Serum total proteins and creatinine levels in experimental gambian trypanosomosis of vervet monkeys

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Although Human African Trypanosomosis presently constitute a major socio-economic problem in several parts of sub-Saharan Africa, conflicting reports on experimental infections appear to be one of the factors limiting the chemotherapeutic control of the disease. Attempt was therefore made to evaluate the effect of two strains of Trypanosoma brucei gambiense on total proteins and other serum biochemical parameters using vervet monkeys as a model. The outcome of both strains in vervet monkeys was traumatic as the monkeys died from infection 12 – 15 weeks post infection while the serum total proteins increased due to increase in serum globulins with resultant fall in the albumin/globulin ratio. Similarly creatinine and fibrinogen levels increased after infection. The study confirms the existence of atypical virulent infections with a resultant early death from T. b. gambiense.

Key words: Serum, total proteins, vervet monkeys, creatinine, Trypanosoma brucei gambiense.

INTRODUCTION

Human African Trypanosomosis (HAT, Sleeping Sickness) is a complex and debilitating disease of man which now poses as an emerging public health crisis in several parts of sub-Saharan Africa (WHO, 1998; Kabayo 2002; Waiswa et al., 2003). The disease has assumed greater global importance with the increase number of cases of infection in tourists returning from sub-Saharan Africa (Osherwitz, 2003; Schwartz, 2003). HAT is described as one of the “most-neglected diseases” (Truc, 2003), not just in terms of drug development, but also in knowledge of the pathology of the disease, and funding of research and control. HAT arising from Trypanosoma brucei gambiense constitute a special problem going by the chronicity associated with the Gambian disease, typically low parasitaemia which makes parasite detection difficult and controversial roles of animal reservoir hosts (WHO, 1998) in the resurgence of sleeping sickness in many parts of West and Central Africa.

Absence of suitable animal research models is one of the most challenging constraints in the understanding of the disease as inoculation of the parasite into human volunteers is unethical. The current understanding of the pathology of T. brucei gambiense rests largely on observations on the pathogenesis of the parasite in laboratory animals (Losos and Ikede, 1972; Poltera, 1985).

Although model studies on the pathology of the disease using T. brucei have been attempted in sheep (Bouteille et al., 1988a, b) and mice (Keita et al., 1997) and in T. b.
**Table 1.** Changes in the serum total protein, creatinine and fibrinogen levels of *T. b. gambiense*-infected monkeys.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-infection</th>
<th>Weeks post-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total Proteins (g/dl)</td>
<td>7.8 ± 0.6</td>
<td>9.5 ± 1.4</td>
</tr>
<tr>
<td>Albumin/Globulin ratio</td>
<td>0.6 ± 0.1:1</td>
<td>0.5 ± 0.5:1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.0</td>
</tr>
<tr>
<td>Fibrinogen (g/dl)</td>
<td>0.25 ± 0.1</td>
<td>0.33 ± 0.0</td>
</tr>
</tbody>
</table>

gambiense*-infected rabbits (Emeribe and Anosa, 1991), observations in many cases varied from the typically chronic nature of *T. b. gambiense* infection in man. In this study, we attempt to evaluate some of the serum biochemical changes induced by *T. b. gambiense*, using the vervet monkey as a model. It is hoped that such findings will enhance better understanding of the pathology of sleeping sickness in man and its control.

**MATERIALS AND METHODS**

A total of four male vervet monkeys (*Cercopithecus aethiops*) having average body weight of 2.8±0.8 kg were used for the study. The monkeys were acclimatized for a period of six months before use. Two of the monkeys were randomly selected and inoculated with NITR/Abraka strain of *T. b. gambiense* while the remaining two were inoculated with IL 3250 strain. Both strains of parasite were obtained from patients suffering from sleeping sickness. A total of 2 x 10^6 parasites were used to infect each of the monkeys intraperitoneally while pre-infection data obtained for four weeks served as control for each animal.

Blood for the separation of serum was obtained fortnightly by venipuncture of the femoral vein using 21 gauge hypodemic needles and 5 ml syringes. About 5 ml of blood for serum was collected in plastic universal bottles. Blood clot was removed from each of the samples, which were then centrifuged for 10 min at 3000 rpm. 3 ml serum was taken from each sample into plastic bijou bottles and stored at –20°C until used. Determination of the serum protein, creatinine and fibrinogen levels was as described by Toro and Ackerman (1975). Data obtained was analysed using analysis of variance (ANOVA) to determine level of significance.

**RESULTS**

The vervet monkeys became parasitaemic 3 to 4 days post-infection (PI). After infection, both strains of *T. b. gambiense* behaved alike in the monkeys and were therefore treated as one group. Changes in the serum total proteins, creatinine and fibrinogen levels of the infected monkeys was as shown on Table 1. The pre-infection value of the total proteins of the animals was 7.8±0.6 g/dl but increased from week 2PI attaining the maximum value of 11.5±2.2 g/dl by week 6 PI (P< 0.05), but returned to pre-infection level by week 10 when the experiment terminated. This was characterized by almost normal albumin level but rise in level of serum globulin (Figure 1). After infection, the serum albumin remained within pre-infection ranges until weeks 8 and 10 when it decreased below pre-infection values.

The post-infection globulin values however increased significantly (P 0.05) from week 2 attaining maximum value at week 8 PI, but declined slightly by week 10. This was characterised by sharp drop in the albumin/globulin ratio from 0.6±0.1:1 before infection to the value of 0.3±0.1:1 by week 10 (Table 1). The pre-infection serum creatinine value of the monkeys was 1.2±0.1 mg/dl. After infection it increased slightly from week 4PI attaining the highest value of 1.4±0.3 mg/dl (P 0.05) and there after declined slightly to 1.3±0.2 mg/dl by week 10. The Fibrinogen concentration of the animals increased after infection with a maximum of 132% increase by week 8PI.

**DISCUSSION**

Elevations in the serum total proteins of the monkeys after infection support earlier observations in *T. b. rhodesiense* infection of man (Wellde et al., 1989a) and cattle (Wellde et al., 1989b) and in goats infected with *Trpansoma congoense* (Witola and Lovelace, 1997; Ndoutamia et al., 2002). The total protein is either normal, increased or decreased in African trypanosomosis (Anosa, 1988). This increase was due to increase in serum globulin. The serum albumin was

**Figure 1.** Serum albumin and globulin levels of *T.b. gambiense* infected monkeys.
however decreased leading to the fall in the albumin/globulin ratio of the infected monkeys. These observations were also similar to those of Wellde et al. (1989a) in patients with Rhodesian Sleeping Sickness in Lambwe Valley endemic area of Kenya and cattle experimentally infected with the human infective T. b. rhodesiense (Wellde et al., 1989b). Increase in the serum globulin in both cases was due to increase in gamma-globulin level which also led to the drop in the albumin/gama-globulin ratio. On the contrary serum total proteins along with albumin and albumin/globulin ratio were depressed in T. brucei infected goats which suggested hepatic dysfunction (Adejinmi and Akinboade, 2000). Even though the albumin levels of the monkeys did not differ from the pre-infection ranges in the first six weeks of infection, hypoalbuminaemia was observed in the last three weeks of the observation period. Whereas hypoalbuminaemia may result from plasma dilution or decreased protein synthesis due to hepatic dysfunction, these decreases may have marked the setting in of hepatic pathology in the monkeys.

Hyper gammaglobulinaemias in African trypanosomosis on the other hand is usually associated with the increase in immunoglobulin M (IgM) which is a consistent finding in trypanosomosis of man and animals (Anosa, 1988). Mean serum IgM levels in Rhodesian Sleeping Sickness patients were similarly elevated to nearly three fold those of control population (Wellde et al., 1989a).

Increase in the fibrinogen level of T. b. gambiense -infected vervet monkeys also confirmed earlier observation in T. b rhodesiense infected patients in the Lambwe Valley endemic area of Kenya (Wellde et al., 1989a). As an acute phase protein, fibrinogen levels are believed to be elevated in inflammation conditions although over-production could probably occur in response to a relatively low level coagulopathy (Wellde et al., 1989a). Increase in the serotonin level from week four of the infection in the vervet monkeys was previously demonstrated in monkeys infected with T. b. rhodesiense, particularly in the first week of infection (Sadun et al., 1973), in Rhodesian Sleeping Sickness infected patients (Wellde et al., 1989a) and goats infected with T. brucei brucei (Adejinmi and Akinboade, 2003). This was associated with damage to host tissues as well as renal and hepatic malfunction. Normal creatinine levels were however reported in human T. b. rhodesiense infection (Barret-Connor et al., 1973).

Changes in the Serum total proteins and creatinine level suggest that T. b. gambiense was pathogenic to vervet monkeys leading to various stages of tissue damage as the monkeys later died between week 12 and 15 PI. Although the observations of serum proteins of the monkeys in this study by themselves are not enough to confirm hepatic malfunction, hypoalbuminaemia demonstrated in the last three weeks of the infection suggest that hepatic pathology was probably associated with T. b. gambiense infection in the vervet monkeys. This is at variance with the observations of Yesufu (1971) who reported that vervet monkeys infected with T. b. gambiense looked healthy and added weight even though parasites could still be demonstrated in the blood one year after infection.

Interstitial activities of trypanosomes in the tissues of man leading to severe inflammatory reactions and malfunctioning of several organs have been reviewed by Poltera (1985). The outcome of infection with strains of T. b. gambiense used in this study suggests that such pathological changes occurred in the vervet monkeys. WHO (1998) reported the existence of Type II T. b. gambiense which produces an acute rhodesiense-like disease syndrome. The outcome of experimental Gambian trypanosomosis in the monkeys therefore suggests that early chemotherapeutic management of Gambiense Sleeping Sickness in man is necessary to avert an early death from T. b. gambiense infecting atypical strains.

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