



Ovine Theileriosis Enhances Cardiovascular Disease Biomarkers in Naturally Infected Sheep (Ghezel breed) in West Azerbaijan, Iran

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Abstract

This study was aimed to evaluate the plasma levels of cardiovascular disease biomarkers in naturally infected theileriosis in sheep (Ghezel breed). *Theileria* species are known to be ruminant blood parasites and involves deleterious effects in the livestock. Blood samples were collected from 30 selected sheep (Ghezel breed), naturally infected with theileriosis (infected group) and same number non-infected ones. Hematological parameters and the plasma concentrations of cardiac troponin I (cTnI), creatine kinase-MB (CK-MB) and homocysteine (Hcy) were determined in all samples. The results revealed that significant increase ($P>0.01$) in the level of cTnI, CK-MB, and Hcy concentrations in infected sheep compared with non-infected ones. In addition, cardiovascular biomarkers levels increased with aging and parasitemia rate ($P<0.01$). In conclusion, theileriosis provides evidence of the progression of cardiovascular biomarkers by aging and following elevation of parasitemia rate in Ghezel breed sheep and seems that further attention should be paid on this issue.

Introduction

Theileria species are known as important tick-born protozoan parasites that infect wild and domestic animals (Col and Uslu, 2007). Theileriosis has been reported from North Africa, Southern Europe, Asia and India (Razmi et al., 2009), and has been considered in Iran since long time ago, as a fatal disease of sheep and goats that imposes heavy losses due to mortality and decreased production in affected animals (Khaki et al., 2015). Theileriosis is a progressive lymphoproliferative disease, and is characterized by fever, enlargement of peripheral lymph nodes, progressive anemia, lachrymation, diarrhea or constipation and dyspnea (Omer et al., 2003; Radostitis et al., 2007).

Troponins are known as a highly conserved polypeptide (O'Brien et al., 2006; O'Brien, 2008), which forms in three isoforms, one of which is Cardiac troponin I (cTnI) (O'Brien, 2008; Razavi et al., 2015). cTnI is uniquely expressed in the myocardium and is very sensitive serum biomarker of physical or metabolic myocardial damage with the specificity of 100% (Boswood, 2009; Hamacher et al., 2015). On the other word, an increase in serum CK-MB activity is generally

related to damage of the myocardium and skeletal muscles. Evaluation of CK-MB levels has also been the good biomarker for the enzymatic diagnosis of acute cardiac injury (Leonardi et al., 2008; Philips et al., 2003), if the involvement of skeletal muscle injury not be matter on that case.

Homocysteine (Hcy) is a thiol-containing amino acid which is produced from the metabolism of methionine. In this metabolism, folate and vitamin B12 contribute the methylation of Hcy to methionine and their deficiencies are initial determinants for its elevation. High concentration of Hcy is involved as a risk factor for atherosclerosis, cell damage, endothelial cell injury in experimental animals (Lai and Kan, 2015) and also cardiovascular diseases in human (Nazifi et al., 2012). Hyperhomocysteinemia induces endothelial dysfunction and promotes the development of cardiovascular diseases (Ganguly and Alam, 2015). Increased Hcy could also exert pathological effects by promoting oxidative stress (Perna et al., 2003) which has been indicated as a mechanism involved in the induction of cardiovascular diseases.

Recent studies have revealed that theileriosis could increase plasma levels of troponin I, creatine kinase-MB (CK-MB) and Homocysteine (Hcy) in cattle (Fartashvand et al., 2013; Rezavi et al., 2015). However, there is not enough study about ovine theileriosis. The aim of this study was to evaluate the concentration of cTnI, CK-MB and Hcy in the serum of sheep theileriosis in Ghezel breed to obtain a clear information on the occurrence of cardiac damage in ovine theileriosis.

Materials and Methods

Animals and Parasitological Examination

Present study was conducted in the northwest of Iran (West Azerbaijan Province). In this region, ovine theileriosis is common. Thirty fat-tailed sheep (Ghezel breed), 1-2 years old suffered from acute ovine theileriosis were chosen from southern farms of West Azerbaijan province (North West of Iran). These suffered animals were examined for the presence of ticks, clinical signs including lymph nodes enlargement, hyperthermia (39.9-40.4), anorexia and icterus. Blood samples were collected via the marginal vein of ear for observation of parasite into the erythrocytes and blood smear staining was performed with Giemsa solution 5%. Microscopic examination in the immersion objective (X100) showed Piroplasms form of *Theileria* into red blood cells and were classified as three types parasitemia rate (<2, 2-4 and >4 %). The same number sheep from similar breed without any clinical or paraclinical signs of theileriosis and other diseases were selected as the healthy group.

Blood Sampling and Analysis of Biochemical and Hematological Parameters

Blood samples (ten milliliter) were gathered from all ones through jugular vein and transferred into EDTA-contained tubes. Two milliliter were allocated for hematological analysis and all hematological parameters was automatically determined by "Exigo cell counter, Sweden" within 4 hour of collection. Remained blood

samples were centrifuged at 6000 RPM for 10 minutes at room temperature and plasmas were obtained and kept frozen (-25°C) until analysis. The concentration of cTnI was measured by Elisa kit (Cobas) and creatine kinase-MB was determined by the colorimetric method in plasma (Pars azmoon Co. kits, Tehran, Iran; using a Hitachi-917 Auto analyzer, Japan). Finally, Hcy levels was measured by spectrophotometer (Pars azmoon Co.kits, Tehran, Iran; using a UV/Visible spectrophotometer, Spekol 1500, Germany).

Statistical Analysis

Statistical analysis was performed for all of the data completed during the study. The Mean \pm SD and determination of variation between the data points were carried out with Student's t - test with SAS v9.1 (SAS Institute Inc., Cary, NC, USA). ANOVA system was used to compare the data in the same group. The significance level was specified at $P < 0.01$.

Results

The results are demonstrated in Table 1-4. In the analysis of plasma parameters in the infected animals, when compared with controls, it was found that there was statistically significant difference ($P > 0.01$) in the mean plasma activity of cTnI, CK-MB and Hcy based on parasitemia rate and age. In CBC (Complete Blood Count), low levels of RBC (Red Blood Cell), PCV (Packed Cell Volume) and Hb (Hemoglobin) were observed in the infected groups in comparison with control one. In terms of leukocytes, considerable elevation of lymphocytes (lymphocytosis) and absence of significant alterations in neutrophil were revealed in this study. It is worth mentioning that, during enhancement of parasitemia rate and age, lymphocytosis was more severe.

Table 1. Hematological parameters in infected sheep and control one based on parasitemia rate.

	Parasitemia (%)	RBC ($\times 10^{12}/L$)	PCV (L/L)	Hb (g/dL)	WBC ($\times 10^9/L$)	Neutrophil ($\times 10^9/L$)	Lymphocyte ($\times 10^9/L$)
Control	0 (n=30)	9.86 \pm 0.11	0.271 \pm 0.002	11.02 \pm 0.06	9.89 \pm 0.16	2.45 \pm 0.14	7.22 \pm 0.9
Infected	<2 (n=8)	7.31 \pm 0.1†	0.189 \pm 0.001†	9.34 \pm 0.07†	12.48 \pm 0.14†	2.55 \pm 0.06	8.95 \pm 0.13†
	2-4 (n=15)	5.42 \pm 0.05†	0.138 \pm 0.004†	7.15 \pm 0.02†	14.56 \pm 0.15†	2.64 \pm 0.05	10.57 \pm 0.18†
	>4 (n=7)	4.12 \pm 0.2†	0.112 \pm 0.002†	4.84 \pm 0.11†	14.98 \pm 0.11†	2.8 \pm 0.07	10.86 \pm 0.11†

Data are expressed as mean \pm standard deviation, †significantly different from the control group ($P < 0.01$).

Table 2. Hematological parameters in infected sheep and control one based on age.

	Age	RBC ($\times 10^{12}/L$)	PCV (L/L)	Hb (g/dL)	WBC ($\times 10^9/L$)	Neutrophil ($\times 10^9/L$)	Lymphocyte ($\times 10^9/L$)
Control	(n=30)	9.86 \pm 0.11	0.271 \pm 0.002	11.02 \pm 0.06	9.89 \pm 0.16	2.36 \pm 0.13	7.22 \pm 0.9
Infected	1 year (n=6)	8.16 \pm 0.2 [†]	0.212 \pm 0.001 [†]	8.79 \pm 0.06 [†]	13.27 \pm 0.11 [†]	2.42 \pm 0.11	8.73 \pm 0.11 [†]
	2 years (n=18)	5.87 \pm 0.03 [†]	0.155 \pm 0.003 [†]	6.18 \pm 0.03 [†]	15.76 \pm 0.09 [†]	2.75 \pm 0.12	10.23 \pm 0.12 [†]
	3 years (n=4)	5.05 \pm 0.01 [†]	0.121 \pm 0.002 [†]	5.29 \pm 0.04 [†]	16.84 \pm 0.12 [†]	2.38 \pm 0.09	10.69 \pm 0.11 [†]
	4 years (n=2)	4.11 \pm 0.3 [†]	0.102 \pm 0.001 [†]	4.15 \pm 0.08 [†]	17.43 \pm 0.06 [†]	2.81 \pm 0.14	10.97 \pm 0.08 [†]

Data are expressed as mean \pm standard deviation, [†]significantly different from the control group (P<0.01).

Table 3. cTnI, Hcy and CK-MB in infected sheep and control one based on parasitemia rate.

	Parasitemia (%)	cTnI (ng/mL)	Hcy (mg/dL)	CK-MB (U/L)
Control	0 (n=30)	0.018 \pm 0.002	1.75 \pm 0.21	127.56 \pm 12.78
Infected	<2 (n=8)	0.081 \pm 0.005 [†]	4.29 \pm 0.88 [†]	289.25 \pm 54.62 [†]
	2-4 (n=15)	0.129 \pm 0.004 [†]	6.13 \pm 1.09 [†]	429.37 \pm 39.55 [†]
	>4 (n=7)	0.182 \pm 0.012 [†]	7.19 \pm 1.16 [†]	509.27 \pm 71.59 [†]

Data are expressed as mean \pm standard deviation, [†]significantly different from the control group (P<0.01).

Table 4. cTnI, Hcy and CK-MB in infected sheep and control one based on parasitemia rate.

	Age	cTnI (ng/mL)	Hcy (mg/dL)	CK-MB (U/L)
Control	(n=30)	0.018 \pm 0.002	1.75 \pm 0.21	127.56 ^a \pm 12.78
Infected	1 year (n=6)	0.07 \pm 0.018 [†]	4.14 \pm 1.03 [†]	276.08 ^b \pm 66.71 [†]
	2 years (n=18)	0.088 \pm 0.017 [†]	5.23 \pm 1.14 [†]	409.07 ^c \pm 83.09 [†]
	3 years (n=4)	0.105 \pm 0.013 [†]	6.51 \pm 2.15 [†]	414.55 ^c \pm 78.53 [†]
	4 years (n=2)	0.131 \pm 0.021 [†]	8.50 \pm 1.90 [†]	465.18 ^d \pm 142.19 [†]

Data are expressed as mean \pm standard deviation, [†]significantly different from the control group (P<0.01).

Discussion

Ovine tropical theileriosis caused by *T. lestoquardi* is one of the most prevalent diseases of sheep in Iran (Hashemi-Fesharki, 1988). Theileriosis causes significant economic losses as well as reduces milk and meat production (Kinnaid et al., 2013; Omer et al., 2003). In this study, during parasitemia progression and increasing age of infected sheep, a significant decrease ($p \leq 0.01$) in the RBC, PCV and Hb were occurred which can evidence a progressive anemia in infected animals in both parasitemia rate and age indices. There are some evidences that *Theileria* induces oxidative stress which is

involved in pathogenesis of anemia in theileriosis (Nazifi et al., 2012; Perna et al., 2003; Rezaei and Dalir-Naghadeh, 2006). This reduction in ovine theileriosis might be ascribed to macrophages-mediated erythrocytes destruction in the spleen, lymph nodes and reticulo-endothelial system (Mehta et al. 1988; Rayula and Hafeez, 1995; Singh et al., 2001). On the other word, Nazifi et al. in 2012 demonstrated deleterious effect of Hcy on erythrocytes in malignant ovine theileriosis that can emanate to anemia. Hence, in present study, the other reason of anemia could be attributed to high level of Hcy which demolish erythrocytes and leading to

anemia. In respect of leukogram, lymphocytosis was occurred in the infected ones in comparison with control group which may be attributed to proliferation of lymphocytes in the lymphoid organs (Modi et al., 2015). In addition, the intra-lymphocytic schizonts may involve in the stimulation of lymphoblasts and causes proliferation of lymphocytes and subsequently this mechanism can play important role in occurrence of lymphocytosis (Yamaguchi et al., 2010) which may explain high lymphocytosis during elevation of parasitemia rate. It is consistent with study of Stockham et al., 2000. Razavi et al. (2015) denoted lymphocytosis and unaltered neutrophil count in sheep with theileriosis rather than control ones which is in accordance with present study. However, in Razavi et al. (2015) study, there was not considerable correlation among neutrophil and lymphocyte of circulation with parasitemia rate elevation in sheep theileriosis. In contrary, a pan leukopenia was reported in cattle theileriosis in Saudi Arabia (Omer et al., 2003) and Ghanem et al. (2013) demonstrated same results (pan leukopenia) in the Egyptian water buffaloes with theileriosis which is not in accordance with our study.

Our study revealed that during increasing of parasitemia rate and elevation of age in sheep theileriosis, pivotal enhancement ($P \leq 0.01$) of CK-MB, cTnI and Hcy occurred in plasma levels in compared to healthy ones. It is postulated that following long-term anemia and its-mediated tachycardia, oxygen consumption of myocardium is elevated and is emanated to increased level of plasma cTnI and CK-MB activity in the theileriosis ones (Miranda et al., 2006; Zellweger et al., 2003). The recent result might affirm our hypothesis that anemia-induced hypoxia and vasculitis may participate in the elevation of cTnI and CK-MB in this study and it is speculated that reduced PCV during aging may cause to an elevation of them. Protozoan diseases-mediated cardiomyocyte damage is very scarce (Hervas et al., 1998). However, some hemolytic infectious diseases such as babesiosis (Lobetti, 2005; Lobetti et al., 2002), equine piroplasmosis (Diana et al., 2007), dog trypanosomiasis (Andrade et al., 1994), and dog ehrlichiosis (Diniz et al., 2008) involve in this issue. Since, the presumptive role of electrolyte and acid-base imbalance, hypoxia, endothelial injury, DIC (Disseminated Intra vascular Coagulation), and inflammatory process in the heart arrhythmias and elevation of cTnI have been reported in horse piroplasmosis (Diana et al., 2007) hence, it is possible that resembling mechanisms may involve in the cardiomyocyte damage in malignant ovine theileriosis.

One of the other presumptive reason of cardiomyocyte damage could be ascribed to endothelial

damages, vasculitis and thrombosis (Hervas et al., 1998). One of common factors of endothelial damages and thrombosis is attributed to high level of Hcy. Hcy is known as an intermediate product in the normal metabolism of methionine and is able to provoke oxidative stress (Perna et al., 2003). It is involved in cardiovascular diseases and endothelial cell damage (Jacobsen, 2000). Moreover, Hcy-mediated endothelial injury has been determined in animal studies (Thambyrajah and Townend, 2000). Chillemi et al. (2004) demonstrated considerable concentration of Hcy during acute infection of *Plasmodium falciparum* and ascribed it to imbalance in the folate cycle due to decreased availability of vitamin B12 and NADPH (Nicotinamide Adenine Dinucleotide Phosphate) caused by enhanced oxidative stress. Folate has an essential role in methionine-Hcy metabolism and nucleic acid synthesis (Wagner, 1995). Also, *Plasmodium falciparum* utilizes the host plasma folate for division and proliferation. (Chango and Abdennabi-Najar, 2011). Since a link between Hcy metabolism and folate has been determined, the noticeable rise of Hcy may suggest failure of methylation of Hcy to methionine due to folate shortage caused by excessive consumption of folate through *T. lestoardi* during schizont stage of theileriosis. Hcy concentration was high during high parasitemia rate and increasing of age. In line with our results, Nazifi et al. (2012) reported a significant positive correlation between severity of parasitemia and level of Hcy in the theileriosis ones.

In conclusion, results of this study suggest that occurrence of cardiovascular disease in Ghezel breed with naturally infected theileriosis which possesses close connection by aging and severity of parasitemia.

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