Kafkas Univ Vet Fak Derg
18 (4): 647-651, 2012
DOI:10.9775/kvfd.2012.6169

RESEARCH ARTICLE

The Determination of Oxidative Stress by Paraoxonase Activity, Heat Shock Protein and Lipid Profile Levels in Cattle with Theileriosis [1]

Vecihe TURUNÇ * Tünay KONTAŞ AŞKAR **

[1] *The study was summarized from the master thesis of the first author*

* Institute of Health Sciences, University of Mustafa Kemal, TR-31040 Hatay - TURKEY
** Department of Biochemistry, Faculty of Science, University of Karatekin, TR-18100 Çankırı - TURKEY

Makale Kodu (Article Code): KVFD-2012-6169

Summary

Theileriosis is a common illness that cause large economic losses. The aim this study was to define the oxidative status by determining the changes in levels of malondialdehyde, heat shock protein, paraoxonase activity and lipid profile (triglycerides, total cholesterol, very low-density lipoprotein, low-density lipoprotein, high-density lipoprotein) in cattle with tropical theileriosis. In the study, 15 healty cattle and 15 cattle with tropical theileriosis, between 1-3 years old, were used. Plasma malondialdehyde ($P<0.001$) and heat shock protein 27 ($P<0.01$) levels were determined significantly higher in cattles with Theileriosis than the control group. On the other hand, serum paraoxonase level was significantly lower than the control group. According to lipid profile analyses, while serum trigliserid ($P<0.001$) and very low-density lipoprotein ($P<0.01$) levels were found significantly higher in cattles with theileriosis, serum total cholesterol ($P<0.001$), low-density lipoprotein ($P<0.001$) and high-density lipoprotein ($P<0.001$) levels were found significantly lower in cattles with theileriosis than the control group. In conclusion, serum lipid profile could play role in pathogenesis of theileriosis because of the biochemical changes, such as degeneration of liver and increased levels of lipolysis for compansate the energy requirement which was increased due to theileriosis. In addition, determination of serum paraoxonase activity and heat shock protein 27 levels may be useful tools in the determination of oxidative stress in tropical theileriosis.

Keywords: *Cattle, Theileriosis, Oxidative stress, Paraoxonase, Heat shock protein27, Lipid profile*

Theileriosisli Sığırlarda Oksidatif Stresin Paraoksonaz Aktivitesi, Isı Şok Protein ve Lipid Profili Düzeyleri ile Belirlenmesi

Özet

Theileriosis, yaygın olarak görülen ve büyük ekonomik kayıplara neden olan bir hastalıktır. Bu çalışmada; theileriosisli sığırlarda, akut dönemde oksidatif stres etkisinin ısı şok protein düzeyleri, paraoksonaz aktivitesi ve lipid profili analizi (trigliserid, total kolesterol, çok düşük dansiteli lipoprotein, düşük dansiteli lipoprotein, yüksek dansiteli lipoprotein) ile ortaya konulması amaçlanmaktadır. Çalışma, 1-3 yaş arasında 15 sağlıklı ve 15 theileriosisli sığırda gerçekleştirilmiştir. Theileriosisli sığırlarda plazma malondialdehit ($P<0.001$) ve ısı şok protein 27 ($P<0.01$) düzeyleri kontrol grubuna göre yüksek bulunmuştur. Serum paraoksonaz düzeyi ise, kontrol grubuna göre düşük ($P<0.001$) bulunmuştur. Yapılan lipid profili analizlerinde ise, Theileriosisli sığırlarda kontrol grubuna göre serum trigliserid ($P<0.001$) ve çok düşük dansiteli lipoprotein ($P<0.01$) düzeyleri yüksek bulunurken, total kolesterol ($P<0.001$), düşük dansiteli lipoprotein ($P<0.001$) ve yüksek dansiteli lipoprotein ($P<0.001$) düzeyleri ise düşük bulunmuştur. Sonuç olarak, Theileriosiste artan enerji ihtiyacının karşılanması için yağların lipolizinin artması ve karaciğer hasarının oluşması gibi çeşitli biyokimyasal değişimler nedeni ile plazma lipid profili theileriosisin patogenezinde rol oynayabileceği düşünülmektedir. Ayrıca serum paraoksonaz aktivitesi ve ısı şok protein 27 düzeyinin belirlenmesi, tropikal theileriosisde oksidatif stres etkisinin belirlenmesinde yararlı bir indikatör olabileceği kanısına varılmıştır.

Anahtar sözcükler: *Sığır, Theileriosis, Oksidatif stres, Paraoksonaz, Isı şok protein 27, Lipid profili*

İletişim (Correspondence)
+90 376 2181123
tunaykontas@yahoo.com

INTRODUCTION

Tropical Theileriosis which leads to high morbidity and mortality in cattle, is a disease caused by *Theileria annulata*. The disease has lympho-proliferative character and transmitted by acarid species of the genus *Hyalomma*. Tropical theileriosis is widespread in countries which located in subtropical climate such as South Europe, North Africa, Middle East, Middle Asia, India, South Russia and Turkey. It causes high level of mortality in culture breeds and high economic losses in animal breeding [1].

Clinical symptoms of the disease appear as fever (in the first days, 41-42ºC), rise in pulse and respiration, irregular swelling of lymph node, sometimes haemoglobinuria, conjunctiva and petechial bleeding in mucosa. In the last period of disease, haemolytic anaemia, icterus and leucopaenia appear due to the destruction of lymphocytes [2].

Membranes that cover the cells and cell organelles, contain large amount of unsaturated fatty acids. Oxygen molecule has high affinity to unsaturated fatty acids in cell membranes. Binding of oxygen molecules to double bounds of unsaturated fatty acids, causes lipid peroxidation [3]. Lipid peroxidation is a very harmful chain reaction and is associated with oxidative stress in cells and lipoproteins. Oxidative stres directly damages the membrane structure and other cell compounds and indirectly produces reactive aldehydes like malondialdehyde (MDA) [4,5]. Erythrocyte membrane is vulnerable to lipid peroxidation because of its richness for polyunsaturated fatty acids [6].

Oxidative stress increrases the synthesis of heat shock proteins (HSPs), also called stress proteins. Heat shock proteins are a group of proteins that are present in all cells of all life forms. The synthesis of these proteins are induced when a cell undergoes various types of stresses like heat, cold, oxygen deprivation, etc. Heat shock proteins have many functions for the protection of cells under stres conditions [7].

Paraoxonase (PON) [aryldialkylphosphatase (EC 3.1.8.1)] is a calcium-dependent esterase that was initially identified by its hydrolysis of aromatic carboxylic esters and organophosphorus insecticides [8]. It has been suggested that PON may play a protective role under oxidative stress. Paraoxonase was found to use efficiently not only lipoprotein-associated peroxides (including cholesteryl linoleate hydroperoxides), but also hydrogen peroxide (H_2O_2). Para oxonase also inhibits the accumulation of peroxynitrite-generated oxidized phospholipids by its ability to hydrolyze phosphatidylcholine (PC) core aldehydes and PC isoprostanes to yield lysophosphatidylcholine [9].

Diagnosis of *Theileria* infection is done by clinical symptoms, microscopical examination and serological tests [10]. However, it is important to determine the porter animals and the prognosis of disease with specific biochemical parameters for prevention of economic losses in country. Besides various studies related to theileriosis, the researchs concerning oxidative stress are limited. In the present study, the levels of heat shock protein 27 (HSP 27) and paraoxonase (PON) enzyme with high-density lipoprotein (HDL) besides MDA will be determined for the demonstration of the oxidative stress, occurs in acute infection of cattles with theileriosis.

MATERIAL and METHODS

In the summer periods of 2006-2007, 30 Holstein cattle, between 1-3 years old, referred to the clinic of Veterinary Medicine Faculty, Mustafa Kemal University, Turkey, were used as the material for this study. The animals were divided into 2 groups: 15 healthy cattle (control group) and 15 cattle naturally infected with *Theileria annulata* (patient group). The animals were selected to proper groups after examination of Giemsa stained thin blood smears. The cows showed symptoms suggestive of *T. annulata* infection, including anorexia, anaemia, fever, icterus, and haemoglobinuria.

The blood samples were collected from the control and patient group on the day of diagnosis into tubes with heparin for plasma and into tubes with coagulant free for serum. The blood samples were centrifuged for 10 min at 3.000 rpm for the separation of plasma and 10 min at 2.000 rpm for the separation of serum samples. Then plasma and serum samples were kept frozen (-20ºC) in 1.5 ml microtubes until analysis. MDA analysis were done immediately after the separation of plasma. Samples were analyzed for MDA, HSP 27, PON1 enzyme activity and lipid profile (triglycerides, total cholesterol, VLDL, HDL and LDL).

The determination of MDA in plasma samples were done with the method of Yoshoiko et al.[11]. This method is based upon reaction of MDA, one of aldehyde products and thiobarbituric acid (TBA, Merck). In the study, plasma HSP 27 level was determined using Biosource (U.S.A) HSP-27 enzyme-immunoassay kit. The determination of basic PON enzyme activity in serum samples were done with the method of Eckerson et al.[12]. This method is based on the hydrolyses of paraoxon substrate enzymatically by PON1 enzyme to diethylphosphate and p-nitrophenol.

Triglyceride, total cholesterol, HDL analyses in serum samples were performed on an automated analyzer (Roche, DP Moduler System, Tokyo, Japan) using commercial test kits and LDL and VLDL levels were calculated by Friedewald formulae (LDL = Total cholesterol – [(HDL) + (Triglycerides /5)], and VLDL = Triglycerides/5) [13].

For the statistical evaluation of the results, SPSS 11.0 for windows packet program was used. Statistical differences between groups were evaluated by using student t-test. Data were presented as mean ± standard error (± SE) and $P<0.05$ was considered as significant.

RESULTS

Table 1 shows the changes in MDA, HSP27, PON activity and lipid profile (triglyceride, total cholesterol, VLDL, LDL, HDL) levels between the control and patient groups. Plasma MDA (*P<0.001*) and HSP 27 (*P<0.01*) levels in the patient group were found significantly higher than in the control group. On the other hand, serum PON activity in the patient group have been found significantly lower when compared to the control group (*P<0.001*) *(Table 1)*.

In the present study we also determined the lipid profile related with PON activity, which includes serum triglyceride, HDL, LDL, and VLDL cholesterol levels of cattle with theileriosis in the control group and in the patient group. The patient group exhibited a significant increase in serum triglyceride (*P<0.001*) and VLDL cholesterol levels (*P<0.01*), compared to the control group. On the other hand, the patient group had significantly lower total cholesterol, HDL cholesterol and LDL cholesterol levels when compared to the control group (*P<0.001*).

Table 1. *Changes in plasma MDA, HSP27 levels, serum PON activity and lipid profile levels in the control and patient groups*
Tablo 1. *Kontrol ve hasta gruplarının MDA, HSP 27, PON aktivite ve lipid profili değişimleri*

Parameters	Control Group (n=15)	Patient Group (n=15)
MDA (µmol/L)	15.23±2.33	38.10±2.71*
HSP27 (ng/ml)	2.52±0.21	4.12±0.35¶
PON (U/L)	66.65±13.37	32.34±9.77*
Triglyceride (mg/dl)	30.7±4.3	59.2±9.8*
Total cholesterol (mg/dl)	184.8±39.4	104.5±20.1*
VLDL (mg/dl)	5.2±0.7	8.5±1.1¶
LDL (mg/dl)	114.3±22.4	52.0±12.9*
HDL (mg/dl)	77.6±2.0	42.8±12.1*

Data are expressed as mean ± standart error of the mean values
**,¶,†* *Significantly different from the control group (P<0.001, P<0.01 and P<0.05, respectively)*

DISCUSSION

The present study confirmed and extended the results of preliminary studies [14-16] that showed oxidative stress occurs depending on the increase of reactive oxygen species production in many infections and parasitic diseases. In tropical theileriosis high fever, inflammation, oxidative stress and cellular injury conditions occur, and these conditions lead to formation of compound structured aldehydes such as MDA [17]. Rezaei and Dalir-Naghadeh [6] reported that the level of MDA which is one of the lipid peroxidation products was increased in cattle with theileriosis. In the present study, high MDA levels in the patient group is a result of lipid peroxidation and oxidative stress which occurs in damaged erythrocytes due to parasitaemia.

High fever, inflammation, cellular injury conditions seen in most inflammatory diseases, like theileriosis, also leads to increase in stress protein expression [7]. Heat shock protein 27 is a stress protein with antioxidant effects. However, it was reported that increases in HSP27 production was seen in vascular diseases, hypertermia, apoptosis and various types of cancers [18], but we could not found in the literature any information about HSP27 values in cattle with theileriosis. Under stress conditions, HSP 27 takes role in protection of cells independent from ATP against oxidative stress. This protein shows it's antioxidant effect by increasing intracellular glutathion level, and decreasing iron quantity inside the cell [19,20]. The increase in HSP27 levels in the patient group may be related with the increase in synthesis of the protein in host under stress factors, such as high fever, inflammation, etc. that occur by the stimulation of host immun system with parasite's heat shock proteins in this study.

However, there is no information in literature about the alteration of PON level in parasitic diseases, recent studies reported PON as an antioxidant enzyme, that decreases oxidative stres by preventing the formation of free radicals like peroxides and aldehydes in many diseases [21,22]. Paraoxonase may act to hydrolyze specific lipid peroxide(s) or serve as a target for peroxides. The PON free sulfhydryl group cysteine 284 was shown to be required for its protection against lipid peroxidation [9]. Paraoxonase1 is associated with HDL in plasma and the ability of HDL-associated PON to hydrolyze H_2O_2 (in addition to peroxides) may thus play an important role in eliminating potent oxidants [23]. In the present study, serum PON activity was found low in the patient group. The decrease in PON enzyme activity in cattle with theileriosis may be related with the oxidative stres occurs in disease depending on the erythrocyte disruption due to parasitaemia.

Furthermore, related with PON, we also determined

the levels of serum triglyceride, total cholesterol, VLDL, LDL and HDL cholesterol in cattle with theileriosis. In the present study, we determined that the serum triglyceride and VLDL levels were high in cattle with theileriosis. Serum triglyceride concentration may increase, remain unchanged, or decrease in different types of acute conditions [24]. Singh et al.[25] and Çöl and Uslu [1] found decrease in levels of serum triglyceride and total cholesterol in cattle with theileriosis. Cunha et al.[26] and Aşkar et al.[27] reported that serum triglyceride level is high in cattle with babesiosis, however, total cholesterol levels is low. High triglyceride and VLDL cholesterol levels in the patient group may be associated with increased adipose tissue lipolysis stimulating hepatic production of triglycerides and VLDL cholesterol and a defect of triglyceride removal from circulation.

In acute infections, blood parasites use host's blood glucose for their energy need, and this situation partially leads to formation of hypoglycemia in the host. Therefore body lipids are used for gluconeogenesis to provide host energy deficiency. It is an essential mechanism for provision of host high energy need, occurs in the acute phase response of the infection [28]. But liver damage occurs with the usage of body lipids for gluconeogenesis. Therefore, cholesterol could not be synthesized enough, and this is the reason of low total cholesterol levels in cattle with theileriosis.

Lipoproteins such as HDL and LDL include cholesterol in their structures. In normal conditions, LDL cholesterol transports cholesterol, phospholipids, and lipid-soluble vitamins from the liver to extra-hepatic tissues. And HDL cholesterol plays a major role in the reverse transport of cholesterol from peripheral tissues to the liver. Several types of acute conditions are associated with a marked decrease in HDL cholesterol [29]. Furthermore, HDL is associated with PON enzyme and takes role in prevention of oxidative stress that occurs during infection. Paraoxonase was suggested to contribute to the antioxidant protection conferred by HDL on LDL oxidation [30]. In the present study, we determined that the serum LDL and HDL cholesterol levels are low in the patient group. Low levels of LDL and HDL cholesterol in the patient group may be related with the decrease in synthase of total cholesterol levels because of liver damage occurs during acute infection. Also the decrease in HDL cholesterol levels with PON enzyme activity in the patient group can be an indicator of the antioxidant defence against oxidative stress. Similar changes in serum lipid profiles have also been reported in cattles with babesiosis [27], and in humans with severe leptospirosis [31].

In the present study, we demonstrated the changes in MDA, HSP 27, PON enzyme activity and lipid profile in acute phase of theileriosis. Determined abnormalities in lipid profile suggest that plasma lipid profile could play role in patogenesis of theileriosis because of the biochemical changes, such as degeneration of liver and increased levels of lipolysis for compansate the energy requirement which was increased due to theileriosis. On the other hand, investigation of acute disease in vivo, using a natural process to show oxidative stres, supported the importance of HSP 27 and PON activity in the antioxidant defence against oxidative stres in theileriosis. Therefore, paraoxonase activity and heat shock protein 27 levels may be useful tools in the determination of oxidative stress in tropical theileriosis.

REFERENCES

1. Çöl R, Uslu U: Changes in selected serum components in cattle naturally infected with *Theileria annulata. Bull Vet Inst Pulawy*, 51, 15-18, 2007.

2. Osman SA, Al-Gaabary MH: Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes *(Bubalus bubalis)* in Egypt. *Vet Parasitol*, 146, 337-340, 2007.

3. Serarslan G, Altuğ E, Kontas T, Atik E, Avcı G: Caffeic acid phenetyl ester accelerates cutaneous wound healing in a rat model and decreases oxidative stress. *Clin Exp Dermatol*, 32, 709-715, 2007.

4. Romero FJ, Bosch-Morell F, Romero MJ: Lipid peroxidation products and antioxidants in human disease. *Environ Health Persp*, 106, 1229-1234, 1998.

5. Clarkson PM, Thompson HS: Antioxidants: What role do they play in physical activity and health? *Am J Clin Nutr,* 72, 637-646, 2000.

6. Rezaei Asri S, Dalir-Naghadeh B: Evaluation of antioxidant status and oxidative stres in cattle naturally infected with *Theileria annulata. Vet Parasitol*, 142, 179-186, 2006.

7. Aşkar TK, Ergün N, Turunç, V: Isı şok proteinler ve fizyolojik rolleri. *Kafkas Univ Vet Fak Derg*, 13 (1): 109-114, 2007.

8. Juretic D, Tadijanovic M, Rekic B, Simean-Rudolf V, Reiner E, Baricic M: Serum paraoxonase activities in hemodialyzed uremic patients. *Clin Sci*, 42, 146-150, 2001.

9. Aviram M, Rosenblat M, Bisgair CL: Paraoxonase inhibits high density lipoprotein (HDL) oxidation and preserves its functions: A possible peroxidative role for paraoxonase. *J Clin Invest*, 101, 1581-1590**,** 1998.

10. Dumanlı N, Aktaş M, Çetinkaya B, Çakmak A, Köroğlu E: Prevalence and distribution of tropical theileriosis in eastern Turkey. *Vet Parasitol*, 127, 9-15, 2005.

11. Yoshoiko T, Kawada K, Shimada T: Lipit peroxidation in maternal and cord blood and protective mechanism aganist actived-oxygen toxicity in the blood. *Am J Obstet Gynecol*, 135, 372-376, 1979.

12. Eckerson HW, Romson J, Wyte C: The human serum paraoxonase polymorphism: Identification of phenotypes by their response to salts. *Am J Human Genet*, 35 (2): 214-227, 1983.

13. Friedewald WT, Levy RI, Frederickson DS: Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, 18, 499-502, 1972.

14. McCord JM: Human disease, free radicals and the oxidant/antioxidant balance. *Clin Biochem***,** 26, 351-357, 1993.

15. Grewal A, Ahuja CS, Singha SPS, Chaudhary KC: Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of cross-bred cattle naturally infected with *Theileria annulata. Vet Res Commun,* 29 (5): 387-394, 2005.

16. Ece A, Kelekçi S, Kocamaz H, Hekimoğlu A, Balık H, Yolbaş İ, Erel Ö: Antioxidant enzyme activies, lipid peroxidation, and total antioxidant status in children with Henoch-Schönlein purpura. *Clin Rheumatol*, 27, 163-167, 2007.

17. Yagi K: Lipid peroxides and human diseases. *Chem Phys Lipids*, 45 (2-

4): 337-351, 1987.

18. Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fugua SA: Biological and clinical implications of heat shock protein 27.000 (Hsp27). *J Nation Cancer Instute*, 85 (19): 1558-1570, 1993.

19. Ferns G, Shams S, Shafi S: Heat shock protein 27: Its potential role in vascular disease. *Int J Exp Pathol*, 87 (4): 253-274, 2006.

20. Ergönül S, Aşkar TK: Anaplasmosisli sığırlarda ısı şok protein (HSP), malondialdehit (MDA), nitrik oksit (no) ve interlökin (IL-6, IL-10) düzeylerinin araştırılması. *Kafkas Univ Vet Fak Derg*, 15 (4): 575-579, 2009.

21. Aviram M, Rosenblat M: Paraoxonases and cardiovascular diseases: Pharmacological and nutritional influences, *Curr Opin Lipidol*, 16, 393-399, 2005.

22. Çelik M, Gülcü F, Ozan G, Gürsu MF: Paraoksonaz 1 ve arilesteraz aktivite düzeyleri. *Turk J Biochem*, 30 (2): 194-199, 2005.

23. Aviram M: Does paraoxonase play a role in susceptibility to cardiovascular disease. *Mol Med Today*, 5, 381-386, 1999.

24. Chiang AN, Hwang B, Shaw GC: Changes in plasma levels of lipids and lipoprotein composition in patients with Kawasaki disease. *Clin Chim Acta*, 260, 15-26, 1997.

25. Singh A, Singh J, Grewal AS, Brar RS: Studies on some blood parameters of crossbred calves with experimental *Theileria annulata* infections. *Vet Res Commun*, 25, 289-300, 2001.

26. Cunha BA, Crean J, Rosenbaum G: Lipid abnormalities in babesiosis. *Am J Med*, 108, 758-759, 2000.

27. Aşkar TK, Salmanoğlu B, Çakmak A, Beşkaya A: Relative changes in serum lipid-lipoprotein and trace element levels in cattle babesiosis. *Medycyna Weterinaria*, 64 (9): 1104-1106, 2008.

28. Adamu S, Ige AA, Jatau ID, Neils JS, Useh NM, Bisalla M, Ibrahim NDG, Nok AJ, Esievo KAN: Changes in the serum profiles of lipids and cholesterol in sheep experimental model of acute African trypanosomosis. *Afr J Biotechnol*, 7 (12): 2090-2098, 2008.

29. Carpentier YA, Scruel O: Changes in the concentration and composition of plasma lipoproteins during the acute phase response. *Curr Opin Clin Nutr*, 5, 153-158, 2002.

30. Gülcü F, Gürsu FM: Paraoksanaz ve aril esteraz aktivite ölçümlerinin standardizasyonu. *Turk J Biochem*, 28 (2): 45-49, 2003.