Turk. J. Vet. Anim. Sci.
2007; 31(2): 85-89

Research Article

Investigation of Some Biochemical Parameters and the Antioxidant System in Calves with Dermatophytosis

Mahmut KARAPEHLİVAN[1,*], Erdoğan UZLU[2], Necati KAYA[1], Orhan KANKAVİ[3],
Kerem URAL[4], Mehmet CİTİL[2]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars - TURKEY

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars - TURKEY

[3]Department of Biochemistry, Burdur Faculty of Veterinary Medicine, Akdeniz University, 15100, Burdur - TURKEY

[4]Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, 06110, Ankara - TURKEY

Received: 13.09.2005

Abstract: The study included 10 clinically healthy calves (group 1) and 10 clinical cases of dermatophytosis (group 2). The influence of dermatophytosis on some serum biochemical profiles and the antioxidant system was investigated. Serum malondialdehyde (MDA), reduced glutathione (GSH), uric acid, nitric oxide (NO), total sialic acid (TSA), lipid-bound sialic acid (LBSA), total protein (TP), and albumin levels were measured in groups 1 and 2. Calves in group 2 had significantly higher ($P < 0.001$) MDA, NO, TSA, and LBSA levels, and a higher $P < 0.01$ TSA:TP ratio; however, GSH and uric acid levels were significantly lower ($P < 0.001$). There was no significant difference between the groups in total protein and albumin. These findings suggest a relationship between dermatophytosis, the antioxidant system, and lipid oxidation.

Key Words: Antioxidant system, calf, dermatophytosis, MDA, nitric oxide, sialic acid

Dermatofitozis'li Buzağılarda Bazı Biyokimyasal Paremetreler ve Antioksidan Sistemin Araştırılması

Özet: Bu çalışmada, klinik yönden sağlıklı 10 adet sağlıklı (Grup 1) ve 10 adet dermatofitozisli (Grup 2) buzağı kullanıldı. Dermatofitozis hastalığının serum biyokimyasal profil ve antioksidan sistem üzerine etkileri araştırıldı. Grup 1 ve Grup 2'deki hayvanlarda serum malondialdehit (MDA), redükte glutatyon (GSH), ürik asit, nitrik oksit (NO), total sialik asit (TSA), lipit bağlı sialik asit (LBSA), total protein (TP) ve albümin düzeyleri ölçüldü. Dermatofitozisli buzağılarda (Grup 2) MDA, NO, TSA, LBSA düzeyleri $P < 0.001$ ve TSA/TP oranında $P < 0,01$ düzeyinde istatistiksel olarak anlamlı artışlar, GSH ve ürik asit düzeylerinde ise $P < 0,001$ düzeyinde istatistiksel olarak anlamlı düşüşler tespit edildi. Ancak TP ve albümin düzeylerinde istatistiksel farklılık gözlemlenmedi. Bu sonuçlar dermatofitozis hastalığı ile antioksidan sistem ve lipit oksidasyonu arasında bir ilişkinin olduğunu göstermektedir.

Anahtar Sözcükler: Antioksidan sistem, buzağı, dermatofitozis, MDA, nitrik oksit, sialik asit

Introduction

Ringworm, or dermatophytosis, is an infection of domestic animals caused by various fungi, including *Trichophyton* spp. and *Microsporum* spp. *Trichophyton verrucosum*, *T. mentagrophytes*, and *T. megnini* are the common fungi involved in bovine dermatophytosis (1). The disease is widespread and economically important. It is also a major public health problem. Infection is usually limited to the cornified layers of skin (2,3). Following infection, skin response is mild to severe, depending on various factors, such as the host's reaction to the metabolic products of the fungus, virulence of the infecting fungus, anatomic location, and environmental factors. Crowded housing, as is seen in winter housing, increases the incidence of the disease (2-5).

*E-mail: mkarapehlivan@hotmail.com

Sialic acid (SA), an acetylated derivative of neuraminic acid, is widely distributed in mammal tissue and it is an important structural component of biological membranes (6). Sialic acid concentration increases rapidly following inflammation and injury; therefore, the detection of SA, particularly lipid-bound sialic acid (LBSA) levels, may be a valuable indicator for the diagnosis and/or prognosis of inflammatory diseases (7-9).

There is a scarcity of information on the biochemical changes in dermatophyte infection in calves, especially regarding oxidative stress and antioxidant enzymes during natural dermatophytosis. The aim of the present study was, therefore, to evaluate oxidative status in dermatophytic calves based on the determination of malondialdehyde (MDA), reduced glutathione (GSH), and uric acid levels, and also to investigate possible relationships between dermatophytosis and other relevant indicators, such as nitric oxide (NO), total sialic acid (TSA), lipid-bound sialic acid (LBSA), total protein (TP), and albumin in calves.

Materials and Methods

The study included 10 calves (aged 10-12 months) suffering from dermatophytosis (group 2) and 10 clinically healthy calves (group 1) from a farm in Kars, Turkey, during the winter housing season. Diseased and healthy calves were kept in different barns on the same farm. All calves in both groups were subjected to similar management conditions. A month before the collection of blood samples, none of the animals received topical or systemic drugs for the treatment of dermatophytosis or for any other disease. Caution was also taken to ensure that all the animals selected received the same diet before sampling. All animals on the farm also received anthelmintic drug treatment at the beginning of winter housing and 2 weeks later. A complete physical examination, including general condition, respiration, heart rate, and rectal temperature, was performed on all animals. Cattle are mainly fed a commercial concentrate during winter in Kars (10). No specific inquiry was made as to whether the study animals received vitamin-mineral supplementation.

Blood samples were collected from the jugular vein of all the animals into plain tubes and immediately carried to the laboratory. Sera were collected by centrifugation at 3000 x g for 10 min at room temperature and kept frozen (–25 °C) until analysis. All serum samples were analysed within 1 month of collection.

After cleaning the area with a cotton swab soaked with 70% ethyl alcohol, plucked hairs and scraped scales obtained from 10 calves were examined for fungal agents by direct microscopy in 10% potassium hydroxide and lactophenol (11). Samples were inoculated on mycobiotic agar (selective media of *Trichophyton* agars-DIFCO). The plates were incubated at 28 °C for 2-6 weeks and regularly examined for colony formation. In an attempt to identify the pathogenic fungi, macroscopic and microscopic examinations were carried out and the appearance of fungal growth, colony morphology, colour, shape, size, and colony reverse side morphology were examined (1).

Serum TSA and LBSA levels were measured colorimetrically according to the methods detailed by Sydow (1985) (12), and Katopodis and Stock (13), respectively. Serum MDA concentration was determined by the thiobarbituric acid (TBA) reactivity method (14). NO was determined according to the method of Cortas and Wakid (15). GSH content was measured according to the method of Beutler et al. (16). All other parameters (serum uric acid, total protein, and albumin) were determined on a spectrophotometer (Shimadzu UV-1201, Japan) using commercial kits (Bio-Merieux, France).

Statistical analysis was performed using SPSS (SPSS, Chicago, IL, USA). Normal distribution of the data was determined using the Anderson-Darling normality test. Values were expressed as mean ± standard deviation. The Duncan-ANOVA test was used to compare the parameters between the groups. The significance level was set at $P < 0.005$.

Results

Clinical examination indicated ringworm infection. This was supported by culture examination. Culture examination revealed *T. verrucosum* as the causative fungus. Classification of ringworm lesions was made. The concentration of biochemical parameters related to disease activity in the dermatophytic and control animals are shown in the Table. MDA, NO, TSA, and LBSA levels ($P<0.001$) and TSA:TP ratio ($P<0.01$) were significantly higher in group 2, while mean GSH and uric acid values ($P<0.001$) were lower in comparison to group 1. There was no significant difference between the groups in total protein or albumin values (Table).

Table. The effects of dermatophytosis on antioxidant and biochemical parameters in calves.

Values are expressed as mean ± SD.

Parameters	Control (n = 10)	Dermatophytic (n = 10)
MDA (µmol/l)	4.92 ± 0.18	6.64 ± 0.56 **
NO (nmol/ml)	6.44 ± 1.14	11.51 ± 1.93 **
GSH (mg/dl)	68.18 ± 12.23	49.58 ± 5.75 **
Uric acid (mg/l)	8.93 ± 0.69	5.95 ± 0.73 **
Alb (g/dl)	3.31 ± 0.55	4.02 ± 0.50 Ns
TSA (mg/dl)	54.41 ± 4.41	77.42 ± 6.49 **
LBSA (mg/dl)	20.83 ± 2.59	33.14 ± 1.91 **
TP (g/dl)	6.99 ± 0.78	7.49 ± 0.80 Ns
TSA/TP	7.83 ± 0.84	10.40 ± 1.30 *

Group 1: control; Group 2: calves with dermatophytosis.
** $P < 0.001$, * $P < 0.01$
Ns: Not significantly different between groups.

Discussion

Dermatophytosis, a zoonotic disease, is common at the time of weaning and occurs throughout the year with a higher incidence during the winter housing season (17). The infection causes oxidative stress leading to alterations in homeostasis (18).

In pathological conditions, the human body usually has adequate reserves against the production of free radicals, which are produced during metabolism (19). However, when free radical generation exceeds the antioxidant production capacity, oxidative stress occurs in lactating cows (20). Recently, there has been considerable interest in oxidative stress caused by reactive oxygen species (ROS) and its involvement in disease processes (21). Oxygen-derived ROS have an important role in severe infections (22). It has been well recognised that defence against mycotic infection depends upon the oxidative killing of micro-organisms, as exhibited during Candida infections that require several immune cells' cooperation through candidacidal mechanisms involving ROS (23). However, to the best of our knowledge, no previous study has focused on establishing the relationship between oxidative damage and dermatophytosis in cattle. Formation of free radicals and subsequent lipid peroxidation may be caused by dermatophytosis through the production of ROS following skin damage (24).

The endogenous antioxidant system contains catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase as enzymatic antioxidants, as well as non-enzymatic antioxidants in skin, including glutathione, uric acid, and α-lipoic acid (24). Oxidative stress may be monitored using several biomarkers (20) and total antioxidant status (TAS) has been reported to be a single measure providing relevant information on antioxidant status (25). Yet, TAS has not received any attention in the clinical oxidative stress profile (20). Furthermore, none of the tests that are used for the evaluation of lipid peroxidation (26) have been standardised, making laboratory comparisons difficult (20). In the present study, as TAS levels were not measured, antioxidant status was evaluated by measuring MDA, GSH, and uric acid levels. The significant rise in MDA levels and the significant decrease in the GSH and uric acid levels in calves suffering from dermatophytosis may reflect the work of defence mechanisms against lipid peroxidation during oxidative stress in dermatophytosis. The high level of MDA in dermatophytic calf blood serum indicated an advanced peroxidative process in cell membranes.

Uric acid is an antioxidant molecule, like serum albumin and bilirubin. It is normally present in all tissue compartments and changes in its level may suggest pathology. Previously, it has been reported that albumin and uric acid, along with ascorbic acid, provide major contributions to TAS in humans (27). Reduced serum uric acid may help to explain the importance of uric acid as an

antioxidant in calves with dermatophytosis, as uric acid level in group 2 calves was low in this study.

NO is known as a potent vasodilator and an endothelial-derived relaxing factor (28). NO is synthesised by endothelium (29). NO plays a role in a variety of biological processes, including neurotransmission, immune defence, and the regulation of cell death (apoptosis). Moreover, NO may act as a mediator of inflammatory processes by stimulating production of pro-inflammatory eicosanoids. In inflammatory conditions, NO production increases and causes tissue injury by reacting with superoxide to yield peroxynitrite, a powerful toxin (30). In the present study, the significant rise in NO levels may have been related to the pathogenesis of dermatophytosis in the group 2 calves.

Recent studies have reported the concentration of SA in humans and animals in a number of diseases in which the underlying pathology is tissue damage, tissue proliferation, polymerisation, or inflammation (7,8). Earlier studies reported a significant increase in TSA and LBSA levels in Behçet's disease and human dermatophytosis. A rise in TSA and LBSA was attributed to the release of SA from cell membranes into circulation (8,31). This might have also been the case in the dermatophytic calves in this study, as TSA and LBSA levels, and the TSA:TP ratio were markedly increased.

Our results suggest a possible relationship between antioxidant imbalance, lipid oxidative breakage, and dermatophyte infection; however, in order to more definitively delineate the pathogenesis of dermatophyte infection, further studies are necessary. The determination of oxidative stress may require that clinicians treating the disease should include antioxidative drugs in their treatment regime.

References

1. Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R.: Clinical Veterinary Microbiology 1st Ed. Wolfe Publishing, London, UK. 1994; 1164-67.
2. Gudding, R., Lund, A.: Immunoprophylaxis of bovine dermatophytosis. Can. Vet. J., 1995; 36: 302-306.
3. Parker, W.M., Yager, J.A.: Trichophyton dermatophytosis - a disease easily confused with pemphigus erythematosus. Can. Vet. J., 1997; 38: 502-505.
4. Pier, A.C., Ellis, J.A., Mills, K.W.: Development of immune response to experimental bovine *Trichophyton verrucosum* infection. Vet. Dermatol., 1993; 3: 131-138.
5. Rybnikar, A.: Cross-immunity in calves after vaccination against trichophytosis. Acta Vet. Brno, 1992; 61: 189-194.
6. Schauer, R.: Chemistry, metabolism and biological functions of sialic acids. Adv. Carbohydr. Chem. Biochem., 1982; 40: 131-234.
7. Citil, M., Gunes, V., Karapehlivan, M., Atalan, G., Marasli, S.: Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticulo peritonitis. Rev. Med. Vet., 2004; 155: 389-392.
8. Güner, İ., Ersöz, B., Bayındır, O., Öztürk, G., Tunçok, S.: Bazı deri hastalıklarında serum total ve lipid bağlı sialik asit düzeyleri. Ege Tıp Derg., 1992; 31: 203-205.
9. Motoi, Y., Kimura, Y., Wakamatsu, H., Shimbayashi, K.: Determination and clinical evaluation of sialic acid and mucoprotein in bovine blood. Nat. Inst. Anim. Hlth., 1984; 37: 643-649.
10. Erdoğan, H.M., Çitil, M., Güneş, V., Saatci, M.: Dairy cattle farming in Kars district, Turkey: I. characteristics and production. Turk. J. Vet. Anim. Sci., 2004; 28: 735-743.
11. Halley, L.D., Standard, P.G.: Laboratory Methods in Medical Mycology, 3rd Ed. pp 41-57. US Department of Health, Education and Welfare, Center of Disease Control, Atlanta, USA, 1973.
12. Sydow, G.: A simplified quick method for determination of sialic acid in serum. Biomed. Biochim. Acta, 1985; 44: 1721-1723.
13. Katopodis, N., Stock, C.C.: Improved method to determine lipid bound sialic acid in plasma or serum. Res. Commun. Chem. Pathol. Pharmacol., 1980; 30: 171-180.
14. Yoshioka, T., Kawada, K., Shimada, T., Mori, M.: Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am. J. Obstet. Gynecol., 1979; 135: 372-376.
15. Cortas, N.K., Wakid, N.W.: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin. Chem., 1990; 36: 1440-1443.
16. Beutler, E., Duron, O., Kelly, B.M.: Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 1963; 61: 882-888.
17. Radostits, O.M., Blood, D.C., Gay, C.C.: Veterinary Medicine, 8th Ed. Bailliere Tindall, London, UK, 1994, 381-390.
18. Metodiewa, D., Koska, C.: Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotoxicol. Res., 2000; 1: 197-233.
19. Roth, E.: Oxygen free radicals and their clinical implications. Acta Chir. Hung., 1997; 36: 302-305.
20. Castillo, C., Hernandez, J., Bravo, A., Lopez-Alonso, M., Pereira, V., Benedito, J.L.: Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J., 2005; 169: 286-292.

21. Thomas, C.E., Kalyanaraman, B.: Oxygen Radicals and the Disease Process. Harwood Academic Publishers, Amsterdam, the Netherlands, 1997.

22. Erel, O., Kocyigit, A., Avci, S., Aktepe, N., Bulut, V.: Oxidative stress and antioxidative status of plasma and erythrocytes in patients with vivax malaria. Clin. Biochem., 1997; 30: 631-639.

23. Vazquez-Torres, A., Balish, E.: Macrophages in resistance to candidiasis. Microbiol. Mol. Biol. Rev., 1997; 61: 170-192.

24. Koca, R., Armutcu, F., Altinyazar, H.C., Gurel, A.: Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. Clin. Exp. Dermatol., 2004; 29: 406-409.

25. Ghiselli, A., Serafini, M., Natella, F., Scaccini, C.: Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic. Biol. Med., 2000; 29: 1106-1114.

26. Castillo, C., Hernandez, J., Lopez-Alonso, M., Miranda, M., Benedito, J.L.: Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. Arch. Tierz., 2003; 46: 227-233.

27. Wayner, D.D., Burton, G.W., Ingold, K.U., Barclay, L.R., Locke, S.J.: The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochim. Biophys. Acta, 1987; 924: 408-419.

28. Kolo, L.L., Westfall, T.C., Macarthur, H.: Nitric oxide decreases the biological activity of norepinephrine resulting in altered vascular tone in the rat mesenteric arterial bed. Am. J. Physiol. Heart Circ. Physiol., 2004; 286: 296-303.

29. Keilhoff, G.: Foreword basic research on nitric oxide (NO). Cell. Mol. Biol. (Noisy-le-grand), 2005; 51: 245.

30. Carrillo-Vico, A., Lardone, P.J., Naji, L., Fernandez-Santos, J.M., Martin-Lacave, I., Guerrero, J.M., Calvo, J.R.: Beneficial pleiotropic actions of melatonin in an experimental model of septic shock in mice: regulation of pro-/anti-inflammatory cytokine network, protection against oxidative damage and anti-apoptotic effects. J. Pineal Res., 2005; 39: 400-408.

31. Dogan, H., Pasaoglu, H., Ekinciler, O.F., Tatlisen, N.: A comparative study of total protein, total and lipid associated serum sialic acid levels in patients with Behcet's disease and control groups. Acta Ophthalmol. (Copenhagen), 1992; 70: 790–794.