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REGULAR ARTICLE

Hyperlipidaemia in trypanosomiasis of naturally infected horses: possible cachexia–anorexia syndrome?

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Abstract Trypanosomiasis caused by *Trypanosoma evansi* commonly produces wasting disease with signs of emaciation and cachexia mainly at the end stage. The present study was conducted to explore the possible hyperlipaemia or hyperlipidaemia and its association with cachexia–anorexia in equine trypanosomiasis. Out of the fifteen confirmed animals, none of the plasma sample was opaque. There was a significant increase in plasma triglyceride, total cholesterol and blood urea nitrogen and a highly significant increase in low-density lipoprotein (LDL) levels. A mild increase in high-density lipoprotein (HDL) and very low-density lipoprotein levels were observed, while the relative percentage of HDL and LDL was altered with high significance. A moderate increase in triglyceride and highly significant increase in LDL might be the reasons for retention of appetite and lipolysis. Possible protein breakdown and presence of lipolysis might be the reasons for cachexia in equine trypanosomiasis.

Keywords Hyperlipidaemia · Cachexia · Low-density lipoprotein · Blood urea nitrogen

Introduction

Trypanosomiasis, popularly known as Surra and caused by *Trypanosoma evansi*, persists to be endemic in northern and eastern India and arid parts of Rajasthan (Singh et al. 1995; Singh et al. 2004; Laha and Sasmal 2008; Ranjithkumar et al. 2011). The organism commonly produces wasting disease with a protracted clinical course and is most severe and most frequently diagnosed in horses and camels (Ventura et al. 2000; Sellon 2007). The fall in the body weight of animals is an indication of the wasting nature of trypanosomiasis (Audu et al. 1999). The disease is characterised by energy deficit and loss of depot fat and muscle mass, probably associated with an increase in gluconeogenesis (Igbokwe 1995). In an experimental *T. evansi*-infected sheep model, a fall in total plasma protein concentrations suggests the increased protein breakdown or urea loss (Audu et al. 1999). Significant increase in urea and significant decrease in glucose of blood prove the higher metabolic rates in *T. evansi*-infected camels (Gutierrez et al. 2005). *Trypanosoma congolense*-infected sheep showed higher plasma triglycerides and nonesterified fatty acids (Katunguka-Rwakishaya et al. 1999). An increase in total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglyceride levels was observed, while a decrease in high-density lipoprotein (HDL) was reported in several rabbit model *Trypanosoma brucei brucei* infections (Rouzer and Cerami 1980; Nakamura 1998; Orhue and Nwanze 2006). Further, Biryomumaisho et al. (2003) reported the increased level of plasma-free fatty acids in trypanosome-infected animals. Clinical signs of emaciation and cachexia were followed after fever and anaemia in *T. evansi*-infected animals (Brun et al. 1998).

Cachexia represents a complex metabolic end state characterised by progressive weight loss, depletion of skeletal muscle mass, loss of adipose tissue, systemic inflammation and modulation of appetite (Rydén and Arner 2007; Tan et al. 2011). The cachexia–anorexia syndrome involves metabolic and immune changes and is associated with

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triacylglycerolaemia, lipolysis and acceleration of protein turnover (Plata-Salaman 2000). The metabolic alterations include hypertriglyceridaemia, increased hepatic secretion of very low-density lipoproteins, increased de novo fatty acid synthesis and a futile cycle of fatty acids between liver and adipose tissues (Tisdale 2004). The total body fat loss associated with cachexia is mainly a product of increased lipolysis rather than decreased fat synthesis or lipogenesis (Delano and Moldawer 2006). Various factors have been associated with hyperlipaemia in horses and ponies, and parasitism is one among them (Bergero and Nery 2008). Severe hypertriglyceridaemia (SHTG) occurs in inappetent and clinically ill horses without evidence of serum opacity. The development of SHTG may be associated with aphagia, azotaemia or a systemic inflammatory response or a combination of these factors (Dunkel and Mckenzie 2003). Anorexia to good appetite was reported in *T. evansi*-infected horses by different authors at different stages of the disease (Wernery et al. 2001; Tuntasuvan et al. 2003; Sellon 2007).

To our knowledge, very little/no research has been conducted on the cachexia of trypanosome-infected horses and its association with appetite. Considering all these, the present study was carried out to rule out the possibility of hyperlipaemia or hyperlipidaemia and its possible association with cachexia–anorexia syndrome of trypanosome-infected horses.

Materials and methods

Study place

Indian native horses (Kathiawari breed) brought to the Referral Veterinary Polyclinic, IVRI, and horses from Auguri-Tanda, a nearby village, were chosen for this study. Outbreak of Surra occurred during October and November months of 2010, i.e. immediately after the southwest monsoon. The horses were mostly between 3 and 8 years in their age and varied widely because most of them were purchased from local markets and/or animal fairs. Mixed farming was followed by the farmers and most of the affected animals were used for draught purpose with unsatisfactory standards of feeding and other health care activities. Six apparently healthy animals from Indian Army Cantonment, Bareilly campus, acted as controls. The health status was assessed by clinical, haematological and parasitological examination and through records.

Sampling and diagnosis

All the horses in the village (Auguri-Tanda, UP, Northern India) were thoroughly screened for *T. evansi* infection. They were also screened for other cachectic diseases like babesiosis and equine infectious anaemia. Fifteen animals were found positive for trypanosomiasis. Diagnosis of *T. evansi* was based on demonstrating the parasite by stained blood smear, haematocrit centrifugation and mice inoculation test (OIE 2004). Eight animals were found positive by examination of Giemsa-stained blood smears, while the remaining, with mice inoculation test. Blood samples were collected from jugular vein and shifted to dipotassium EDTA and heparinized glass tubes. The samples were primarily used for routine haematological and oxidant/antioxidant indices estimation (Ranjithkumar et al. 2011). The leftover plasma samples were stored at −20 °C until use.

Biochemical assays

Plasma samples were analysed for triglyceride concentration by glycerol-phosphate oxidase *p*-aminophenazone method and total cholesterol (TC) was estimated by cholesterol oxidase *p*-aminophenazone method using a UV/visible spectrophotometer. The HDL concentration was measured by accelerator-selective detergent method and LDL concentration by selective detergent method as per the manufacturer's guidelines. The VLDL concentration was calculated using the formula $TC-(HDL+LDL)$ as per Asadi et al. (2006). All the chemicals were purchased from Span Diagnostics, Surat, India.

Statistical analysis

The results were reported as means ± SE for both control and infected groups. The data has followed the normal distribution. Data were analysed statistically using independent *t* tests. $P<0.001$ was considered as statistically highly significant, and $P<0.05$ was considered as statistically significant (Snedecor and Cochran 1994).

Results

Six animals in our study were severely emaciated, i.e. at the end stage of the disease. Around 73 % (11/15) of animals in our study showed variable appetite to inappetence. None of the plasma samples in our study were opaque. When compared to the controls, significant increase in the triglyceride, total cholesterol, blood urea nitrogen (BUN) ($P<0.05$) and a highly significant increase in LDL ($P<0.001$) levels were noticed (Table 1). A mild increase in HDL and VLDL levels was observed, while the relative percentage of HDL and LDL altered with high significance.

Discussion

The native breeds of Indian horses were well adapted to different agro-climatic zones and varying terrains of India

Table 1 Comparison of plasma lipid profile and biochemical parameters in Kathiawari horses infected with *T. evansi* and healthy horses

Parameters	Control (n=6)	Infected (n=15)
Triglycerides (mg/dL)	21.73±3.89	95.97±18.41*
Cholesterol (mg/dL)	111.76±13.14	196.77±10.80*
HDL (mg/dL)	64.59±6.20	71.77±6.53
HDL (%)	58.51±1.37	36.36±2.20**
LDL (mg/dL)	34.77±6.84	105.38±6.31**
LDL (%)	29.79±2.68	53.96±2.59**
VLDL (mg/dL)	12.39±1.10	19.62±2.58
VLDL (%)	11.69±1.58	9.78±0.95
BUN (mg/dL)	16.90±1.40	32.15±4.18*

Values are expressed as mean ± standard error

*P<0.05, significant; **P<0.001, highly significant

(Gupta et al. 2005). The majority of equine population (97.96 %), in India, is under landless, small and marginal farmers belonging to socio-economically deprived communities (ICAR 2008), and heavy economic losses were caused by trypanosome infection in these animals (Yadav et al. 2011). Trypanosomes require lipoproteins for their growth because they are lipid auxotrophs (Orhue and Nwanze 2006). They find the lipids from cholesterol esters, cholesterol, LDL, HDL and phospholipids. An increase in total cholesterol, LDL, VLDL and triglycerides with a decrease in HDL was reported in several rabbit model studies of *T. brucei brucei* infection (Rouzer and Cerami 1980; Nakamura 1998; Orhue and Nwanze 2006). The depleted hepatic lipid content was reported in a rat model study (Igbokwe et al. 2009). Significant increase in total cholesterol has also been reported in *T. evansi*-infected pregnant camels (Megahed et al. 2011). In equines, hyperlipidaemia is an elevation of serum triglyceride concentration up to 500 mg/dL without lactescent plasma or fatty infiltration of the liver (Seifi et al. 2002; McKenzie 2011). The present study reveals that the horses infected with trypanosomes were hyperlipidaemic with significant increase in triglyceride and total cholesterol highly significant increase in LDL and highly significant decrease in percentage of HDL. This might be somewhat related to parasite's dependency on lipids for their growth (Rouzer and Cerami 1980; Orhue and Nwanze 2006). A negative energy balance is developed during stress which forces the animal to obtain metabolic requirements from its own tissues, hence, adipose tissue mobilisation with resultant elevation in blood lipoprotein levels (Burden et al. 2011; McKenzie 2011). Further, it has been proved that derangement of lipid metabolism in *T. brucei brucei*-infected animals was associated with the TNF-α induction. Continuous productions with moderate concentrations of serum TNF-α result in cachexia without acute shock, which inhibits the marked increase in trypanosome number (Nakamura 1998). Cachexia in *T. evansi*-infected horses might also be the same reason. Increased VLDL production in the liver is considered to be essential to the development of the disease (Watson et al. 1992a). VLDL is hydrolyzed by lipoprotein lipase (LPL) to form intermediate-density lipoprotein and then by hepatic lipase to low-density lipoprotein (Olson 1998). In hyperlipaemic ponies, the enzymes responsible for catabolism of VLDL and their remnants were increased, i.e. LPL (twofold) and hepatic lipase (threefold) (Watson et al. 1992b). During the first 2 weeks of *T. brucei brucei* infection, the beginning of suppression of lipolytic enzymes was initiated, which results in a high triglycerides, VLDL and rapid increase in LDL (Nakamura 1998). The significant increase in LDL and its percentage in our study might be due to the conversion of increased VLDL as a result of lipolysis and/or abnormal VLDL catabolism. Since the clinical cases were on various stages of disease conditions, it is difficult to speculate the level of the lipolytic enzymes, i.e. increased or decreased. Increased VLDL leading to moderate hypercholesterolaemia accompanied by hypertriglyceridaemia was also reported in ponies (Watson et al. 1992a). Inhibitory effects on lipoprotein lipase by azotaemia (Sato et al. 2002) and decreased uptake of triglycerides into peripheral tissues has already been reported. Further, the increased level of plasma nonesterified and free fatty acids in trypanosome-infected animals (Katunguka-Rwakishaya et al. 1999; Biryomumaisho et al. 2003) also supports the increased lipolysis. The triglyceride values less than 500 mg/dL in trypanosome-infected horses and normal level of VLDL justifies the variable appetite. On the other hand, we can say that the presence of appetite prevents the deterioration of hyperlipidaemic to severe triglyceridaemia. However, in contrast to our findings, Katunguka-Rwakishaya et al. (1999) reported hypolipidaemia, hypophospholipidaemia and hypocholesterolaemia in *T. congolense*-infected sheep. This might be due to very slow rate of hepatic triglyceride secretion as VLDL (Bremmer et al. 1999) and their ketonaemic ability (Bergero and Nery 2008) or ruminants may follow other than lipid metabolism to loss body condition in trypanasomiasis (Katunguka-Rwakishaya et al. 1999). Significant increase in BUN in our study might be due to increased catabolism of protein as described earlier in other trypanosome-infected animals (Audu et al. 1999; Gutierrez et al. 2005; Megahed et al. 2011). Though the levels of plasma cholesterol and BUN were significantly elevated, the increase was less marked in equine trypanosomiasis. The increased levels of BUN from protein breakdown and increased levels of LDL from lipolysis along with TNF-α induction might be the reasons for cachexia in trypanosomiasis.

Conclusion

The present study reveals the presence of hyperlipidaemia in trypanosome-infected horses. A moderate increase in triglyceride and significant increase in LDL, but not VLDL might be the reasons for the retention of appetite and lipolysis. Possible protein breakdown and lipolysis might be the reasons for cachexia in equine trypanosomiasis. On the other way, presence of appetite prevents the further deterioration in trypanosome-infected horses. Possibility of negative energy balance and cachexia–anorexia syndrome may also be suspected in equine trypanosomiasis.

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