

Full Length Research Paper

The biochemical changes induced by natural human African trypanosome infections

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Biochemical evaluation of body fluids gives an indication of the functional state of the various body organs. An evaluation of the biochemical parameters of plasma and cerebrospinal fluid (CSF) in humans infected with *Trypanosoma brucei gambiense* was carried out. A significant elevation ($P < 0.01$) in the plasma values of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were recorded in study participants, while lowered triglyceride levels ($P > 0.01$) were recorded in the infected participants. The levels of plasma electrolyte recorded were within normal reference intervals. Elevated globulin and decreased albumin levels were recorded in infected study participants. Levels of total protein, total bilirubin, Na^+ , Cl^- , HCO_3^- showed no significant increases ($P > 0.05$) during infection. Elevated levels of AST was recorded in the CSF of study participants, the values of CSF enzymes recorded in study were generally lower in trypanosome infected study participants compared to the uninfected participants. Lowered levels of CSF chloride and urea were recorded in the study while the values of potassium, calcium and creatinine in CSF were elevated ($P < 0.05$) in study participants. The results suggest infiltration, inflammation and probable damage of vital body organs resulting from infections with *T. b. gambiense*.

Key words: *Trypanosoma brucei gambiense*, biochemical changes, body fluids, electrolytes, enzymes, plasma, cerebrospinal fluid, human natural infections.

INTRODUCTION

Human African Trypanosomiasis, an endemic disease in 36 sub-Saharan countries is invariably fatal if not treated. Antigenic variation a mechanism by which the parasite evades the host immune system, results in the fluctuating parasitaemia that characterizes the disease. This phenomenon underlies the wide spectrum of systemic dysfunction and the infection of multiple organs by the parasite (Atougua and Kennedy, 2000).

Biochemical changes have also been observed to be associated with trypanosome infection in man and animals and several factors have been found to influence the nature and severity of these changes (Anosa, 1988). These include the strain of the infecting agent and individual variability in susceptibility to infection. Varying observations of biochemical changes have been reported in studies of trypanosome infections in animals. Taiwo et al. (2003) reported elevated levels of total protein, globulin and decrease in cholesterol and glucose levels in sheep experimentally infected with *T. b. brucei* and *T. congolense*. Normal Na, K, Ca and PO_4 levels have been

reported in acute experimental *T. rhodesiense* infections of mice (Raisinghaim et al., 1981; Singh and Gaur, 1983), while hyponatraemia was reported in human *T. b. rhodesiense* infections (Barett-Conor et al., 1973). Abenga and Anosa (2004) reported increased protein, creatinine and globulin levels in monkeys experimentally infected with *T. b. gambiense*. Serum lipids and lipoproteins were also reported to show variations in protozoan infections generally and trypanosomiasis in particular (Diehl and Risby, 1974; Huet et al., 1990). It was reported that Africans living in regions in which malaria and other protozoan infections are endemic commonly have altered serum protein levels with decrease in albumin and raised globulin content of serum (Woodruff, 1959; Barett-Conor et al., 1973; Facer, 1976; Anosa and Kanenko, 1983). The concentrations of proteins in the plasma have been reported to influence the amount in the cerebrospinal fluid (CSF) with a linear relationship between the relative abundance of particular proteins in the CSF compared with that in the serum.

Table 1. Plasma electrolyte and enzyme parameters in Human African Trypanosomiasis.

Parameters	Infected subjects	Endemic negative control	Non-endemic negative control	SI Reference Intervals
Sodium (mmol/L)	134.75±1.41 ^{ab}	139.20±1.24 ^a	136.17±2.01 ^{ab}	135-147
Potassium (mmol/L)	4.58±0.09 ^a	4.52±0.11 ^a	3.7±0.22 ^d	3.5 - 5.0
Chloride (mmol/L)	102.25± 0.5 ^{ab}	103.80±0.74 ^a	102±1.14 ^{ab}	95 -105
Bicarbonate (mmol/L)	22.55±0.47 ^a	23.80±0.49 ^a	22.16±1.01 ^a	22-28
Calcium (mmol/L)	2.11±0.04 ^b	2.27±0.08 ^{ab}	2.08±0.05 ^a	2.1-2.8
Phosphorous (mmol/L)	1.32±0.04 ^a	1.51±0.07 ^a	1.29±0.05 ^a	1.0-1.5
ALT (GPT) (U/L)	80.9±15.53 ^{ab}	48±16.24 ^d	26.0±3.40 ^d	8-20
AST (GOT) (U/L)	219.3±27.65 ^{ab}	140±47.96 ^{ab}	34.67±2.23 ^d	8-20
ALP (GPT) (U/L)	158.8±20.68 ^b	186±26.02 ^{ab}	79.17±10.35 ^{bc}	20-70
GT (U/L)	14.95±2.05 ^{ab}	12.80±1.53 ^{ab}	14.67± 2.35 ^{ab}	4-60

*Values on the same row with different superscripts differ significantly (P<0.05).

However the powerful mechanisms of the blood-brain barrier and the choroid plexus have been known to keep ions constant in the CSF and the extracellular fluid (ECF) of the brain. Bradbury et al. (1963) reported that the electrolytes of the CSF probably mirror those of the ECF of the nervous tissues. The variations in the concentration of these ions in CSF will vary with plasma osmolarity. Injury or diseases of the CNS have been known to result in elevated levels of the enzymes in the CSF (Wilkinson, 1962).

The current understanding of the pathology of *T. b. gambiense* depends largely on experimental infections of animals (Poltera, 1985; Abenga and Anosa 2004). In attempts to understand human infections, data collected from experimental studies have been extrapolated to human situations. The results obtained from such studies vary from the typically chronic *T. b. gambiense* infection in man (Raisinghaim et al., 1981; Singh and Gaur, 1983; Anosa, 1988; Abenga and Anosa, 2005). There exists still, a dearth of information on biochemical changes occurring in natural human trypanosomiasis. This study therefore reports biochemical changes induced in *T. b. gambiense* natural human infections in Nigeria.

MATERIALS AND METHODS

Sample collection

Blood (3 ml) was collected by venipuncture from subjects identified in the prevalence study (unpublished) into EDTA bottles. Plasma was obtained from blood by centrifugation at 2,000 g for 10 min at 27°C. The study controls were made up of five parasite negative subjects each from an endemic and non-endemic area. Cerebrospinal fluid was collected from 4 CSF-trypanosome positive subjects and 4 negative controls, by lumbar puncture under aseptic conditions by medical personnel in hospitals affiliated to the study.

Biochemical analyses

Plasma and cerebrospinal fluid samples were subjected to biochemical analyses. Determination of sodium, potassium and

chloride levels were as described by Schales and Schales (1941). Inorganic phosphate was estimated by the method of Gomori (1942), while calcium, bicarbonate and triglyceride were estimated as described by Toro and Ackerman (1975). Urea concentration was determined by the method of Crocker (1967). Total protein was estimated by the Biuret method and albumin was estimated by the bromocresol green method of Doumas et al. (1971). Globulin was calculated from the difference between total protein and albumin, while the albumin: globulin ratio was determined by the method of Coles (1968). Aspartate transaminase (AST) and alanine transaminase (ALT) activity were estimated by the method of Reitman and Frankel (1957). Gamma glutamine transaminase (GT) and alkaline phosphatase (ALP) activities were determined as described by Frajola et al. (1965). The data obtained was analyzed using Duncan's multiple range tests for variables and correlation Analysis

RESULTS

Results from the study showed a marked elevation of plasma enzyme levels. AST, ALP and ALT in trypanosome positive subjects were significantly (P<0.01) higher than values obtained for the controls (Table 1). There were no significant differences (P>0.05) in the values of the plasma urea, total protein, albumin, globulin, total bilirubin, creatinine, chloride, bicarbonate, inorganic phosphorous and GT between trypanosome positive subjects and the controls. However, increased plasma globulin and decreased albumin levels were recorded in the study. The values of potassium recorded in infected subjects and endemic controls were significantly elevated (P<0.01) compared to values in non-endemic controls. The values of calcium and inorganic phosphorous recorded in control subjects from endemic area were significantly higher (P<0.01) than values obtained from infected and non-endemic control subjects. It was observed that the plasma electrolyte values recorded in the study samples were within the normal reference ranges (Table 1).

Lower cholesterol and triglyceride levels (P>0.01) were recorded in infected subjects while creatinine levels in

Table 2. Mean and SD values of Plasma proteins and metabolite from Human African Trypanosomiasis infections.

Parameters	Infected subjects	Endemic negative controls	Non-endemic negative control	SI Reference Intervals
Total Protein g/L	78.3±2.3 ^a	79± 3.6 ^a	73.5± 3.4 ^a	60 -78g/L
Albumin g/L	33.8± 1.1 ^a	32 ±3.5 ^a	33.8± 1.9 ^a	35- 55g/L
Globulin g/L	44.6± 0.22 ^a	47 ±2.2 ^a	39.7 ±1.7 ^a	23-35g/L
A-G ratio	0.79± 0.04 ^a	0.69 ± 0.08 ^{ab}	0.86± 0.03 ^a	
TotalBilirubin µmol/L	4.87 ±0.26 ^b	4.79 ±0.99 ^b	4.5 ±0.57 ^b	2-17µmol/L
Urea mmol/L	2.54 ±0.23 ^a	1.99±0.16 ^a	2.55 ± 0.44 ^a	1.2-3.0mmol/L
Creatinine mmol/L	66.33±5.89 ^a	61.88±11.16 ^a	60.34 ±6.19 ^a	53-106mmol/L
Cholesterol mmol/L	3.24 ±0.27 ^b	4.47 ±0.89 ^a	3.21 ±0.51 ^b	3.6-6.5mmol/dL
Triglyceride mmol/L	0.87±0.05 ^b	1.09 ±0.21 ^{ab}	1.31 ±0.12 ^{ab}	0.4-1.81mmol/L

* Values on the same row with different superscripts differ significantly (P<0.05).

Table 3. Biochemical profile of Cerebrospinal Fluid from trypanosome infected and uninfected individuals.

Parameters	Infected Subjects	Uninfected Subjects	SI Reference Intervals
Sodium mmol/L	135.6 ±10.5 ^a	128 ± 5.23 ^{ab}	138-150 mmol/L
Potassium mmol/L	4.1± 0.3 ^a	3.88± 0.19 ^a	2.45–3.5 mmol/L
Chloride mmol/L	99.0 ±1.0 ^{ab}	100.5 ±1.55 ^a	118-132 mmol/L
Bicarbonate mmol/L	22.5± 2.5 ^a	21.0± 1.0 ^a	
Calcium mg/L	25.5 ±4.5 ^a	23.8± 3.8 ^a	1.00-1.4 mg/L
Phosphorous mmol/L	0.15 ±0.05 ^a	0.09 ±0.04 ^a	0.4-0.6µmol/L
Urea mmol/L	1.7± 0.09 ^a	1.66± 0 ^a	3.0-6.5mmol/L
Creatinine µmol/L	132.6± 43 ^{ab}	243.1± 98 ^a	50-110µmol/L
Total protein g/L	20± 5 ^a	24.5 ±3.6 ^a	0.15-0.45g/L
Albumin g/L	0.5± 0.1 ^a	0.80± 0.30 ^a	
Globulin g/L	15±4 ^a	16.5± 9.6 ^a	
Total bilirubin g/L	2.5 ±5 ^a	2± 0 ^a	
AST (GOT) IU/L	15.5 ±5.5 ^a	21.75 ±2.25 ^a	0-5 U/L
ALT (GPT) IU/L	5.5± 0.5 ^a	8.0 ±1.47 ^a	6-18 U/L
GT IU/L	2.5± 0.5 ^a	3.5± 0.5 ^a	

*Values on the same row with different superscripts differ significantly (P<0.05).

plasma of infected subjects were higher (P<0.01) than values recorded in controls (Table 2). The total bilirubin values recorded in the study participants were higher than the normal reference ranges. In this study elevated globulin levels and decrease in albumin levels was recorded. Results of the CSF analysis presented in Tables 3 show relatively high (P<0.05) values for most electrolytes, urea and total bilirubin from infected subjects when compared to values recorded from uninfected subjects. Elevated levels of total protein and AST in the CSF of infected subjects were recorded when compared to reference values. Values of enzymes recorded in the CSF of study participants were lower in the infected subjects than in the uninfected subjects. There was no significant difference (P>0.05) between the levels of sodium and bicarbonate recorded in plasma and CSF of parasite positive subjects.

DISCUSSION

Biochemical evaluation of the body fluids gives an indication of the functional state of the various body organs and biochemical changes in body fluids that result from infections depend on the species of the parasite and its virulence (Anosa, 1988). In this study, the elevation of enzyme levels recorded is in agreement with earlier reports from natural and experimental infected animals (Diehl and Risby, 1974; Singh and Gaur, 1983). The results suggest probable infiltration of vital body organs and inflammation particularly of liver, muscles, and kidneys by *T. b. gambiense* at the time of diagnosis. Raised levels of alkaline phosphatase can be seen in inflammatory conditions of the gut and liver, while active hepatocellular damage is reflected by increases in plasma levels of aspartate transaminases (Baron et al.,

1989). Wilkinson et al. (1962) reported that changes in enzyme levels are a good marker of soft tissue damage. They also noted that damage to body cells result in the alteration of membrane permeability and consequent release of enzymes into the extracellular fluid (ECF). Elevated enzyme levels may also result from effect of trypanosome lyses resulting from the host's defense mechanisms (Kennedy, 2004).

The findings in this study showing normal electrolyte levels were also in agreement with the reports of normal values recorded in experimental *T. b. rhodesiense* infections in mice (Moon, 1968). Carlson (1989) noted that normally, the body regulates balances and maintains its fluid composition and volume for normal physiological and biochemical events of life. Though Barrett-Conor et al. (1973) had reported normal creatinine values in human trypanosome infections, results from this study showed elevated levels in natural *T. b. gambiense* infections. This agreed with reports of elevated levels of creatinine in monkeys experimentally infected with *T. b. rhodesiense* and *T. b. gambiense* (Sadun et al., 1973; Abenga and Anosa, 2004). Increased plasma globulin levels were recorded in the study; this is a feature related to tissue destruction. It has also been shown that many Africans have high globulin levels, and tropical parasitic diseases may be responsible for such raised values (Houba et al., 1969)

The role of blood lipids in pathogenesis of trypanosomiasis had been reported (Tizard et al., 1978; Roberts, 1984); decrease in plasma cholesterol levels in sheep during trypanosome infections is known to occur (Katunga-Rwakishaya et al., 1997). Trypanosomes are known to cleave sialic acid from glycoproteins on erythrocyte membranes with the aid of sialidase; they also use erythrocyte sialoglycoproteins for their proliferation and differentiation (Taiwo et al., 2003). A possible outcome of such is hypocholesterolaemia as recorded in this study.

Changes in the electrolyte concentration in CSF provide important information about the disease state in an individual. Katzman and Pappius (1973) reported the similarity in the electrolyte values of both plasma and the CSF because changes occur in the same manner in both fluids since most electrolytes come from the blood. This could explain the similarity in the values of plasma and CSF electrolytes recorded in this study. Also blood-brain barrier tends to keep ions constant in CSF and ECF of the brain. The decreased enzyme levels recorded in infected subjects in the study, maybe due to the fact that CSF transaminase activities are not sufficiently sensitive or reliable to be of practical diagnostic values in the study of disease with neurological disorders (Thompson, 1978). Increase in total protein levels of CSF are associated with inflammatory disorders of CSF and this is the single most useful change in the chemical composition of the fluid, serving as an indication of disease. Also elevated levels

of CSF urea and creatinine reflect changes in their plasma levels.

It can be concluded from the study that though electrolyte levels in infected humans are generally not altered by infections with *T. b. gambiense*, serum levels of cholesterol and triglycerides could be depressed, while enzyme (transaminases) levels were significantly elevated in active human trypanosomiasis infections.

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