. The participants awareness of HAT was 87.2%. Table 1 shows that 27(10.6%) of the sample population (n=254) tested were, CATT positive.

ALL the 27 (100%) CATT postive individuals had malaria parasites in the blood films while 21 (77.8%) had low and 6 (22.2%) moderate grade parasitaemia. They were however clinically asymptomatic. The seropositive individuals were slightly (n=10, 37%) moderately (n=14, 52%) and strongly (n=3, 11%) seropositive. 7 (25.9%) of the seropositives had cervical and anxillary lymphadenopathy and aspirates from these nodes were trypanosome negative. 23 (85.2%) of the 27 individuals were anaemic (PCV <30%) but none was below 24%. Parasitological examination of the blood, lymph node aspirate, buffy-coat and the CSF were all parasitological negative at follow-up. The white blood cells (WBC) and protein levels in the CSF, were normal.

DISCUSSION

CATT reagent has been very useful in screening for HAT in endermic focus of Abraka.[1,3,12].s Besides, it is easy to handle and also easy to train personnel to use it even at short notice. The sensitivity and specificity of CATT are said to be particularly high.[9]. However, we have observed that results from screening programmes have shown some percentage of seropositivity but the parasite positivity is discouraging.[13,14]

From this study we observed that all seropositive individuals were parasitological negative after series of parasitological examinations were performed on the blood smear and lymph node aspirates, before and at follow-up for two years. Admitted that parasites detection in blood smear, lymph node aspirate and CSF using standard diagnostics methods could be difficult. With repeated parasitological assesments, it is capable of improving the chances of finding a trypanosome in an infected individual. More importantly, a patient with sleeping sickness was expected to deteriorate and show obvert clinical signs and symptoms within the 24 months period of follow-up and sleeping sickness cases would become clinically obvious.[15]

It has been reported by Dukes D.G *et al.* that CATT results might be misleading in specific areas as a result of the absence of LiTac 1.3 antigen. Absence of this antigen in *T. b. gambiense* probably in circulation may render CATT not suitable for use in this focus.[16]. CATT has been recommended for screening of populations where the prevalence of HAT is less than 5% and the positive predictive value remains too low.[15]. Hence, the need for parasitological confirmation becomes inevitable.

Abraka is a known active sleeping sickness endemic focus[17] and may not have access to the highly sensitive serological tests such as immunofluorescence or enzymelinked immunosorbent assay due to high cost and skill required. These tests are generally used in non-endemic developed countries to screen individuals with suggestive clinical features or previous exposures.[18;19]. Since serological tests are not 100% sensitive, it was recommended to search for trypanosome in individuals with negative serological tests who have strong clinical suspicion of HAT.[20].

The inability of WHO to eradicate HAT in subSahara Africa could possibly be due to unreliability of CATT, which is the commonest and the most 'reliable' screening tool. Many seronegative individuals may continue to constitute reservoirs of infection in their communities [20].

From this study, it was observed that all seropositive individuals had malaria parasitamia. This could be indicative of cross-reactivity. This finding calls for further research to establish its implication on the reliability of the CATT. Based on the finding from this study, we conclude that CATT is not specific enough for HAT within Abraka sleeping sickness endemic focus. The observed outcome might be attributed to the Gambian type of the disease as compared to the Rhodescian type that possess the major LiTac 1.3 antigen use for the CATT reagent. To this end, more reliable screening methods should be sort to enable the various HAT control programmes have the desired objectives.

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