**Role of Serodiagnosis of Human African Trypanosomiasis (Sleeping Sickness)**

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**Abstract**

Human  African  trypanosomiasis  (HAT) or  sleeping  sickness,  is  caused  by  *Trypanosoma  brucei  gambiense*,  which  is  a chronic form of the disease present in western and central Africa and by *Trypanosoma brucei rhodesiense*, which is an acute disease located in eastern and southern Africa.

**Objective:** To evaluate the microscopical, serological (CATT; CIATT) and PCR.

**Methods:** A population of 200 serum samples from 3 displaced health centres around Khartoum state was examined usig different techniques blood films, CATT, CIATT test and PCR on whole blood and similar analysis in their cross reactivity which  resulted  in

 the  detection  of  the  false  positive  cases  of  sleeping  sickness  from  patients  with  malaria, leishmaniasis, Filarial  (*W.  bancrofite*) and  negative  cases  show  in  *Toxoplasma  gondii* and  hydatid  disease  from  non-endemic  areas  of sleeping sickness.

**Results:**  Showed  that  from  200  serum  samples  there  were  121  male  and  79  female  detected  (17.35%  and  24.05%,

 respectively) and by CIATT detected 7.0% and 6.0%, the result show more positive cases by CATT than CIATT in displaced people  in  Khartoum  state.  However  parasitological  examination  did  not  show  the  present  of  the  parasites.  But in  PCR  test from 37 blood samples there were 4 cases positive, 10.8%.

**Conclusion:** By serological analysis the CATT shows more positive cases than CIATT and can be used interchangeably with the old format of the CATT test. It is highly suitable for use in peripheral health facilities in HAT-endemic countries.

**Keywords:** HAT, CATT, CIATT, Microscopic, PCR, Non-endemic areas

**Introduction**

**African trypanosomiasis**; also known as **sleeping sickness**, is an insect-borne [parasitic](https://en.wikipedia.org/wiki/Parasitic) [disease](https://en.wikipedia.org/wiki/Disease) of humans and other animals. Is a deadly disease caused by subspecies of *Trypanosoma brucei* (Protozoa, Kinetoplastida)—*T.b. gambiense* and *T.b. rhodesiense*—transmitted to humans through the bite of insect vectors of the genus *Glossina* (tsetse flies); [1]. Checchi [2]. There are two types that infect humans, [Trypanosoma brucei gambiense](https://en.wikipedia.org/wiki/Trypanosoma_brucei_gambiense) (TbG) and [Trypanosoma brucei rhodesiense](https://en.wikipedia.org/wiki/Trypanosoma_brucei_rhodesiense) (TbR).TbG causes over 98% of reported cases. Both are usually transmitted by the bite of an infected [tsetse fly](https://en.wikipedia.org/wiki/Tsetse_fly) and are most common in rural areas. Initially, in the first stage of the disease, there are fevers, headaches, itchiness and joint pains. This begins one to three weeks after the bite.  Weeks to months later the second stage begins with confusion, poor coordination, numbness and trouble sleeping. [3].  Diagnosis is via finding the parasite in a [blood smear](https://en.wikipedia.org/wiki/Blood_smear) or in the fluid of a lymph node. [3].   A [lumbar puncture](https://en.wikipedia.org/wiki/Lumbar_puncture) is often needed to tell the difference between first and second stage disease. [3].

Preeminent in this discovery was David Bruce, who, while working in Zululand on a wasting disease of cattle known as *nagana*, identified trypanosomes in the blood of affected cattle [4,5].He then established experimentally that healthy game animals were host reservoirs of the disease, which was transmitted by the bite of the tsetse fly to domestic animals, which then became ill [5]. In 1899 the causative parasite was identified as Trypanosoma brucei, and in 1902 Everett Dutton first identified, in a European patient, a subspecies of trypanosomes called Trypanosoma brucei gambiense [5] that is now recognized as the cause of West African sleeping sickness. In 1903, Aldo Castellani, working with Bruce, identified trypanosomes in the blood and cerebrospinal fluid (CSF) of a patient with sleeping sickness [5], and in 1910 J.W.W. Stephens and H.B. Fantham first described Trypanosoma brucei rhodesiense [5], which is now recognized as causing East African sleeping sickness. There are characteristic differences between the biology and clinical features of T.b. gambiense disease and T.b. rhodesiense disease, probably due to a greater adaptation of the *gambiense* parasite to humans.

**Geographical distribution**

African trypanosomiasis is confined to tropical Africa between latitudes 15°N and 20°S, or from north of South Africa to south of Algeria, Libya, and Egypt [6] The prevalence of African trypanosomiasis outside this area varies by country and region. In 2005, major outbreaks occurred in Angola, the Democratic Republic of Congo, and Sudan. [8]In the Central African Republic, Chad, Congo, Côte d’Ivoire, Guinea, Malawi, Uganda, and Tanzania, African trypanosomiasis remains a major public health problem [7-9].

Human African trypanosomiasis (HAT) is a neglected tropical disease that occurs in sub-Saharan Africa, within the distributional limits of the tsetse fly vector. Two forms of the disease exist. The slow-progressingform, caused by Trypanosoma brucei gambiense, is found in Western and Central Africa. The faster progressing form, caused by T. b. rhodesiense, is found in Eastern and Southern Africa [8]Foci due to *T. b. gambiense* have been described in the Greater Equatoria Region bordering the Central African Republic [10]. Democratic Republic of the Congo [14, 15].  And Uganda. HAT caused by *T.b. rhodesiense* has been reported from areas of Jonglei state (Akobo County) bordering Gambella in Ethiopia [11], although since 1984 no HAT cases have been reported from either Gambella or Jonglei [20].In South Sudan, the main vector of the disease is *Glossina fuscipes*, but *G. tachinoides*, *G. pallidipes*, and *G. morsitans* have also been found in the Greater Equatoria Region [12, 13]**.**

Nine counties in Equatoria are endemic for HAT: Tambura, Ezo, Yambio, Maridi, Ibba, Mundri, Yei, Kajo Keji, and Magwi, representing a total population of 1.8 million according to the 2008 census ([Figure 1](http://europepmc.org/articles/PMC3362634/figure/pntd-0001541-g004/)). The 15,754 new cases reported from 692 locations between 2000 and 2009 have been mapped, and detailed epidemiological information on the disease was provided down to the village level. Eight hospitals and five primary health care centers had the capacity for diagnosis and treatment at one time or another between 2000 and 2010, covering all the HAT-endemic counties.



**Figure (1) Areas reporting cases of HAT in South Sudan.**

Period 2000–2009. doi:10.1371/journal.pntd.0001541.g0

**Epidemiology**

The epidemiology of Gambian trypanosomiasis is reviewed in detail. The long duration of infection in human hosts with cycles of intermittent parasitaemia, the vectors' feeding habits and the intensity of human-fly contact are the major determinants of the dynamics of transmission of this parasite. The development of immunity may lead to a reduction in the fraction of the population that is susceptible to infection and the burning out of epidemics after 20 to 30 years. So far, the acquired immune deficiency syndrome pandemic has had no impact on the epidemiology of Gambian trypanosomiasis. A brief review of the epidemiology of Rhodesian trypanosomiasis highlights the differences from Gambian trypanosomiasis that, to some extent, explain its lower propensity to cause epidemics: it is a more aggressive disease that rapidly kills its human host, and its transmission involves mostly domestic and game animals, humans being in most circumstances an accidental host. The various methods and strategies for the surveillance and control of both diseases are reviewed [14, 15]**.**

**Transmission**

The trypanosomes causing HAT are classically transmitted by the bite of blood sucking tsetse flies (Diptera, genus Glossina). T. b. gambiense can also be transmitted congenitally [16]**.** Other routes of transmission are possible but poorly documented and considered extremely rare (sexual, laboratory accidents, blood transfusion, organ transplantation [17]**.**The principal mode of transmission of HAT is vector-borne by tsetse fly. The tsetse fly is considered as a cyclical vector as the transmission requires a transformation process of the parasite in the fly. The disease is mostly transmitted through the bite of an infected tsetse fly but there are other ways in which people are infected.  In the case of high parasitemia, the mechanical transmission of the infection by tsetse flies could be possible but very rare and it has already been described for *T. b. rhodesiense*. [[18]**.**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4130665/#b91-clep-6-257)

**Pathophysiology and Etiology**

When tsetse flies bite an infected mammalian host, they ingest trypanosomes with the host's blood. After several differentiation steps in the fly, trypanosomes reach the salivary glands. With every subsequent bite, the infected tsetse fly injects trypanosomes with the saliva (19).

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**Figure (2) life cycle**

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream(1) Inside the host, they transform into bloodstream trypomastigotes(2), are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission(3), The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host (4, 5). In the fly’s midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission(6), leave the midgut, and transform into epimastigotes(7), The epimastigotes reach the fly’s salivary glands and continue multiplication by binary fission(8), The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

At the inoculation site, because of the localized inflammatory reaction, a local chancre may form, which spontaneously resolves within days to weeks. Trypanosomes multiply and spread to the lymphatic system and bloodstream. In the bloodstream, the surface of trypanosomes is entirely covered with variant surface glycoprotein (VSG), which forms a coat of identical proteins. The host mounts an effective antibody response to the VSG, resulting in opsonization (marking of the antigen for destruction by phagocytes) and parasite clearance. Trypanosomes are capable of evading this immune response by antigenic variation: they spontaneously switch their VSG coat, which is then no longer recognized by existing antibodies. [20,27]**.**

Eventually, the trypanosomes cross the blood-brain barrier and enter the CNS. They cause extensive meningoencephalitis, infiltration of white matter with inflammatory cells, perivascular cuffing, and astrocyte and macrophage stimulation. The site of invasion and the inflammatory reaction determine the appearance and type of neuropsychiatric symptoms. Brain invasion is accompanied by a strong intrathecal immunoglobulin response. [28]**.** Production of cytokines (IL-10, IL-6) and chemokines (CXCL-8, CXCL-10, and CXCL-13) in the CNS seems to be related to progression of the disease and severity of the neurologic signs [23, 26]**.**If untreated, patients usually progress to coma, severe organ failure, and eventually death[27-28]**.**

    **Symptoms:**

Following the bite of the infected fly (both male and female can transmit infection), the parasite multiplies in the lymph and the blood of the person bitten, causing unspecific symptoms and signs such as headaches, fever, weakness, pain in the joints, lymphadenopathy, and stiffness. People who become infected may or may not show signs of illness immediately, but over time the parasite crosses the blood-brain barrier and migrates to the central nervous system. Here it causes various neurological changes which include the sleep disorder (hence the name “sleeping sickness), deep sensory disturbances, abnormal tone and mobility, ataxia, psychiatric disorders, seizures, coma and ultimately death.

In the case of *T.b. rhodesiense* infections, the disease is acute, lasting from a few weeks to several months while in *T.b. gambiense* infections the disease is chronic, generally progressing slowly over several years. If left untreated, the infection worsens, and death may occur in several months to several years **(22)**.

**Diagnostic Methods**

Diagnosis of sleeping sickness is based on parasitological laboratory diagnosis blood for wet; thick and thin films stained by Giemsa stain is used for detection and identification even in the hands of well trained technologist; and serological There are two field tests for diagnosis of the disease, namely Card Agglutination Test for Trypanosomiasis CATT for diagnosis of T. b. gambiense disease; the other is the Card Indirect-Agglutination Antigen. Test for Trypanosomiasis (CIATT) for the diagnosis of T. b. gambiense and T. b. rhodesiense disease PCR examinations. Serological tests Card Agglutination Test for Trypanosomiasis (CATT) The test was carried out as described byMagnus et al. [29]**.** On the other hand, the sensitivity of the screening test can be improved by adding a Ca2+/Mg2+chelating agent (i.e. Na-EDTA) to the reaction thus avoiding a complement mediated prozonephenomenon [28, 29]**.** Is a fast and simple agglutination assay for detection of *T. b. gambiense*-specific antibodies in the blood, plasma, or serum of HAT patients. Reported sensitivity of CATT on undiluted blood varies from 68·8% to 100% [22]**;** Boatin(29),Chappuis[30]**,** Fairlamb[31]**,** andBu ̈scher**,(32),** reported specificity ranges between 83·5% and 99·3%and reproducibility is good (Kappa coefficient = 0·84) [31]**.** A preliminary evaluation of the Tryp Tect CIATT showed a high sensitivity compared to other parasitological techniques [34], but the results of subsequent studies raised strong doubts about its specificity [35]**.**

**Biological Parameters**

Biological blood parameters such as increased sedimentation rate and low hematocrit reflect the systemic chronic inflammation present in HAT patients and are therefore nonspecific. Thrombocytopenia is generally mild or absent, and features of disseminated intravascular coagulopathy are not found [36]**.** Liver transaminase levels, bilirubin, and renal function tests are usually within normal limits or slightly elevated [37]. Protein measurements usually show decreased albumin and increased immunoglobulin concentrations, especially of IgM (8). Low serum C3 levels and split C3 products can be found, reflecting complement activation**.** The following studies may also be considered: Computed tomography (CT) of the head; Magnetic resonance imaging (MRI) of the head and Electroencephalography (EEG)

**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 200 blood samples for serum separation were collected aseptically in anticoagulation and plain container, from the three health centers of displaced people’s camps who originality from endemic areas in Southern Sudan according to the willingness of the people for sampling (Convenience sampling methods). Around Khartoum state (Elsalam Medical center in central Mandela- Mayo, Dar Elsalam Jabel Awella Medical Health Center and Wed Elbasher Omdurman Medical Health Center). Was taken to the National Health Laboratory for examination. After obtaining the informed consent of patients, a questionnaire was filled for each individual and blood samples. The first step is to conduct blood and other tests. A microscope is used to confirm the presence of the agent.

**PARASIUTOLOGICAL DIAGNOSIS**

Parasitological examination Wet thick and thin blood films. In wet blood films, 5 to 10 μl of finger prick blood is placed on a slide and examined microscopically (magnification, ×400) under a cover slip. Trypanosomes can be seen moving between the erythrocytes (the movement of the surrounding erythrocytes often attracts attention). Despite its very low sensitivity, with a detection limit as high as 10,000 trypanosomes/ml, corresponding to 1 parasite/200 microscope fields, this method is still used in some centers because of its low cost and simplicity.

 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. Giemsa- or Field's-stained thin blood films have a similarly low sensitivity. Examination of 20 μl of stained blood in a thick blood film slightly improves sensitivity, with a detection threshold of around 5,000 trypanosomes/ml. It is the technique of choice for blood examination only when no centrifuge is available [37]**,** although it is quite time-consuming (10 to 20 min per slide) and requires expertise to recognize the parasite, which is frequently deformed in this preparation. Apart from trypanosomes, other parasites such as microfilaria and *Plasmodium* can be detected.

**Serological diagnosis**

**Reagents: 1. CATT reagent**

Stabilized freeze saline solution of fixed by formaldehyde-fixed and Coomassie-blue-stained bloodstream-form of T. b. gambiense.  The antigen suspension was divided into two equal volumes. One volume was processed with the standard CATT lyophilisation medium, divided over separate vials each containing 1.05 ml, and freeze dried. For the other a different lyophilisation medium was used: the volume was divided over separate vials each containing 0.375 ml and freeze dried. Each vial is reconstituted with 2.5 ml of CATT buffer and allows the performance of at least 50 tests. The freeze dried antigen and control sera should be kept at between 4 - 8С0. The shelf-life of antigen, control sera and buffer is 1 year, when refrigerated at below 10 С0. The reconstituted reagents can be used for up to7 days when stored at 4 - 8С0, or up to 8 hours under field non- refrigerated conditions. The antigens suspension must not be frozen. Each vial of the reagent sufficient for 50 tests.

**2. CATT buffer**

Phosphate buffered saline solution (PBS, PH 7.2) for reconstitution of CATT preservative: sodium azide (0.1%)

**3. Positive control serum:** To be reconstituted with 0.5ml of CATT buffer

**4. Negative control serum:** To be reconstituted with 0.5ml of CATT buffer

**CATT procedure**

1. One drop (about 45µl)well homogenized CATT reagent was placed on each area of the card 5 µl serum spread the reaction mixture using a stirring rod.
2. Mix well with stirring rod and left for 5 minutes on rotator.
3. Reading results after the 5 minutes depended on the intensity of agglutination.

**Card Indirect Agglutination Test for Trypanosomiasis (CIATT)**

**Specimen:** The test is best performed on serum

1. CIATT reagent
2. tris buffer
3. Positive control serum
4. Negative control serum

**Principle of the test:**

The CIATT reagent is a suspension of latex particles which have been sensitized with a specific monoclonal antibody against an invariant, internal antigen which is common to both T. b. gambiense and T. b. rhodesiense following the procedure described by Chappuis et al [38]**.**

**IHA (CIATT) Method:-**

**Procedure:**

1. Introduced serum:              5 µl
2. Add CIATT (IHA) reagent: 100 µl
3. Mix well with rotator or by hand for 2-3 hours after that read this is quantitative method but qualitative method is in micro titration plates of polystyrene with v bottom wells diluted the serum and positive control 1/7 (1:8) respectively in tris buffer PH 8.0 (50 µl serum: 350 µl buffer) into well1 A – Hand 50 µl of tris buffer solution into the remaining wells with the exception of negative control diluted 1:16 into well A12.

**PCR:**

The polymerase chain reaction (PCR) has been proposed for diagnosis, staging and post-treatment follow-up of sleeping sickness but no large-scale clinical evaluations of its diagnostic accuracy have taken place yet

**Other investigation tests for cross-reaction:-**

\*. Visceral leishmaniasis 30 blood samples were collected from Gaderif hospital.

\*. Serum of positive malaria parasites also from Gaderif hospital.

\*. ICT for lymphatic filariasis the 200 samples.

\*. Toxoplasmosis 15 serum samples.

\*. Hydatid disease 15 serum samples.

**Result:**

**Microscopic examination**:

 All the 200 blood films were negative by wet, thick and thinfor detection of trypomastigote.

**Serological examination**:

 Using CATT on 200 serum samples from the three health centers of  displaced people’s camps in Khartoum state, who originated from endemic areas in southern Sudan, showed 40 positive cases (20.0%) and 14 positive cases (7.0%) respectively. However, a microscopic examinations using wet, thick and thin blood film gave negative results in all samples (Table 1). Positive cases were found statistically to be higher in males than females (7.0%, 6.0% respectively (P= 0.840).

A comparison between CATT and CIATT for diagnosis of sleeping sickness was made on 200 serum samples from displaced people’s camps the result showed 40 positive cases for CATT (20.0%) but only 14 positive cases were detected by CIATT (7.0%) (The intensity of reaction is given in (Table 1 and 3). 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 (3.0% and 6.0% respectively), W. bancrofti 200serum sample (2.5%); and positive cases of toxoplasmosis and hydatidosis gave negative result. Statistically CATT and CIATT were found similar in the detection of the false positive cases from patients with malaria and leishmaniasis from non-endemic areas, while negative results in Toxoplasma gondii and hydatid disease.

**Card Agglutination Test for Trypanosomiasis (CATT)**

The result was assessed as following:-

1. Positive reaction +++……………………………(very strong agglutination)
2. Positive reaction ++……………………………..(strong agglutination)
3. Positive reaction +……………………………….( still good agglutination)
4. Negative reaction \_................................................( absence agglutination)

Table 1: show the CATT positive reaction according to the age group (20.0)

|  |  |  |
| --- | --- | --- |
| Age group | Total  | CATT |
|  |   | No  | percentage |
| 10 -19 | 4 | 0 | 0.0 |
| 20 – 29 | 27 | 7 | 26.0 |
| 30 – 39 | 65 | 14 | 22.0 |
| 40 – 49 | 59 | 12 | 20.0 |
| 50 - 59 | 25 | 5 | 20.0 |
| > 60 | 20 | 2 | 10.0 |
| Total  | 200 | 40 | 20.0 |

P. Value = 0.840

**Mandella Health Centre:**

Human African Trypanosomiasis surveys were conducted using Card Agglutination Test for Trypanosomiasis (CATT) in displaced people’s camps in Khartoum state, who originated from endemic areas in southern Sudan, the results show that18(15%) positive out of 120 serum samples from the **Mandella** health center. The titer (1:10/ 9.2%, 1:20 /3.3%, 1:40/ 2.5%). however Parasitological examination of all blood film stained by Giemsa stain did not show any parasite., and dried blood samples on filter paper done by CATT and CIATT reagent gave 19 positive result samples; the prevalence rate 15.8%. This requires a smaller quantity of reagents than that needed for the CATT on serum samples and the cost is therefore reduced the results seem less reliable than for the CATT on serum, but this depends on the technique used. Large scale evaluation is necessary to assess the reliability of CATT on dried blood samples in different epidemiological situation.

**Dar Elsalam Health Centre at Jabel Aula:**

Serological CATT in Dar Elsalam the second health centre in Jabel Aula is currently made only at the National Public Health laboratory. 73 serum samples gave 22 positive result at 5.8% in serial dilution  (1:10 /13.6% , 1:20 /10.9%, and 1:40 show 5.4%).  However no variation observed in intensity of reaction between Mandella Health Centre and Dar Elsalam Health Centre at Jabel Aula.

**Wad Elbasher Health Centre at Ombada:-**

The third Health Centre in Omdurman at Ombada Wad Elbasher 7 serum samples and blood film gave negative result.

**Blood-impregnated filter paper by CATT:**

The CATT can be performed with blood-impregnated filter paper (FP). From a total of 200 blood samples there were 37 cases positive (prevalence rate of (18.5%) as seen from the results the T.b. gambiense dried samples on filter papers be tested by CATT. This requires a smaller quantity of reagents than that needed for CATT on serum samples and the cost is therefore reduced the results seem less reliable than for the CATT on serum, but this depends on technique used.





Fig. 1A: shows CATT titre



Fig. 1B: shows CATT titre



Fig. 2: shows CATT by age group in the study group

**(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).

The result is to be read against a white background or with the aid of a mirror.

Complete agglutination of the cell                                                   Positive

Agglutination with the ring formation                                             Weekly positive

       Sedimentation cell                                                                       Negative.

Card Indirect-Agglutination Antigen Test for Trypanosomiasis (CIATT) was made on 200 serum samples from displaced people’s camps. Only 14 positive cases were detected by CIATT (7.0%) (The intensity of reaction is given in (Table 2). There was dilution of the reagent 1+7 (1:8) from 1:2, till 1:512 go on titer until 1:4096 is the significant titer before is cut-off (1:512), there were 14 samples. The difference between two tests there were variations between positive cases detected by CATT and CIATT in displace people in Khartoum state. CATT detected more positive cases than CIATT (20.0%, 14.0% respectively). However parasitological examination did not show the presence of parasites.

Table 2: show the CIATT positive according to the gender (7.0% in male and 6.0% of female).

|  |  |  |
| --- | --- | --- |
| Sex | Total  | CIATT |
|  |   | No  | Percentage % |
| Male  | 121 | 9 | 7.0 |
| Female  | 79 | 5 | 6.0 |
| Total  | 200 | 14 | 7.0 |



Fig. 4: shows CIATT positivity by age in the study group

Table 3: show the comparison between CATT (20.0); CIATT (7.0%) and filter paper (19.0%)    according to the age group

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age group | Total  | CATT | CIATT | Filter paper |
|  |   | NO | Percentage % | NO | Percentage % | NO | Percentage % |
| 10 -19 | 04 | 0 | 0.0 | 0 | 0.0 | 0 | 0 |
| 20 – 29 | 27 | 7 | 25.0 | 2 | 7.0 | 5 | 18 |
| 30 – 39 | 65 | 14 | 22.0 | 2 | 3.0 | 14 | 21 |
| 40 – 49 | 59 | 12 | 20. 0 | 7 | 12.0 | 11 | 18 |
| 50 - 59 | 25 | 5 | 20.0 | 3 | 12.0 | 5 | 20 |
| > 60 | 20 | 2 | 10.0 | 0 | 0.0 | 2 | 10 |
| Total  | 200 | 40  | 20.0 | 14  | 7.0 | 37 | 19 |

Table 4: show comparison between CATT (20.0%); CIATT (7.0%) and filter paper (19.0%) according to the gender

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sex | Total  | CATT | CIATT | Filter paper  |
|  |   | No  | Percentage% | No  | Percentage % | NO | Percentage % |
| Male  | 121 | 21 | 17.3% | 9 | 7.4 | 19 | 15.7 |
| Female  | 79 | 19 | 24.0% | 5 | 6.3 | 18 | 22.8 |
| Total  | 200 | 40 | 20.0% | 14 | 7.0 | 37 | 18.5  |

Prevalence rate by sex was found to be higher in males compared to females; in males (60.5%) and in females (35.0%). More positive cases were detected by serological screening in age group 30-39 years (33.0%) than in other groups 10-29 years and from 40-60 years.

**Polymerase chain reaction (PCR)**

**Result:** from 73 blood samples that collected from Dar Elsalam Jabel Aula there were 37 samples for PCR there were 4 positive the prevalence rate was 10.8% A simple and rapid test, the card indirect agglutination trypanosomiasis test (CIATT) is described, for detecting circulating antigens in persons suffering from Trypanosoma brucei gambiense and T. b. rhodesiense infection by latex agglutination. As sera from 30 (3.0%) patients with malaria, 30 (6.0%) with visceral



Fig.: 6: minicircle PCR profile for trypanosome DNA- electrophoresis-generating Discreet banding patterns ranging from 200-800 base per; lane 2-7 denoted pattern (A), while lane 3-6 dented pattern (B.); negative control (-); ,positive control(+). Molecular weight marker (M)123 bp

**Other investigation tests for cross-reaction:-**

A simple and rapid test, the CATT and card indirect agglutination trypanosomiasis test (CIATT) for detecting circulating antigens in persons suffering from Trypanosoma brucei gambiense and T. b. rhodesiense infection by latex agglutination. As sera from patients with malaria and leishmaniasis from non-endemic areas of sleeping sickness (3.0% and 6.0% respectively), and W. bancrofti 200serum sample (2.5%); while 15 positive serum samples of toxoplasmosis and hydatidosis gave negative result.

From Statistically CATT and CIATT were found similar in the detection of the false positive cases from patients with malaria and leishmaniasis from non-endemic areas, while negative results in Toxoplasma gondii and hydatid disease.

 Table 5: show the cross- reaction of diseases for CATT reagent

|  |  |  |
| --- | --- | --- |
| Disease  | NO examine  | CATT |
|  |   | No  | Percentage% |
| Leishmania | 30 | 2 | 6.0 |
| Malaria  | 30 | 1 | 3.0 |
| Filarial  | 200 | 5 | 2.5 |
| Toxoplasma  | 15 | 0 | 0 |
| Hydatid disease | 15 | 0 | 0 |
| Total  | 290 | 8 |  2.75 |



Fig. 3. Showing ICT test of W. bancreofti done by serum of patient  trypanosoma



Fig. 6: Shows the cross reaction of antitrypanosonmiasis antibodies (by the ICT) with antigens of W. bancrofite in the different age groups

**Discussion**

Human African Trypanosomiasis or sleeping sickness has been a public health problem in Sudan throughout most of the 20th century. Endemic foci of T. b. gambiense are found in southern Sudan**.** The disease in Sudan has been mainly of the Gambian type transmitted mostly by Glossina fuscipes fuscipes [40]. The latest civil unrest has resulted in a massive resurgence of the disease in the republic of Southern Sudan **,** in the provinces of Western and Eastern Equatoria. When a bilateral Belgian-Sudanese trypanosomiasis treatment and control programme was implemented. This effort brought the disease under relatively good control within a few years. However trypanosomiasis programme collapsed in 1990 during the Civil war. Sleeping sickness treatment in Southern Sudan remained available at a single site the La Rangu Hospital in Yambio country. As treatment drugs were in limited supply staff members were unpaid and transport to the hospital was not available, there was virtually no active case detection in Southern Sudan for more than 8 years**.**

High seroprevalence of sleeping sickness using CATT was recorded in Khartoum state. However, Khartoum 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 (9(7.0%) and5 (6.0%), 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 (Table 2). As seen from the results there was a variation between positive cases detected by CATT and CIATT (20.0%and 7.0%, 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**.**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 [41]**.** However, CATT has a number of operational limitations that hinder its large-scale implementation, especially in basic health facilities in remote areas, including the need for specialized equipment, electricity and refrigeration. The sensitivity and specificity of CATT have also been reported to be sub-optimal in a number of settings [42]**.** Moreover, recent report has suggested that CATT is not effective in all T. b. gambiense endemic foci [43]**.** 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**.** The finding was previously confirmed by 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 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(44). 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 [27, 45]. 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, but in toxoplasma gondii and hydatid disease give negative result [14, 18, 46, 50].

Polymerase chain reaction (PCR) test evaluated on DNA extracted from 37 positive blood samples which were collected from Dar Elsalam health center in Jabal Awlia by CATT samples were negative when tested parasitologically. In the 37 blood samples there were 4 cases positive by PCR. The prevalence rate was l0.8%. Since there is evidence that some of the unconfirmed CATT positive individuals are indeed infected lowering the detection limit of the PCR method [9, 51, 56].

**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 [40, 56, 60]

**Competing interests**

The authors declare that they have no competing interests.

**Author’s Contribution**

**AAMA Author’s Contribution**

**AAMA** was collected blood from displaces area, examined microscopic for the trypomastigote, and serological analysis. MEH analyzed the final manuscript; MEI is director of the lab. Was helped with her experience.

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