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SHORT COMMUNICATION

Status of Lipid Peroxidation, Cell Destruction and the Antioxidant Capacity in Foals with Lower Respiratory Tract Disease

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Summary

The aim of this study was to demonstrate the total serum malondialdehyde (MDA) levels for lipid peroxidation status, total serum sialic acid (SA) levels for cell destruction level, serum ceruloplasmin (CP), albumin and uric acid (UA) for the antioxidant capacity in foals with lower respiratory tract disease. The material of the study was consisted of 8 foals as the study group, with lower respiratory tract disease and 8 healthy foals as the control group, aged between 5.5±1.5 months. The results revealed a significant difference (P<0.05), between the groups for serum MDA, CP, SA and UA levels, but a difference had not been determined for serum albumin level. In conclusion, it was determined that serum MDA, SA, CP and UA levels may be useful parameters as an indicator of lipid peroxidation, cell destruction and antioxidant capacity in foals with lower respiratory tract disease.

Keywords: *Malondialdehyde, Sialic acid, Uric acid, Ceruloplasmin, Foal*

Alt Solunum Yolu Hastalıkları Olan Taylarda Lipit Peroksidasyon, Hücresel Hasar ve Antioksidan Kapasite Durumları

Özet

Bu çalışma ile alt solunum yolu hastalıkları şekillenen taylarda lipit peroksidasyon düzeyini belirlemek amacı ile serum malondialdehit (MDA), hücresel hasar seviyesi için serum siyalik asit (SA) ve serum antioksidan kapasiteleri için serum seruloplazmin (CP), serum ürik asit (UA) ve serum albumin seviyelerinin değerlendirilmesi amaçlanmıştır. Çalışmada deney grubunu yaşları 5.5±1.5 aylık olan alt solunum yolu hastalığı şekillenen 8 adet tay oluştururken, kontrol grubunu ise 8 adet sağlıklı tay oluşturmuştur. Çalışma ve kontrol grupları arasında serum MDA, CP, SA ve UA seviyeleri bakımından istatiksel olarak farklılık (P<0.05) gözlenirken, serum albumin seviyeleri bakımından gruplar arasında farklılık gözlenmemiştir. Sonuç olarak; serum MDA, SA, CP ve UA seviyelerinin alt solunum yolu hastalığı şekillenen taylarda lipit peroksidasyon düzeyi, hücresel hasar seviyesi ve antioksidan kapasitenin belirlenmesinde faydalı parametreler olabileceği tespit edilmiştir.

Anahtar sözcükler: *Malondialdehit, Siyalik asit, Ürik asit, Seruloplazmin, Tay*

INTRODUCTION

Respiratory diseases are common in young horses, especially in foals between 1 and 6 months of age, who frequently present with lower airway infections. Lower respiratory tract infections in young foals continue to be a major problem for the horse industry and constitute a source of economic loss in terms of mortality cost of treatment and prophylaxis, growth and performance retardation and loss of value [1]. Pneumonia is one of the main causes of disease and death in foals aged between 32 and 180 days [2]. Diagnosis of pneumonia is based on clinical examination. Laboratory examinations of blood chemistry are needed for improved diagnosis, treatment and prognosis and

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some of these are thought to be total serum MDA, CP, total serum SA, albumin and UA concentrations as being cell destruction and response indicators. In infection status due to invasion of microorganisms, a significant increase in free radical production occurs [3]. Evidence exist that free radicals and free radical secretion products are important mediators in inflammation [4]. Most affected compounds are the lipids, when the free radicals are overproduced. Unsaturated phospholipids and cholesterol which are present in membrane structure, can easly get in reaction with free radicals. Lipid peroxidation (LPO) is the chain of events starting with the reaction of unsaturated fatty acids in membrane phospholipids with oxygen radicals, and resulting with the formation of lipid hidroperoxides [5]. MDA is a breakdown product that is frequently quantified as a measure of lipid hydroperoxides and is accepted as the indicator of elevating oxidative stress in the body [6]. Within the last couple of decades, interest has focused on the potential use of the acute phase proteins (APPs) as indicators of the presence, degree and time course of inflammation, because these proteins are released in large quantities into the blood stream in response to infection and tissue injury [7]. CP, the major copper transporting protein in plasma and lung epithelial lining fluid, has significant antioxidant capacity through its scavenging of reactive oxygen species and equine serum CP is an acute-phase reactive protein increased in the intermediary or later phase of acute inflammation [8]. SA are often involved in important cell surface communications and considered as part of the acute-phase response in infection processes indicating cell destruction. Particularly, SA is present in normal serum of human beings and animals that their content in serum has been changed in various diseases [9,10]. Albumin is the most abundant serum protein produced by the liver and is considered among the plasma antioxidants. In clinical practice, the serum level of albumin continues to serve as an important marker for the presence, progress or improvement of many diseases, even though it is the complex end result of synthesis, degradation or loss, and distribution between intra and extravascular space [11]. Serum UA, like other plasma antioxidants such as albumin, bilirubin, or vitamins A, C and E, is a powerful free-radical scavenger and increases in response to acute oxidative stress [12]. Lower respiratory tract diseases of the foal is a well documented subject, despite there is no study describing the changes in MDA, UA, CP and SA levels in this disease, according to our investigations. The aim of this study was to demonstrate the LPO status, cell destruction level and antioxidant capacity in foals with lower respiratory tract disease.

MATERIAL and METHODS

Animals

The material of the study was consisted of 8 purebred Arabian foals with different gender as the study group, with lower respiratory tract disease from the Sultansuyu Studfarm of General Directorate of Agricultural Enterprises (TIGEM), Malatya, and 8 healthy purebred Arabian foals aged between 5.5±1.5 months with different gender as the control group, which were chosen within the routine 3 months check ups. The animals were treated according to the Animal Care and Use Regulation (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose 1996).

Clinical Examination and Blood Sampling

Accurate diagnosis of lower respiratory tract infection in each foal was made by clinical examinations and general clinical signs, which consisted of slight increase in respiratory rate and a mild fever in the body temperature (38.7-40.5°C, up to 41.0°C), tachypnea, and increased effort of breathing characterized by nostril flaring and increased abdominal effort. Cough and bilateral sero-mucous nasal discharges were the common clinical findings of the ill foals. Depression, crackles and wheezes at thoracic auscultation especially in the vesicular region of the thorax were recorded in the the lower respiratory tract diseased foals. All of the foals detected with infections within apparently acute onset. Only neutrophilic leukocytosis with or without monocytosis was seen in the complete blood count of the affected animals. Venous blood (10 ml) was obtained from the jugular vein and was collected into a plain tube. Blood samples were centrifuged at 3.000 g for 10 min at room temperature. Serum were separated and stored at -25°C until analysis.

Biochemical Analysis

Serum MDA levels were measured using thiobarbituric acid reaction according to the method described by Yoshioka et al.[13]. Serum CP analysis were performed by spectrophotometric method (clombo) using PPD (P-fenilendiamin diklorid). Serum total SA level was measured according to Warren's [14] thiobarbituric acid method with spectrophotometry. Serum was kept with 0.1 N sulphyric acid at 80°C for 1 h, for the bound SA to get free. Calibration curve was prepared using SA at various concentrations and serum SA level was calculated with the help of this curve. Serum total SA analyses were repeated two times for each sample and the average of the results were recorded. Serum albumin and UA levels were measured using commercial kits (Audit, Irland) within the autoanalyser (Autolab, Netherlands).

Statistical Analysis

Student-t test was used for the significance control of the differences between the serum MDA, CP, SA, UA and albumin levels of the control group and the study group [15].

RESULTS

Serum MDA, CP, SA, UA and albumin levels of the control group and the study group are presented in *Table 1*.

Data revealed a significant difference (P<0.05), between the groups for serum MDA, CP, SA and UA levels, but a difference had not been determined for serum albumin levels.

Table 1. *Serum MDA, CP, SA, UA and albumin levels of the control group and the study group*
Tablo 1. *Kontrol ve çalışma grubundaki serum MDA, CP, SA, UA ve albumin seviyeleri*

Parameter	Control Group (n=8)	Study Group (n=8)	t-value
MDA (nmol/mL)	5.81	11.05	4.599
CP (mg/dl)	28.75	36.43	2.902
SA (mg/dl)	20.42	21.43	2.545
UA (mg/dl)	0.50	0.99	2.883
Albumine (g/dl)	2.92	2.98	0.225

DISCUSSION

Elevated MDA levels in humans and animals provide further evidence of massive oxidative stress [4,16]. In recent years, substantial evidence of oxidative stress has been obtained in a number of diseases of farm animals, particularly in pigs, cattle and horses [16-18]. In horses, laminitis, joint diseases, intestinal strangulation, colic symptoms, endotoxaemia, endometritis, urticaria and airway obstruction have been a focus of attention, as well as exercise-related conditions for antioxidant capacities [16,19-21]. No studies have been reached describing the levels of MDA in the lower respiratory disease of the foal, nor in the horse according to our search, but the subject has been studied and an elevation in MDA levels had been well described in humans [22]. Oxidative stress and MDA levels were found to be statistically increased in varying degrees in patients with upper and lower respiratory tract infections [22] and pneumonia [23] in humans, similar to the present study in which significant increases in serum MDA levels have been observed with lower respiratory tract disease in the foal. Of note, it was reported that acute pulmonary neutrophilic inflammation in horses which resolved completely within 2 weeks (without pharmacological treatment), did not induce an increase in markers of oxidative stress), LPO, or lung permeability [24]. This may be due to the fact that exposure of recurrent airway obstruction affected horses to organic dust resulted in acute neutrophilic inflammation, neutrophil degranulation, and ascorbic acid consumption without significant evidence of pulmonary oxidative stress which is seen in lower respiratory tract disease. Art et al.[25] reported a higher degree of oxidation in horses suffering from chronic lower airway inflammation and this data is concordant with MDA elevation in sick foals in our study. Equine serum CP is an acute-phase reactive protein increased in the intermediary or later phase of acute inflammation [8]. Serum CP activity was determined at the early growing stage in foals and it has been stated that CP concentrations peak 7 - 10 days after an inflammatory stimulus and may remain elevated for several weeks [26]. Although inflammation-induced changes in CP had been documented in horses [27], no reports had been found about the CP levels in foals with lower respiratory tract disease or pneumonia. However, CP levels were found to be statistically increased in varying degrees in patients with upper and lower respiratory tract infections in humans [22] and infants in the acute stage of pneumonia [28], nearby sheep with pneumonia [29]. Significantly higher serum CP levels in foals with lower respiratory tract disease are concordant with the mentioned studies above, due to the antioxidant defence against the oxidation resulting from inflammation as suggested by Art et al.[25] whom suggested an increased antioxidant defence, but also a higher degree of oxidation in horses suffering from chronic lower airway inflammation. Generally, decreased serum albumin levels in various animals and human beings at different disease conditions are reported. Hypoalbuminemia in patients with inflammatory diseases such as pneumonia, rheumatoid arthritis, and severe bacterial infection is not an infrequent finding [30]. Significantly decreased serum total protein and albumin levels of cattle infected with *T. annulata* were determined compared to uninfected control cattle [10]. In an adult horse, with *Rhodococcus equi* pleuropneumonia low serum albumin concentrations were determined and hypoalbuminemia was attributed to the formation of a high-protein effusion into the pleural space [31]. Albumin is described as one of the negative acute phase proteins in the horse [7]. Our results are not concordant with the above suggestions, as there were no significant differences in serum albumin levels between healthy and affected foals. As known, hypoalbuminemia is the result of the combined effects of inflammation and inadequate protein and caloric intake in patients with chronic disease. King [32] had been suggested that the chemistry panel should be normal in uncomplicated pneumonia cases, although hypoalbuminemia may occur in severe cases, because of loss of albumin in pulmonary exudates. Present data may be attributed to the extent of disease in our foals, which were not at critical stage and were being uncomplicated cases. Similarly, Kızıl and Kızıl [20] have been reported that a significant increase was determined in serum MDA levels of horses with urticaria, where no differences were noted at the serum albumin levels. A discrete finding had been given by Karapehlivan et al.[9] whom reported slightly higher serum albumin concentrations in calves with pneumonia (31.90±0.64 g/L) than those in healthy calves (28.00±0.68 g/L), but at both groups the levels were within reference levels, so a hyperalbuminemia status can not be not aforementioned. UA level is an important marker in oxidative stress. Recently, serum UA was identified as a strong predictor of mortality in patients with moderate-to-severe chronic heart failure [33]. High UA levels had been reported in severe pulmonary infections [34] and in heaves-affected horses [35]. Similarly, our data revealed high serum UA levels in foals with lower respiratory tract disease, indicating oxidative stres development. Although no

particular document had been found about SA levels in foals with lower respiratory tract disease, an important cause of increased SA has been reported to be bacteria and viruses which play an important role in the development of pneumonia. These agents are known to increase the activity of extracellular neuraminidase and break the bond between SA and the cell membrane [36]. Karapehlivan et al.[9] suggested that serum SA might be a useful parameter as an indicator of inflammation in pneumonia in calves. Similarly, we obtained higher serum SA levels in the affected foals. In this study, lipid peroxidation status, cell destruction level and serum antioxidant capacity were investigated in terms of serum MDA, SA, CP, UA and albumin levels and this is the first documentation of the first four parameters in the lower respiratory tract diseases of the foal according to our investigations. With the present study it was determined that serum MDA, SA, CP and UA levels may be useful parameters as an indicator of lipid peroxidation, cell destruction and serum antioxidant response in foals with lower respiratory tract disease. Free radicals were tended to be evaluated in the present study even they are not specific for the lower respiratory tract disease in foals. For this reason, detection and determination of the free radicals and the oxidative stress in foals with the respiratory tract infections must be evaluated for the correlations with these radicals with the progression of the disase. Further studies are needed to detect these possible correlations.

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