Serum Cardiac Troponin I and Lactate as Prognostic Markers of Canine African Trypanosomiasis

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SUMMARY

The study was aimed at understanding the nature and pattern of serum lactate, cardiac troponin I changes associated with acute Trypanosoma brucei infection of dogs. It also seeks to investigate the usefulness of these biomarkers in monitoring disease progression and predicting mortality. Twenty healthy adult dogs of both sexes were used for the study. All the dogs were intra-peritoneally inoculated with 1ml of phosphate buffered saline diluted blood containing $1\times10^6$ of Lafenwa strain of T. brucei. The serum lactate, cardiac troponin I, potassium, sodium, chloride and bicarbonate were monitored before and at 16 and 24 days after infection. There was a progressive increase in serum values of lactate, cardiac troponin I and potassium at various days after infection. A significant association was found between lactate and heart rate, chloride and heart rate, potassium and cardiac troponin I. This result has shown that there could be myocardial damage due to hypoxia of anemia and hypo-perfusion in canine African trypanosomiasis. The progressive increase in serum lactate and cardiac troponin I could help in predicting severity and outcome of Canine African Trypanosomiasis. The Lactic acidosis may increase ventilation drive and consequently the heart rates of infected dogs.

Key words: Hyperkalemia, Septicemia, Acidosis, Myocardium, Trypanosoma
INTRODUCTION

Human African trypanosomiasis (HAT) also known as sleeping sickness is caused by *T. brucei brucei*, *T. brucei rhodensiense*, and *T. brucei gambiense*. These organisms are also infective for both domestic and wild animals. The disease is of major public health problem in sub-Saharan Africa (Blum et al., 2012). Although there had been reports of cardiac involvement in the disease, the aspect of central nervous system (CNS) involvement has been and is still the priority area for researchers (McCarroll et al., 2015). The growing population of Africans with cardiomyopathies and the possibility of its subtle complication in patients with the cardiac form of HAT is important concern to both clinicians and researchers (McCarroll et al., 2015). The early detection of cardiac involvement in HAT is therefore desirable in order to reduce economic waste and mortality.

In *T. brucei* infections of both man and dog, myocarditis and clinical signs of congestive heart failure have been observed (Morrison et al., 1981; Blum et al., 2007). Among many other markers of myocardial damage such as creatine kinase and its myocardial form (CK-MB), lactate dehydrogenase (LDH) and aspartate amino transferase, cardiac troponin I is preferred for its better sensitivity and specificity (Tsung and Tsung, 1986; Adams et al., 1993). In a disease like canine African trypanosomiasis (CAT), with multiple organ damage (MOD), the high specificity of cardiac troponin I (cTnI) makes it a better alternative to other markers with lower specificity. Although there had been some reports on cardiac troponin assay in human patients with HAT (Blum et al., 2007), there has been little or none on canine patients.

The uses of lactate in predicting severity and outcome of diseases such as equine colic (Adams et al., 1993; Moore et al., 1976), gastric dilatation-volvulus (Papp et al., 1997), canine pyometria (Hagman et al., 2009), and canine babesiosis (Nel et al., 2004) have been reported. Since overwhelming systemic inflammatory responses which often characterize canine babesiosis (Welzl et al., 2001; Matijatko et al., 2010) could be seen also in other septic conditions like acute *T. brucei* infection, therefore, lactic acidosis is also possible in CAT. Studies investigating potential usefulness of lactate as potential marker of severity and outcome of CAT and HAT are scarce.

The striking similarities in pathogenesis and pathology of dogs and human infected with *T. brucei* have been reported (Morrison et al., 1981; Blum et al., 2007). The use of *T. brucei* infected dog as a model in understanding HAT is therefore a priority for researchers and scientists.

The role of dogs as reservoir hosts in human trypanosomiasis is well documented. While dogs could serve as good models in understanding this disease in man, there is also the need to adequately diagnose the disease in the dog population if the disease is to be prevented or controlled in man. In order to formulate rational therapy for the disease in both canine and humans, there is a need for clinicians to adequately predict severity and outcome of the disease in either man or dog. The failure of serological, parasitological and molecular diagnostic
protocols in this regard is the reason for this study. Using the canine model of acute *T. brucei* infection, this study shall attempt to use serial measurements of serum lactate, cardiac troponin I, and some electrolytes to predict the severity and outcome of CAT. It is hoped that when this objective is achieved, it will provide a template on which HAT patients can be evaluated.

**MATERIAL AND METHODS**

**Experimental Animals and Housing**

Institutional approval for this study was granted by the animal ethics committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. Twenty clinically healthy adult Nigerian local dogs sourced randomly from local breeders in Abeokuta comprising 10 males and 10 females (≥1 year old) were used. They were acclimatized to laboratory conditions for two weeks and screened against trypanosome species and other haemoparasites. The dogs received all routine vaccinations against common dog diseases such as canine distemper, leptospirosis, hepatitis, parvo virus enteritis and were all certified free from heart worm. The cardiac health of the dogs was assessed by auscultation and ECG examination. Animals with cardiac abnormalities were excluded from the study. They were all kept in a netted room of 100m² measuring 10mx10m and fed twice daily on cooked local foods and fish diet.

**Experimental Infection**

The infected dogs received 1ml of 1x10⁶ of the parasite in PBS intra-peritoneally each (Ajibola et al., 2016). *T. brucei* obtained from infected cattle slaughtered in Lafenwa abattoir in Abeokuta was passaged in Wistar Albino rats. The parasite was preserved by sub-passaging in donor albino rats. The parasite count was determined according to the method of Herbert and Lumsden, (1976).

**Biochemical Assay**

About 10mls of blood obtained from the cephalic vein and centrifuged at 3000rpm for 15 minutes to obtain serum which is then aliquoted into appropriately labelled serum sample bottles for the immediate analysis of serum lactate, potassium, and sodium, chloride and cardiac troponin I concentration. These analytes were assayed before infection and on days 16 and 24 post infection in each of the dogs. A commercial ELISA kit (East-biopharm, Hangzhou, China) based on biotin double antibody sandwich technology was used for determination of cardiac troponin I according to manufacturer’s instruction. Serum lactate level was determined spectrophotometrically according to the method of Noll (1988) as stated in the manufacturers test kit manual of Megazyme International, Ireland. Serum sodium was determined by modification of the methods of Maruna (1958) and Trinder (1951) as described in the TECO kit (USA). The determination of serum potassium concentration was done according to the method of Teri and Sesin (1958) as described in the TECO kit manual (USA). The serum level of bicarbonate was determined spectrophotometrically according to the method of Forrester *et al.* (1976) as described in the test kit manual of Chema diagnostic,
Italy. The serum chloride concentration was also determined by spectrophotometry.

Statistics

All data were expressed as mean ± standard deviation. Differences within parameters during the course of the disease were evaluated by repeated measure ANOVA. The association between any two parameters was evaluated using regression analysis. The tests were declared significant when p < 0.05. All statistical tests were done using SPSS version 16.

RESULTS

The infection became patent in the dogs on the fifth day after infection. All the dogs included in the study showed arrhythmia form the eighth day post infection. A dog showed clinical signs of congestive heart failure such as testicular edema, corneal opacity, and lethargy on the twenty-fourth-day post infection. At the termination of experiment, the infected dogs were treated with 3.5mg/kg diminazene aceturate intramuscularly. All the dogs responded to treatment and survived.

The result of the serum biochemical studies is shown in Table 1. The mean serum sodium levels before infection (166.98±36.98) were not significantly different from the mean serum sodium levels of infected dogs on day 16, (159.60±8.20) and day 24(150.33±5.68). The mean serum chloride levels of infected dogs on days 16(97.82±1.64) and 24(95.44±4.21) were also not significantly different from their levels before infection (93.89±1.54).

The serum bicarbonate, lactate, troponin-I, and potassium levels of dogs’ pre-infection were significantly different from infected dogs.

The cardiac troponin I level at 24 days post infection (0.14±0.02) was significantly higher than the values obtained before infection (0.04±0.03) and at 16 days post-infection (0.06±0.04).

The mean serum lactate level of dogs’ pre-infection (0.037±0.026) was significantly lower than serum lactate levels of infected dogs at days 16(0.21±0.03) and 24(0.23±0.04).

The serum potassium levels of T. brucei infected dogs on day 24 (7.69±0.74) were significantly higher than values obtained before (5.56±0.43) and at 16 days post-infection (6.04±0.81).

Although there was no significant difference in the mean values of bicarbonate on days 16 and 24, the serum bicarbonate levels of infected dogs on day 16(8.70±1.98) and day 24(8.67±2.91) post-infection were significantly lower than the value obtained pre-infection (10.34±1.88).

As seen in Table 2, potassium had a positive and significant correlation with cardiac troponin ((p = 0.04 r = 0.53) but its correlation with bicarbonate was negative but equally significant (p = 0.02, r = - 0.60). The heart rate had a positive and significant correlations with both lactate (p = 0.02 r = 0.69) and chloride (p= 0.04, r = 0.62).
TABLE I: Variation in Serum Biochemical Indices of *T. brucei* Infected dogs

| Biochemical indices       | Pre-infection | 16dpi | 24dpi |
|--------------------------|---------------|-------|-------|
| Cardiac troponin-I (ng/ml) | 0.04±0.03<sup>a</sup> | 0.06±0.04<sup>b</sup> | 0.14±0.02<sup>c</sup> |
| Lactate (g/L)            | 0.03±0.02<sup>a</sup> | 0.21±0.03<sup>b</sup> | 0.23±0.04<sup>ab</sup> |
| Potassium (mEq/L)        | 5.56±0.43<sup>a</sup> | 6.04±0.81<sup>a</sup> | 7.69±0.74<sup>b</sup> |
| Sodium (mEq/L)           | 166.98±36.84 | 159.60±8.20 | 150.33±5.68 |
| Bicarbonate (mmoles/L)   | 10.34±1.88<sup>a</sup> | 8.70±1.98<sup>ab</sup> | 8.67±2.91<sup>ab</sup> |
| Chloride (mEq/L)         | 93.89±1.54   | 97.82±1.64 | 95.44±4.21 |

Values are expressed as mean ± SD, values along the same row with different superscript are significantly different (p<0.05). dpi means days post-infection.

TABLE II: Coefficient of Correlation between Some Plasma Biochemical Indices and Electrolytes

| Correlation            | Regression coefficient(r) | P values |
|------------------------|---------------------------|----------|
| Potassium and troponin | 0.53                      | 0.04     |
| Potassium and lactate  | 0.12                      | NS       |
| Potassium and bicarbonate | -0.60                | 0.03     |
| Heart rate and lactate | 0.69                      | 0.02     |
| Heart rate and chloride | 0.62                   | 0.04     |
| Chloride and lactate   | 0.47                      | NS       |
| Chloride and troponin  | -0.05                     | NS       |
| Chloride and bicarbonate | -0.41                | NS       |
| Lactate and bicarbonate | -0.50                   | NS       |
| Bicarbonate and troponin | -0.39                 | NS       |

NS ; Not significant
DISCUSSION

This study reports a marked serial increase in mean serum canine troponin I level of *T. brucei* infected dogs. The serial increases in cardiac troponin I value of the infected dogs reflects the extent of disease severity and may be used to predict severity. A similar finding has been reported in other related septicemic conditions such as canine babesiosis (Lobetti *et al.*, 2012; Lobetti, 2005), canine Ehrlichiosis (Diniz *et al.*, 2008). The observed increase in cardiac troponin level of infected dogs is attributable to myocardial necrosis and infarction (Ricchiuti *et al.*, 1998). These two conditions are known to characterize acute *T. brucei* infection in dogs (Morrison *et al.*, 1981). The mechanism of myocardial cell death has been linked to tissue hypoxia. Anemia which is regular feature of acute *T. brucei* infection in dogs is capable of causing anoxic damage to the cardiomyocytes. Increased cardiac troponin I value has been reported in conditions like cardiomyopathies and congestive heart failures (Oyama and Sisson, 2004).

Another important finding of this work is the *T. brucei* induced hyperlactemia. High plasma lactate levels have been variously reported in septicemic conditions such as canine babesiosis (Nel *et al.*, 2004) and human malaria (Phillips 1989). This may have resulted from anemic hypoxia which produces anaerobic metabolism in muscle tissues and consequently metabolic acidosis. The pyruvate produced from glycolysis is often converted to lactate under anaerobic conditions (Ganong, 1975). It has been found that survival rates of dogs admitted to hospital for various medical emergency conditions is dependent on plasma lactate levels of patients at presentation to the hospital (Lagutchik *et al.*, 1998). Blood lactate measurements are good prognostic indicators in equine colic (Moore *et al.*, 1976), canine pyometria (Hagman *et al.*, 2009) and Gastric Dilatation-Volvulus (Papp *et al.*, 1997). The outcome of Canine African Trypanosomiasis (CAT) as in canine babesiosis can also be predicted from serial measurement of serum lactate. Starting from the sixteenth to the twenty fourth day of infection, the marked increase in serum lactate levels of infected dogs makes it a potential diagnostic marker of acute canine trypanosomiasis. The increased lactate levels associated with disease progression suggest the potential usefulness of lactate as a prognostic marker for CAT. Although there is a more than 100%-fold increase in the lactate profile of infected dogs in this study, the serum lactate levels of the dogs pre and post infection were still within normal physiological range reported for the species.

The low level of bicarbonate has no bearing with the rising serum lactate of infected dogs and so metabolic acidosis observed in this study may due to its loss in kidney.

Alterations in serum potassium were also reported for infected dogs. The septic nature of CAT appears to cause hypoxia and subsequently myocardial injury which could to increased serum potassium concentration. The serum chloride and lactate levels of the infected dogs were probably the reason for the heart rate changes observed in this work. Sine lactate is produced under anaerobic metabolism, its accumulation in serum could
improve the heart rate by increasing the ventilatory drive of the infected dogs.

This study has thus confirmed the usefulness of the combined usage of serum lactate, potassium and cardiac troponin I in monitoring CAT progression and predicting severity of infection.

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