

## Serum Biochemical Changes in Experimental Gambian Trypanosomosis. II. Assessing Hepatic and Renal Dysfunction

Jerry Ngutor ABENGA<sup>1,\*</sup>, Victor Onwubalili ANOSA<sup>2</sup>

<sup>1</sup>Department of Veterinary Pathology and Microbiology, College of Veterinary Medicine, University of Agriculture, P. M. B. 2373, Makurdi - NIGERIA

<sup>2</sup>Department of Veterinary Pathology, University of Ibadan, Ibadan - NIGERIA

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**Abstract:** Serum biochemical changes were assessed to determine hepatic and renal function in 4 vervet monkeys experimentally infected with *Trypanosoma brucei gambiense*, the causative agent of sleeping sickness in West and Central Africa. Parameters examined included serum bilirubin, blood urea nitrogen (BUN), and cholesterol levels. Significant increases in the total bilirubin and BUN levels occurred from the second week post-infection (PI) ( $P \leq 0.05$ ) and remained so until the monkeys died of infection, at 12 to 15 weeks PI. Serum cholesterol levels increased from the fourth week to the eighth week of infection. These changes suggested early hepatic and renal pathology, and dysfunction in the monkeys, which were thought to be associated with the early death of the primates. These observations were at variance with the typically mild and chronic nature of *T. brucei gambiense* in humans. It was concluded that early hepatic and renal damage in trypanosomosis is an important obstacle to the success of chemotherapy of sleeping sickness in humans, as the drugs themselves are thought to be hepatotoxic and nephrotoxic. Furthermore, characterisation of atypically virulent *T. brucei gambiense* strains may be necessary in distinguishing such strains from *T. brucei rhodesiense* and preventing the spread of Rhodesian sleeping sickness.

**Key Words:** Serum biochemistry, trypanosomosis, human, monkeys

### Introduction

Human African trypanosomosis (HAT, sleeping sickness) is still endemic in several parts of sub-Saharan Africa, where it constitute a major health hazard in endemic countries (1,2). The disease, though described as one of the most neglected (3), has in the last decade assumed greater global importance following resurgence in endemic areas and reports of increasing number of infections among tourists returning from tropical Africa (4,5). Sleeping sickness arising from *Trypanosoma brucei gambiense* constitutes a special problem going by the debilitating nature of the Gambian disease, typically low parasitaemia, which makes parasite detection difficult, and the controversial roles of animal reservoir hosts (6,7). The absence of suitable animal research models is one of the most challenging constraints in the understanding of the pathology arising from Gambian sleeping sickness and distinguishing this from the cytotoxic side effects of drugs during treatment (8).

This study was therefore aimed at assessing the occurrence of hepatic and renal pathology (dysfunction) arising from Gambian trypanosomosis using vervet monkey as a model. This is with the view of enhancing proper clinical management and chemotherapy of sleeping sickness in humans.

### Materials and Methods

Four male vervet monkeys (*Cercopithecus aethiops*) having average body weight of  $2.8 \pm 0.8$  kg were used for the study. Two of the monkeys were randomly selected and inoculated with the NITR/Abraka strain of *T. brucei gambiense* isolated from Abraka, Nigeria, during an outbreak of the disease in the area (9,10). The remaining 2 monkeys were inoculated with the IL3250 strain similarly obtained from patients suffering from sleeping sickness. A total of  $2 \times 10^6$  parasites were used to infect each of the monkeys intraperitoneally, while

\* E-mail: jnabenga@yahoo.com

pre-infection data obtained for 4 weeks served as the control for each animal.

Procedures for obtaining blood from the monkeys, separation of serum, and handling were as previously reported (11,12). Parameters determined included total bilirubin, cholesterol, and blood urea nitrogen (BUN) as previously described (13). The data obtained were analysed using analysis of variance (ANOVA) to determine the level of significance.

**Results**

After infection, both strains of *T. brucei gambiense* behaved alike in the monkeys and were therefore treated as one group. Changes in the serum total bilirubin, BUN, and cholesterol levels were as summarised in the Table.

The total bilirubin level of the *T. brucei gambiense* infected primates increased above pre-infection values, ranging from 188.0% to 268.1% increases in the second and fourth week post-infection (PI), respectively (Figure 1). Thereafter, the values declined only slightly but remained above pre-infection values until the tenth week ( $P \leq 0.05$ ).

The direct bilirubin levels increased in the first week 6 weeks of infection, ranging from 145.9% by week 2 to 250.0% by week 6 PI. This was followed by a sharp drop to pre-infection values by weeks 8 and 10. The serum cholesterol levels of the monkeys also increased from the fourth week of infection, the increase ranging from 28.12% in the fourth week to 34.33% in the eighth week PI (Figure 2.). Increases in the BUN level of the *T. brucei gambiense*-infected monkeys ranged from 29.7% at week 2 PI to 187.9% and 166.2% by weeks 8 and 10, respectively (Figure 2,  $P \leq 0.05$ ).

**Discussion**

The pattern of increase in total bilirubin level in the monkeys following infection with *T. brucei gambiense* supported earlier observations in experimental *T. brucei* infection of dogs (14) and rabbits (15), and was due to increases in direct bilirubin levels. The observations in the vervet monkeys, however, differed from those reported by Welde et al. (16) in *T. brucei rhodesiense*-infected sleeping sickness patients in the Lambwe valley of Kenya, where serum bilirubin levels of infected people did not

Table. Summary of serum bilirubin, BUN, and cholesterol levels of vervet monkeys before and after infection with *T. brucei gambiense*.

	Pre-infection values	Terminal values post-infection
Bilirubin (Total) (mg/100 ml)	0.2 ± 0.0	0.5 ± 0.0*
Bilirubin (Direct) (mg/100 ml)	0.1 ± 0.1	0.1 ± 0.0
Cholesterol (mg/dl)	121.3 ± 8.2	125.5 ± 40.4
Blood urea nitrogen (mg/dl)	29.8 ± 2.4	75.5 ± 14.5*

Note: \*:  $P \leq 0.05$

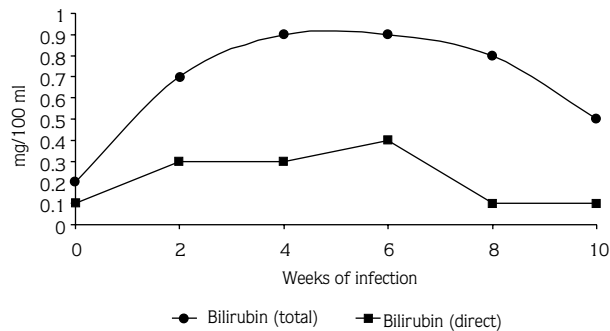


Figure 1. Sequential changes in serum bilirubin levels of monkeys after infection with *T. b. gambiense*.

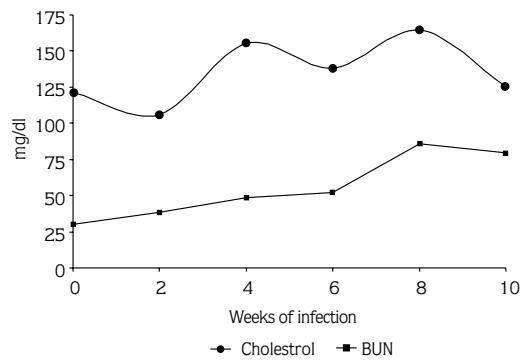


Figure 2. Sequential changes in serum cholesterol and BUN levels of monkeys after infection with *T. b. gambiense*.

differ statistically from those of the control population. The increases in serum bilirubin levels in the primates were suggestive of haemolytic anaemia in the monkeys due to *T. brucei gambiense* infection, as earlier observed by Anosa (17) or obstructive jaundice as previously reported in *T. brucei*-infected rabbits (15). Hypercholesteraemia demonstrated in the monkeys after infection was higher than that observed in *T. brucei*-infected goats (18) and rabbits (15). Hypercholesteraemia has been associated with hepatic malfunction resulting from impairment of liver lipid metabolism (18) in African trypanosomiasis. In contrast, hypocholesteraemia was the case in cattle experimentally infected with human infective *T. brucei rhodesiense* (19) and in goats infected with *T. brucei* (20).

Elevation in the BUN level in the *T. brucei gambiense*-infected monkeys was, however, in consonance with the observation reported by Wellde et al. (16) in the Lambwe Valley sleeping sickness patients in Kenya, and in *T. brucei* infection of rabbits (15) and goats (18). Similar increases were reported in acute caprine trypanosomiasis arising from infection with *T. vivax* (21). Elevated serum urea has been associated with kidney diseases such as glomerulonephritis, urinary tract obstruction, and excessive protein catabolism associated with severe toxic and febrile conditions (22). Fever and glomerulonephritis are consistent features of African trypanosomiasis (8) and may have occurred in the vervet monkeys as a result of the infection.

Significantly high serum bilirubin, cholesterol, and urea levels in the *T. brucei gambiense*-infected monkeys suggest that severe renal and hepatic pathology occurred in the monkeys, which resulted in the malfunctioning of these organs and early death of the monkeys 12 to 15 weeks PI.

This is at variance with the chronic nature of *T. brucei gambiense* sleeping sickness in humans (23) and the result of an earlier experimental infection in monkeys (24). This suggests that the strains of *T. brucei gambiense* used in this case were highly virulent and atypical, and supports the reports of deaths associated with the outbreak of sleeping sickness in Abraka focus from where one of the strains was obtained (9,10).

In conclusion, the outcome of *T. brucei gambiense* infection of vervet monkeys confirmed the prominent role of haemolysis in the pathogenesis of anaemia in African trypanosomiasis. Similarly, an early haemolytic crisis coupled with early hepatic and renal dysfunctions associated with atypical *T. brucei gambiense* strains in humans may play roles in the early death of patients suffering from Gambian sleeping sickness. Furthermore, early sequential assessment of renal and hepatic functions of patients under treatment may be essential in preventing death, as most of the trypanocides for both early and late stage treatments themselves have cytotoxic effects. Characterisation of atypically virulent *T. brucei gambiense* strains is therefore necessary in distinguishing these from *T. brucei rhodesiense* and preventing the spread of Rhodesian sleeping sickness to other parts of Africa.

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