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SERUM BIOCHEMICAL CHANGES IN EXPERIMENTAL GAMBIAN TRYPANOSOMOSIS
I. ENZYMES AND ELECTROLYTES

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ABSTRACT

Serum enzyme and electrolyte changes associated with Gambian trypanosomosis (sleeping sickness) were studied in vervet monkeys infected with *Trypanosoma brucei gambiense.*

Although Serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) concentrations of the monkeys increased at various points after infection, the levels of alkaline phosphates (AP) decreased. Similarly, serum sodium and potassium levels increased while those of calcium and phosphate decreased. These changes suggested that hepatic, renal and parathyroid damage are some of the paathological features of Gambian sleeping sickness in man leading to early death of untreated persons.

Key Words: Gambian trypanosomosis, enzymes, electrolytes, *Trypanosoma brucei gambiense*, monkeys.

INTRODUCTION

Human African Trypanosomosis (sleeping sickness) still ravages in several parts of sub-Saharan African despite decades of efforts aimed at control (WHO, 1998; Jannin, 2000). In Nigeria and other countries, the disease now poses as an emerging public health crisis (Airauhi, *et al* 2001; Stich *et al* 2003). African trypanosome infections are generally characterised by haematological and serum biochemical aberrations, the severity of which are often determined by strain of infecting trypanosomes and the host (Anosa, 1988 a and b). In West and Central Africa, a chronic form of the disease caused by *T. b. gambiense* has been described (Scott, 1970).

Lack of adequate information on the factors associated with the pathogenesis of the disease and conflicting reports of observations in experimental animal models have posed an important obstacle to chemotherapy and management of the disease in man (Yesufu, 1971; ISCTRC, 1985; Emeribe and Anosa, 1991; Mahamane *et al* 1997).

This study is therefore an attempt to evaluate some of the serum biochemical changes induced by *T. b. gambiense*, using the vervet monkey as a model.

MATERIALS AND METHODS

Research Animals: A total of four male vervet monkeys (*Cercopithecus aethiops)* weighing between 2.00 and 3.75kg body weight were used for the study. The monkeys were purchased from Kaduna in Kaduna State and Owerri in Imo State of Nigeria and acclimatized for six months before use. During this period the primates were screened and found to be parasitologically negative for trypanosomes. They were also dewormed using Levamisole hydrochloride (Ketrax®, Welcome, Lagos, Nigeria) against round worms, tape worms and hook worms. Broad spectrum antibiotic, 5% Oxytetracycline (Pfizer, Lagos, Nigeria) was also given at the dose of 1.0ml/10kg body weights intramuscularly while multivitamin injection (Tanvit-in®, SKM Pharma, Bangalore, India) was given at the rate of 3.0mls/animal via the intramuscular route. The animals were fed cooked beans, bananas, pawpaw fruits and pawpaw leaves, while water was provided ad libitum. All the animals added weight during acclimatization period.

Trypanosome parasites: Two strains of *T. b. gambiense,* NITR/Abraka and IL3250, all isolated from infected people were used. The parasites were cryopreserved in liquid Nitrogen and sub passaged once into donor mice from where they were used to inoculate the monkeys.

Experimental Design: The monkeys were initially divided into two groups of two animals each. One group was infected with the NITR/Abraka strain while the other group was infected with IL3250 strain. Each group was inoculated with 2 x 10^6 parasites (Lumsden *et al* 1973) intraperitoneally. Preinfection data collected for four weeks served as control for each group. Observations from the two groups were later combined and treated as a single group because of lack of difference between the groups.

Sample Collection and Analysis :Blood for the separation of serum was obtained by venipuncture of the femoral vein using 21 gauge hypodemic needles and 5ml syringes. The concentrations of serum ASAT and ALAT were measured as described by Henry *et al* (1960). The serum AP and electrolyte concentrations wear determined as described by King and Armstrong (1934) and Toro and Ackerman (1975) respectively. Data obtained was Analysed using analysis of variance to determine level of significance.

RESULTS AND DISCUSSION

The serum ASAT concentrations of the monkeys increased sharply after infection from the mean pre-infection value of 38.1 ± 2.2 I.U/L to the value of 66.7 ± 19.7 by week 2 post-infection (PI) and continued to increase there after over the next 8 week period attaining the maximum value of 154.5 ± 8.5 I.U/L by week 10($P<0.05$; Fig.1). The pre-infection serum ALAT concentration of the monkeys was 35.6 ± 3.5 I.U/L. After infection, it increased in the first four weeks attaining a maximums value of 53.5 ± 20.4 ($P< 0.05$) and there after declined slightly below pre-infection value for four weeks and increased there after to 50.0 ±16.0 IU/L by week 10PI. The AP value of the primates was 30.5 ± 2.5 I.U/L before infection. After infection, it showed fluctuating decrease through out the ten week observation period (Fig. 1). The overall changes in the enzyme levels of the monkeys are summarized on Table I.

The serum electrolyte changes in the infected monkeys were as shown on Table II. The pre-infection calcium concentration was 10.0 ± 0.5mg/dl. After infection, the calcium concentration showed fluctuating decrease through out post infection period with maximum decrease to 2.1 ± 0.2mg/dl be week 4Pl ($P< 0.05$). The serum phosphate concentration before infection was 5.1 ± 0.6mg/dl but decreased significantly after

infection ($P< 0.05$) with only slight increases by weeks 6 and 8. Sodium concentration however increased from the 141 ± 9.1mml/L value before infection from week 2 Pl up to week 8 and then declined by week 10Pi ($P> 0.05$).

The serum potassium concentration of the vervet monkeys before infection was 5.0 ± 0.5mmol/L. After infection it increased for the first eight weeks Pl with a maximum increase of 7.3 ± 2.4mmol/L by week 8. It however decreased to 3.4 ± 0.6mmol/L by week 10 Pl ($P< 0.05$).

Elevations in the serum ASAT and ALAT concentrations of the *T. b. gambiense* -infected vervet monkeys supports earlier observations in *T. b. rhodesiense* infection of mice (Moon *et al* 1968), monkeys (Sadun *et al* 1973), man (Wellde *et al* 1989a) and cattle (Wellde *et al* 1989b). Although normal levels of these enzymes were reported in a case of congenital gambian trypanosomosis resulting to death (Olowe, 1975), increases in serum concentration of ASAT and ALAT has been attributed to organ damage involving liver and kidney (Poltera, 1985). The decrease in the level of serum AP in the infected monkeys also agrees with earlier observation of Moon *et al* (1968) in *T. b. rhodesiense* - infected mice. Increases in serum AP reported in *T. brucei* - infected rabbits was attributed to obstructive jaundice (Arowolo *et al* 1988) implying that such changes did not occur in the *T. b. gambiense* infected monkeys.

TABLE I: Summary of Serum enzyme changes in *T. b. gambiense* - infected monkeys.

Serum Enzyme	Pre-infection	Post-infection
Aspartate aminotransferase (I.U/L)	38.1 ± 2.2	119.5 ± 37.9
Alanine aminotransferase (I.U./L)	35.6 ± 3.5	42.3 ± 9.9
Alkaline phosphatase (I.U./L)	30.5 ± 2.5	29.1 ± 1.1

Serum calcium levels decreased in the infected monkeys. These changes were accompanied by decrease in the levels of phosphate ions. Depression of calcium and phosphate levels has been associated with severe damage to the parathyroid (Fiennes *et al* 1946). Similar observations have been reported in *T. brucei* - infected goats (Adejinmi and Akinboade,2000) as well as cattle infected with *T. b. rhodesiense* (Weillde *et al* 1989b). Increases in the levels of sodium level observed in the vervet monkeys after infections was consistent with observations in rabbits infected with *T. brucei* (Goodwin and Guy, 1973) and in acute trypanosomosis of goats due to *T. vivax* infection (Abenga *et al* 2002). Although Barret-Connor *et al* (1973) reported hyponatraemia in humans infected with *T. b. rhodesiense,* hypernatraemia in trypanosomosis has been attributed to renal dysfunction (Carison, 1989; Abenga *et al,* 2002). Increases in the serums potassium level in the *T. b. gambiense* - infected vervet monkeys is also consistent with recent observations of Abenga *et al* (2002) in goats infected with *T. vivax.* This varied from the observations of Adejinmi and Akinboade (2000) who reported depressed serum potassium level in goats infected with *T. brucei.* Increase in potassium correlated with decrease in red blood cell (RBC) values and has been attributed to potassium from damaged RBC and tissues (Ikejiani, 1946).

TABLE II: Mean Serum Electrolyte Values of Monkeys infected with *T.b gambiense*

Electrolyte Parameter	Pre-Infection	Post-Infection				
		Week 2	Week 4	Week 6	Week 8	Week 10
Calcium (mg/dl)	10.0 ± 0.5	6.0 ± 0.3	2.1 ± 0.2	7.2 ± 0.7	8.1 ± 1.8	8.5 ± 1.0
Phosphate (mg/dl)	5.1 ± 0.6	3.6 ± 0.2	1.9 ± 0.2	5.7 ± 0.3	5.2 ± 1.2	2.7 ± 0.7
Sodium (mmol/L)	141.1 ± 9.1	149.0 ± 6.1	151.5 ± 5.0	145.0 ± 4.7	149.7 ± 10.4	132.0 ± 9.7
Potassium (mmol/L)	5.0 ± 0.5	5.8 ± 0.2	6.55 ± 0.9	6.6 ± 0.3	7.3 ± 2.4	3.4 ± 0.6

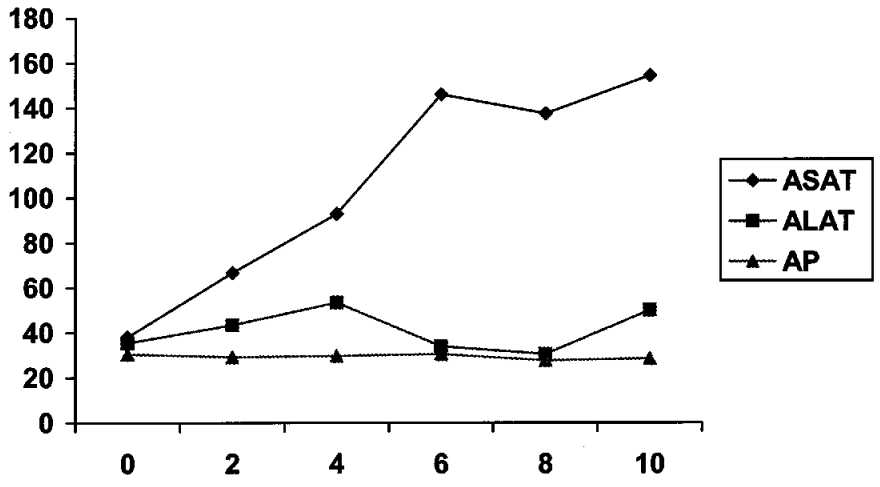

Fig. 1: Serum enzymes in T.b. *gambiense*-infected monkeys

Changes in the serum enzyme and electrolyte levels of *T. b. gambiense* - infected vervet monkeys suggest that hepatic, renal and parathyroid damage occurred in the monkeys and are some of the early pathological features of Gambian trypanosomosis leading to death of infected victims. The course of disease observed in this study was however much severer than the chronic nature of Gambiense sleeping sickness in man and the mild course of *T. b. gambiense* described in monkeys (Yesufu, 1971) and baboons (Kageruka *et al* 1991). The virulent course of disease precipitated by *T. b. gambiense* in the vervet monkeys is believed to have arisen from high antigenic nature of the strains of trypanosome used. This result supports the existence of atypical *T. b. gambiense* strains with resultant Rhodesiense-like virulent course leading to an early death of untreated victims. Molecular characterization and surveillance to determine the distribution of such strains is required for proper planning of control strategies in sleeping sickness endemic countries.

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REFERENCE

Abenga J. N.; Sanda, S. A.; Idowu, T. B. and Lawani, F. A. G. 2002 Effect of acute caprine trypanosomiasis on haemoglobin, urea and serum electrolytes. *Afr. J. Clin. Exp. Microbiol.* 3(1): 45-47

Adejinmi, J. O. and Akinboade, O. A. 2000 Serum biochemical changes in WAD goats with experimental mixed. *T. brucei* and *Cowdria ruminantum* infections. *Tropical Vet.* 18 (1-2): 111-120

Airauhi, L; Unuigbe, E. I. and Airauchi O. D. 2001 Human Sleeping Sickness (SS) in Nigeria: knowledge, attitude and beliefs in a focus in the Abaraka belt, Delta of Nigeria. *Afr. J. Clin Exp. Microbiol.* 2(2):6-9.

Anosa, V. O. 1988a Haematological and biochemical changes in human and animal trypanosomiasis, Part I. *Revue Elev. Med. Vet. Pays. Trop 41* (1):65-78.

Anosa, V. O. 1988b Haematological and biochemical changes in human and animal trypanosomiasis. Part II. *Revue Elev. Med. Vet. Pays. Trop 41*(1) 151-164.

Arowolo, R. O. A.; Elhassa, E. O. and Amure, B. O. 1988 Assessing hepatic dysfunction in rabbits experimentally infected with *T. brucei. Revue Elev. Med. Vet. Pays. Trop. 41*(3): 277-281.

Barret-Connor, E.; Ugoretz, J. and Braude, A. I. 1973 Disseminated intravascular coagulation in trypanosomiasis. *Arch. Int.* 31: 574-577.

Carison, G.P. 1989 Fluid electrolytes and acid base balance. 4th Ed. In: Kaneko, J. J. (ed). Clinical biochemistry of domestic animals. San Diego, California Press Inc: Pp. 678-743.

Emeribe, A. O. and Anosa, V. O. 1991 Haematology of experimental *T. gambiense* infection. II. Erythrocyte and leucocyte changes. *Revue Elev. Med. Vet. Pays. Trop 44*(1): 53-57.

Fiennes, R. N. T. W.; Jones, R. E. and Laws, S. G. 1946 The course and pathology of *T. congolense* disease in cattle. *J. Comp. Path* 56:1-27.

Goodwin, L. G. and Guy, M. W. 1973 Tissue fluids in rabbits infected with *T. brucei. Parasitol. 63*(3): 499-513.

Hendry, R. J.; Chiamori, N; Golub, O. J. and Berkman, S. 1960 Revised spectrophotometric methods for the determination of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic acid dehydrogenase. *Ann. J. Clin. Path. 34:*381-383

Ikejiani, O. 1947 Studies in trypanosomiasis III. The plasma, whole blood and erythrocyte potassium in rats during the course of infection with *T. brucei* and *T. equiperdum J. Parasitol 32:* 379 - 382.

ISCTRC, 1985. Reports and recommendation of the 18th meeting of the Organisation of African Unity Scientific, Technical and Research Commission Harare, Zimbabwe.

Jannin, J. 2000 Actualities de la trypanosomiase humaine. *Med. Trop. 60* (2 suppl): 565 - 567.

Kegeruka, P.; Mangus, E; Bajyana Songa, E.; Nantulya, V.; Jochems, M.; Hamers, R. and Mortelmans, J. 1991 Infectivity of *Trypanosoma (Trypanosoon) brucei gambiense* for baboons (*Papio hamadryas, P. papio). Ann. Soc. Belge Med. Trop. 71:* 39-45.

Lumsden, W. H. R.; Herbert, W. J. and M'neillage, G. J. C. 1973 Technique with trypanosomes. Churchill Livingstone, Edinburgh and London, pp 101-103.

Mahamane Keita, Benard Bouteille, Bertin Enanga, Jean Michael Vallat, Michael Dumas 1997. *T. b. brucei:* A long-tern model of human African trypanosomiasis in mice, meningo-encephalitis, astrocytosis and neurological disorders. *Exp. Parasitol.* 85: 183-192.

Moon, A. P.; Williams, J. S. and Witterspoon, C. 1968 Serum biochemical changes in mice infected with *T. rhodesiense* and *T. dutoni. Exp. Parasitol 22:* 112 - 121.

Olowe, S. A. 1975 A case of congenital trypanosomiasis in Lagos. *Trans. R. Soc. Trop. Med. Hyg. 69*(1): 57-59.

Poltera, A. A. 1985 Pathology of human African trypanosomiasis with reference to experimental African trypanosomiasis and infections of the central nervous system. *Brit. Med. Bull.* 41(2). 169 - 174.

Sadun, E.; Johnson, A.; Nagle, R. and Duxbury, R. 1973. Experimental infections with African trypanosomiasis V. Preliminary parasitological, clinical, haematological, serological and parasitological observations in rhesus monkeys infected with *T. rhodesiense. Am. J. Trop. Med. Hyg. 22:*323-330.

Scott, D. 1970 The epidemiology of Gambian sleeping sickness. In: The African trypanosomiasis (Ed. H. W. Mulligan), Allen and Unwin, London Pp. 614 - 644.

Stich, A; Barrett, M. P. and Krishna, S. 2003 Waking up to sleeping sickness. *Trens Parasitol.* 19(5): 195-197.

Toro. G. and Ackerman, P.G. 1975. Practical Clinical Chemistry. 5th Edition, Little, Browne and Company, Boston.

Wellde, B. T.; Chumo, D.; Reardon, M. J; Mwangi, J. Asenti, A.; Mbwabi, D.; Abinya, A.; Wanyama, L. and Smith, D. H. 1989a. Presenting features of Rhodesian sleeping sickness patients in the Lambwe valley Kenya. *Ann. Trop. Med. Parasit.* 83 (Suppl): 73-79.

Wellde, B. T.; Reardon, M. J.; Kovatch, R. M.; Chumo, D. A. Williams, J. S.; Boyce, W. L.; Hockmeyer, W. T. and Wykoff, D. E. 1989b. Experimental infection of cattle with *T. b. rhodesience. Ann. Trop. Med. Parasitol,* 83 (Suppl. 1): 133-150.

WHO, 1998. Control and surveillance of African trypanosomiasis. World Health Organization Technical Report Series No. 881.

Yesufu, H. M. 1971. Experimental transmission of *T. gambiense* to domestic animals. *Ann. Top. Med. Parasitol.* 65(3): 341-347