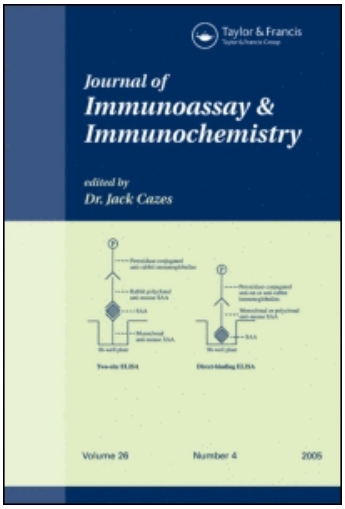

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IMMUNOASSAY OF CIRCULATING TRYPANOSOMAL ANTIGENS IN SLEEPING SICKNESS PATIENTS UNDERGOING TREATMENT.

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(KEYWORDS: Trypanosomal antigens, sleeping sickness, antigen-trapping enzyme-linked immunosorbent assay).

ABSTRACT:

Sera from 99 patients infected with *Trypanosoma brucei rhodesiense* and undergoing treatment, were analyzed for circulating trypanosomal antigens using a sandwich antigen-trapping enzyme-linked immunosorbent assay (ELISA). Trypanosomal antigens were detected in 83 (84%) of the patients. Post-treatment antigen profile in 67 patients showed five distinct patterns: in 48% of the patients antigen levels remained elevated throughout the time of hospitalisation and follow-up; in 31%, antigens dropped to the negative value by the second month; in 7.5%, antigens dropped to the negative level and became elevated afterwards; in 7.5%, antigen levels were negative initially, but later, became elevated and remained so throughout the observation period; in 6%, antigen levels remained below the negative value throughout. All patients who relapsed on follow-up had earlier shown evidence of elevated antigen profile. There were no cases of relapses among 21 patients whose antigen levels dropped subsequent to treatment. This ELISA trypanosome antigen detection test could be useful in evaluating treatment success, when used together with parasitological diagnostic techniques.

INTRODUCTION:

Chemotherapy is currently the most effective way of controlling trypanosomiasis in infected human populations. Treatment regimens are usually lengthy and involve difficult protocols, while drug resistance and parasite relapses are frequent phenomena. The process of treatment is further complicated because there is a required two-year follow-up period with frequent parasitological examinations which involve painful spinal taps. A more rapid, accurate and less painful method of determining the success of chemotherapy that reduces the period of follow-up is therefore urgently required.

In the past, attempts have been made to use serological methods in the assessment of cure by detection of circulating trypanosome-specific antibodies. These have been unsuccessful because antibodies remain in the circulation for long periods even after complete cure (1). On the other hand, parasite-detection techniques often fail to detect cryptic and chronic infections (2). To overcome these shortcomings, trypanosome antigen-detection assays have been developed and are increasingly being used as antigen detecting probes in the diagnosis of human and animal trypanosomiasis (3, 4, 5 and 6).

Recent studies have shown that circulating trypanosomal antigens drop to undetectable levels in less than 30 days following effective treatment of rabbits (3), cattle (7), camels and goats (8) and monkeys (9, 10). Circulating trypanosome antigens can be detected in animal and human clinical samples stored at $-20^{O}C$ for more than 10 years (11). Thus, it is possible that enzyme-linked immunosorbent assay (ELISA) for the detection of circulating trypanosomal antigens could efficiently pinpoint latent parasite infections and thus treatment efficacy. In the present report a polyclonal antibody sandwich ELISA was used for the detection of circulating trypanosomal antigens in *T.b.rhodesiense*-infected patients undergoing treatment at Alupe Hospital in Busia District of Kenya.

MATERIALS AND METHODS.

Patients.

The 99 patients admitted at Alupe Hospital were from Busia District of Kenya, bordering Uganda, and from the Lambwe Valley in Homa Bay District of Kenya. These patients were either referred to Alupe Hospital by the respective District hospitals and Health Centres, or diagnosed during routine sleeping sickness surveillance. Clinical history of these patients included: recurrent fever, lethargy, joint pains, weight loss, back pain, vomiting, abnormal sleeping patterns and incoordination, vision disorders, abnormal appetite and pruritis. In most patients clinical examination revealed evidence of lymphadenopathy, fever, splenomegally, oedema and clinical anaemia . Infrequently, some patients had hepatomegally, abnormal coordination or reflex, dehydration and tachycardia.

Laboratory Examination of Patients.

Blood for serum and parasitological examination was obtained from suspect patients by venipuncture. Cerebro-spinal fluid (CSF) was obtained by lumbar puncture. The presence of trypanosomes in blood samples was demonstrated by examination of wet or stained fixed blood smears, buffy coats, lymph node aspirates, and by rodent sub-inoculation. CSF was examined for leucocyte counts and the presence of trypanosomes under a microscope. All sera and CSF samples were stored at $-20^{o}C$ pending analysis. Control sera included samples from 11 malaria-positive patients and 11 healthy donors from malaria and trypanosome-free areas.

Treatment of Patients.

Patients found positive for trypanosomiasis were hospitalized for periods ranging from 4 to 5 weeks and treated with Suramin (Bayer 205, Antrypol^{R}) for early-stage cases or with melarsoprol (MelB, Arsobal^{R}) and/or difluoromethylornithine (DFMO, Ornidyl^{R} Merrell Dhow) for late-stage cases

according to the regimes described by Wellde *et al* (12) and Bales *et al* (13). Whenever possible, patients were examined clinically on routine follow-up with CSF and blood examination for periods up to 3 years or more. Serum and CSF samples were collected each time and stored at -20°C.

Raising Polyclonal rabbit anti-*Trypanosoma rhodesiense* (RATR) and RATR-Peroxidase conjugate.

T. rhodesiense KETRI 2487, clone KETat1.1, isolated from a patient in Busoga (Uganda) in 1972, was used to raise RATR polyclonal IgG (RATR-IgG). Briefly, five adult male Wistar rats were inoculated intraperitoneally with $1x10^6$ trypanosomes per rat. Trypanosomes were harvested from rat blood at peak parasitaemia as described by Lanham and Godfrey (14). Lysate antigen was prepared by ultrasonication followed by centrifugation according to the method of Rae and Luckins (3).

Polyclonal rabbit anti-*T.b.rhodesiense* IgG (RATR-IgG) was raised in two male Newzealand White rabbits as described by Thalhamer and Freund (15). Horse radish peroxidase (Type VI, Sigma) was conjugated to RATR-IgG by the method of Wilson and Nakane (16) as modified by Henning and Nielsen (17).

Sandwich-ELISA:

RATR-IgG was used to coat micro-ELISA plates (Dynatech Immulon^R USA) at a concentration of 10µg/well in 100µl of carbonate-bicarbonate buffer, pH 9.6. Coated plates were incubated at 37°C for 1 hour and overnight at +4°C. Thereafter, the plates were washed thrice (5 minutes per wash) with 0.01M PBS Tween-20 pH 7.4, before 100µl/well of neat test and control sera were added and plates incubated at 37°C for 1 hour. The plates were washed again and 100µl/well of RATR-peroxidase conjugate added at a dilution of 1:1200. The incubation was repeated at 37°C for 1 hour and plates washed again. Orthophenylenediamine (OPD) was added and plates incubated at room temperature for 30 minutes.

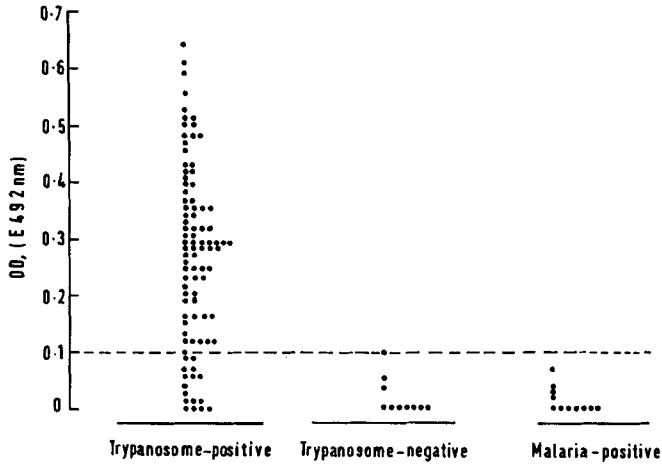

Fig. 1: Scatter diagram of antigen levels in trypanosome-positive, trypanosome-negative and malaria-positive samples. (-------) Cut-off for a positive Ag-ELISA.

The reaction was stopped by adding 50µl/well of 2.0M H_2SO_4, and optical density (OD) readings determined at 492 nm using a Dynatech Micro-ELISA plate reader (MR 580). All serum samples were tested in duplicate.

RESULTS:

Of the 99 patients tested, 83 (84%) showed elevated antigen levels, with OD readings beyond 0.10. None of the sera from malaria-infected patients or trypanosome-free healthy donors showed as readings beyond 0.10 (Fig. 1). Therefore OD reading of 0.10 was used as the cut-off point for a positive antigen-ELISA. Post-treatment antigen profiles in 67 patients sampled for periods of 6 months or more showed five distinct patterns: in 48% antigen levels remained elevated throughout (e.g Fig. 2, patient No. 53 with evidence of parasite relapse); in 31% of the patients, the levels were elevated initially but dropped below the cut-off point (e.g. Fig. 3, patient No. 111); in 7.5% of the patients antigens dropped to negative level and became elevated

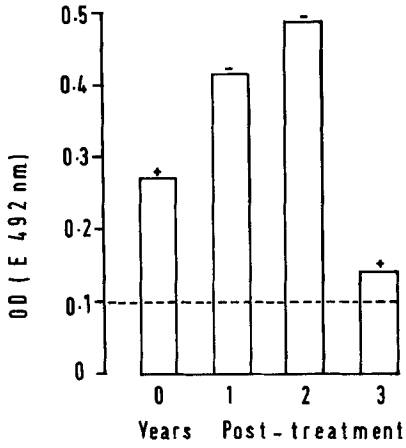

Fig. 2: Antigen profile in patient No. 43. 48% of 67 patients showed this type of profile.(-------) Cut-off for a positive Ag-ELISA; (+) parasites were detected; (-) no parasites were detected.

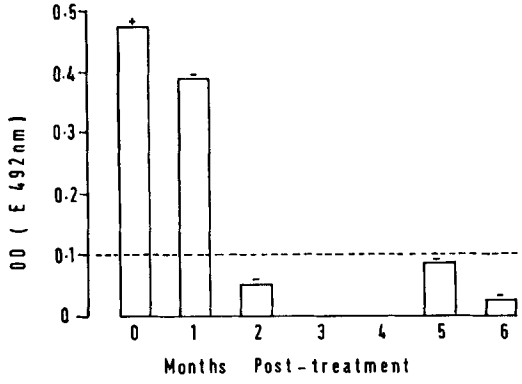

Fig. 3: Antigen profile in patient No. 111. 31% of 67 patients showed this type of profile. (---------) Cut-off for a positive Ag-ELISA; (+) parasites were detected (-) no parasites were detected.

afterwards; in 7.5% antigen levels were negative initially but became elevated later; in 6.0% antigen levels remained negative throughout. Thus, antigens remained elevated in 42 (63%) out of 67 patients despite treatment. All patients who relapsed on follow-up had earlier shown evidence of elevated antigen profile (Fig. 2). There were no cases of relapse among the 21 patients whose antigen levels dropped subsequent to treatment.

DISCUSSION:

The ELISA antigen detection test was positive in most (83 out of 99) of parasitologically positive cases. The failure for the test to pick 16 parasite-positive cases could be due to antigen absorption by the patients' antibodies, or possibly, not enough antigens had been released into circulation at the time of sampling (4). Persistent elevation of circulating antigens in 42 patients despite treatment, may be indicative of chemotherapeutic failure since several studies have shown that following successful treatment, antigen levels fall below detectable range (3, 6, 7, 9). Such a fall to undetectable levels was demonstrated in 21 patients (31%) in the current study.

The present results demonstrate that assays designed to measure circulating trypanosomal antigens may be used to assess treatment success more efficiently than parasitological techniques or tests which depend on antibody detection. Trypanosomes of the ***brucei*** subgroup, being tissue parasites (18), may hide in the CNS and visceral tissues despite their absence in the peripheral circulation. This could explain the inability of the parasitological tests used in the present study to detect parasites in some of the patients, despite elevated antigens levels. Thus, the use of indirect tests like the one described here may be the only practical way of demonstrating the presence of parasites in such patients. Monoclonal or polyclonal antibodies have been used successfully to this end as antigen detecting probes (3, 4). However, since the ELISA antigen detection test may fail to detect a few parasite positive patients, it should never be used in isolation.

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REFERENCES:

1. **Luckins, A. G.** ***Trypanosoma evansi*** in Asia.
Parasitology Today. 1988; 4: 137-142.

2. **Killick-Kendrick, R.** The diagnosis of trypanosomiasis of livestock: A review of current techniques.
Vet. Bull. 1968; 38: 191-197.

3. **Rae P. F. and Luckins, A. G.** Detection of circulating trypanosomal antigens by enzyme immunoassay.
Ann. Trop. Med. Parasit. 1984; 78: 587- 596.

4. **Rae, P. F. Thrusfield, M. V., Higgins, A., Aitken, C. G. G., Johns T. W. and Luckins, A. G.** Evaluation of enzyme immunoassays in the diagnosis of camel (***Camelus dromedarius***) trypanosomiasis: Preliminary investigation.
Epidem. Inf. 1989; 102: 297-307.

5. **Nantulya, V. M.** An antigen detection enzyme immunoassay for the diagnosis of ***Trypanosoma rhodesiense*** sleeping sickness.
Parasit. Immunol. 1989; 11: 69-75.

6. **Olaho-Mukani, W., Munyua, W.K. and Njogu, A.R.**
An enzyme-linked immunosorbent assay (ELISA) for the detection of trypanosomal antigens in goat serum, using a monoclonal antibody.
J. Immunoassay. 1992; 13: 217-227.

7. **Nantulya, V. M. and Lindqvist, K. J.** An antigen detection enzyme immunoassay for the diagnosis of ***Trypanosoma vivax, T. congolense*** and ***T. brucei*** infections in cattle.
Trop. Med. Parasit. 1989; 40: 267-273.

8. **Olaho-Mukani., W.** Production and characterization of monoclonal antibodies to ***Trypanosoma (Trypanozoon) brucei evansi*** and their application as immunodiagnostic regents. PhD. Thesis. University of Nairobi. 1989.

9. **Liu, M. K. and Pearson, T. W.** Detection of circulating trypanosomal antigens by double antibody-ELISA using antibodies to procyclic trypanosomes.
Parasitol. 1987; 96: 277-290.

10. **Liu, M. K., Pearson, T. W., Sayer, P. D., Gould, S. S., Waitumbi, J. N. and Njogu, A. R.** Serodiagnosis of African sleeping sickness in vervet monkeys by detection of parasite antigens.
Acta Trop. (Basel). 1988; 45: 371-330.

11. **Olaho-Mukani, W., Ngaira, J.M. and Mbwabi, D.** Detection of trypanosomal antigens and antibodies in stored samples. **In: Kinoti, S.N., Omuse J.K. and Kofi-Tsepko W.M., (eds).** Recent Advances in Medical Research. 1989; 61-64.

12. **Wellde, B. T., Chumo, D. A, Reardon, M. J. *et al.*** Treatment of Rhodesian Sleeping Sickness in Kenya.
Ann. Trop. Med. Parasit. 1989; 83: 99-109.

13. **Bales, J. D. J. Harrison, S. M., Mbwabi, D. L and Scheter, P. J. .** Treatment of arsenical refractory Rhodesian sleeping sickness in Kenya.
Ann Trop. Med. Parasit. 1989; 83, 111-114.

14. **Lanham,S.M. and Godfrey,D.G.** Isolation of Salivarian Trypanosomes from man and other mammals using DEAE-cellulose.
Exp. Parasitol. 1978; 23:521-534.

15. **Thalhamer, J. and Freund, J.** Cascade Immunization: A method of obtaining polyspecific antisera against crude fractions of antigens.
J. Immunological Methods. 1984; 66: 245-251.

16. **Wilson, M. B. and Nakane, P. K.** Recent developments in periodate method of conjugating horseradish peroxidase (HOP) to antibodies 215-224. In: Immunofluorescence and Related Staining Techniques.
New York. Elsevier/North Holland Biochemical Press.

17. **Henning, D. and Nielsen, K.** Peroxidase-labelled monoclonal antibodies for use in enzyme immunoassay.
J. Immunoassay. 1987; 81: 297-307.

18. **Losos, G. J. and Ikede, B. O.** Review of the pathology of the disease in domestic and laboratory animals caused by ***Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense*** and ***T. gambiense.***
Vet. Path. 1972; 9: 1-50.