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Research Article

Determination of Some Acute Phase Proteins, Biochemical Parameters and Oxidative Stress in Sheep with Naturally Infected Sheeppox Virus

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Abstract

The aim of the present study was to determine oxidative stress and acute phase response in naturally infected sheep with poxvirus. For this purpose, 20 poxvirus infected and 10 clinically healthy sheep were used in the study. In these animals, alkaline phosphatase (ALP), aspartate aminotransferase (AST) activity, haptoglobin, ceruloplasmin, urea, creatinine, albumin, iron (Fe), malondialdehyde (MDA) and reduced glutathione (GSH) concentrations were determined. The present study determined that the concentrations of haptoglobin, ceruloplasmin, urea, creatinine, MDA and ALP, AST activity were significantly increased, and albumin, Fe and GSH concentrations were significantly decreased in the sheeppox virus infected group compared to the control group. Results of this study indicated that oxidative stress and acute phase response develop in sheep infected with poxvirus.

Keywords: *Acute phase proteins, Biochemical parameters, Sheeppox*

Poksvirüs İle Doğal Enfekte Koyunlarda, Bazı Akut Faz Proteinler, Biyokimyasal Parametreler ve Oksidatif Stresin Belirlenmesi

Öz

Çalışma poksvirüs ile doğal enfekte koyunlarda, oksidatif stres ve akut faz cevabı belirlemek amacıyla yapıldı. Çalışmada 20 adet çiçek hastalığı ile enfekte ve 10 adet klinik olarak sağlıklı koyun kullanıldı. Hayvanlarda alkalen fosfataz (ALP), aspartat aminotransferaz (AST) aktivitesi, haptoglobin, seruloplazmin, üre, kreatinin, albümin, demir (Fe), malondialdehit (MDA) ve redükte glutatyon (GSH) konsantrasyonları belirlendi. Çalışmada, poksvirus ile enfekte koyunlar ile kontrol grubundaki hayvanlar karşılaştırıldığında haptoglobin, seruloplazmin, üre, kreatinin, MDA değerleri ile ALP ve AST aktivitelerinin yükseldiği, albümin, Fe ve GSH konsantrasyonunun ise kontrol grubuna göre düştüğü belirlendi. Bu çalışmada poksvirus ile enfekte koyunlarda akut faz yanıtın oluştuğu ve oksidatif stresin geliştiği kanısına varıldı.

Anahtar sözcükler: *Akut faz proteinler, Biyokimyasal parametreler, Koyun çiçeği*

INTRODUCTION

Sheeppox is a serious, often fatal, disease characterized by widespread skin eruption and lung lesions in sheep and goats. It is caused by a virus belong to poxviridae virus family and *Capripoxvirus* genus. The disease is common in southeastern Europe, Africa and Asia, including Turkey [1-3]. Clinical signs vary from mild to severe, depending on

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host and viral factors. Fever and a variable degree of systemic disturbance develop. It is clinically manifested as conjunctivitis, rhinitis, and multiple non-vesicular swellings in skin and mucous membranes, respiratory distress, and death. Widespread skin lesions are seen on the muzzle, ears, and areas free of wool. In severe cases, lesions can also spread to the lungs, liver, kidneys and secondary pneumonia is commonly seen [1,3-6].

The acute phase response (APR) is a systemic reaction of the body to local or systemic disturbances in association with a wide variety of disorders, including infection, trauma, inflammatory diseases, various neoplasms and immunological disorder. Acute phase proteins (APP) are circulating blood proteins primarily synthesized by the liver in a few hours in response to inflammatory stimulus [7-10]. Acut phase proteins are defined as positive APP, which serum concentrations increase and negative APP, which serum concentrations decrease in response to APR [11,12]. Synthesis of APP is induced by proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and they are released into the bloodstream as long as inflammatory stimulus persists. Acute phase proteins are considered as a part of the innate host defense system, and its quantification provides valuable clinical information in the early identification of inflammation or injury, diagnosis, monitoring health status and prognosis of various diseases. Furthermore, these proteins have been shown to useful differential diagnosis of clinical and subclinical diseases, bacterial and viral infections [9,10]. Acute phase proteins have been studied widely in human medicine, especially as biomarkers of inflammatory processes, various infections, to diagnose and monitor the outcome of the treatment of diseases. However, they have been relatively under-utilised in veterinary medicine. In veterinary medicine, APPs have been identified as useful diagnostic biomarkers of diseases in dogs, cats and cattle. However, the valuable of APPs as indicators of inflammatory conditions, monitoring animal health and detection of diseases in veterinary clinical practice, especially in farm animal medicine is not well-documented [11,12].

It is known that free radicals play an important role in the pathogenesis of various infectious diseases [13], and that poxvirus causes an increase in free radicals, affecting the membrane phospholipids and thus causing damage to cells and tissues [14]. In the organism, there is a balance between oxidants and antioxidants, and when the balance shift in favour of oxidants, oxidative stress develops. Development of oxidative stress results in increased formation of free radicals and lipid peroxidation [15]. Malondialdehyde (MDA) is an important indicator of lipid peroxidation and is used as an indicator to determine the severity of tissue damage [16,17]. The system preventing damage caused by free radicals in organism is called the antioxidant (Reduced Glutathione 'GSH') system [18].

It is well-known that the main lesions occur in the skin in poxvirus disease, and that many organ or system functions such as liver, kidney, respiratory and circulatory system are impaired as a result of systemic inflammatory response [4]. Acute phase proteins are synthesized from the liver as a consequence of APR in many viral, bacterial and parasitic diseases [9,10]. Therefore, the aim of the present study is to determine whether or not oxidative stress and APR develop in the naturally infected sheep with poxvirus.

MATERIAL and METHODS

Twenty, 1 to 2-year-old, naturally poxvirus-infected Morkaraman sheep and 10 clinically healthy sheep were used. In poxvirus infected sheep, fever, loss of appetite, rhinitis, respiratory problems, and pox lesions under the tail, in the eyelids, axillae, mucous membranes and in in between the back legs were observed. From these sheep, poxvirus was isolated and identified in the research laboratory (Erzurum Veterinary Control Institute-Turkey). The research work was carried out with the approval of the Institutional Ethics committee of Kafkas University, Faculty of Veterinary Medicine (KAU 2016/75). All sheep were routinely examined and the lesions, rectal temperatures, heart rates and respiratory rates were recorded. Blood samples were collected into the tubes with and without anticoagulant from the jugular veins. Serum ceruloplasmin concentrations were determined spectrophotometric method, developed by Colombo and Ricterich [19], while serum haptoglobin concentrations were determined by methods of Skinner et al.[20]. Whole blood GSH and serum MDA concentrations were measured using spectrophotometric methods as described by Beutler et al.[21], and Yoshioka et al.[22], respectively (UV-1201, Shimadzu, Japan). Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) activities, urea, creatinin, albumin, and Fe concentrations were determined using colorimetric commercial test kits (IBL, Turkey) according to the manufacturer's instructions (Epoch, Biotek, USA).

Statistical Analysis

SPSS for Windows 20.0 was used for the statistical analyses. The distributions of the data obtained from the groups were shown as normal distribution according to the Kolmogorov-Smirnov test. Therefore, Student's t-test was used for the comparison of the groups.

RESULTS

In infected animals, fever (39.85±0.20°C), conjunctivitis, rhinitis, nasal discharge (serous and/or mucopurulent), lacrimation, depression, anorexia, swelling in the hairless areas (under the tail, eyelids, axillae etc.), coughing and coarsening of vesicular respiratory sounds, increase in heart rate (98.0±3.5/min), rapid and shallow respiration (30.0±1.00/min.) were observed. There were no abnormal

***Table 1.** Acute phase proteins, biochemical and oxidative stress parameters in control and sheep with naturally infected Sheeppox virus. Data are presented as mean±standard error (X±SEM)*

Parameters	Control	Infected	P
Haptoglobin (g/L)	0.138±0.008	0.647±0.020	P<0.01
Ceruloplasmin (mg/dL)	13.05±0.89	18.23±0.93	P<0.05
Albumin (g/dL)	3.26±0.15	2.45±0.16	P<0.01
ALP (U/L)	62.78±1.67	99.56±4.72	P<0.05
AST (U/L)	57.36±5.09	91.45±6.65	P<0.05
Urea (mmol/L)	6.87±0.33	8.96±0.43	P<0.01
Creatinin (µmol/L)	96.52±3.17	194.57±3.58	P<0.01
Fe (µg/dL)	116.08±8.26	72.12±3.26	P<0.01
MDA (nmol/mL)	1.89±0.17	2.87±0.24	P<0.01
GSH (mg/dL)	85.42±4.60	69.27±2.17	P<0.01

clinical findings in control group. In these animals, the mean rectal temperature, heart rate and respiratory rate were 38.9±0.03°C, 76.0±1.5/min. and 22.0±0.50/min. respectively. Poxvirus were also isolated and identified in the samples collected from infected sheep.

In the study, blood concentrations of haptoglobin (P<0.01), ceruloplasmin, ALP and AST activities (P<0.05), urea, creatinine and MDA (P<0.01) were significantly high in sheeppox virus infected-animals compared to those of control animals. While, blood levels of albumin, Fe and GSH (P<0.01) were significantly lower in infected group than that of control group *(Table 1)*.

DISCUSSION

In agreement with literature [1,2,14], swelling in the hairless areas (under the tail, eyelids, axillae etc.), conjunctivitis, rhinitis, lacrimation, depression, nasal discharge (serous and/or mucopurulent) and anorexia etc. were found on clinical examination of sheep with naturally infected sheeppox virus.

The acute phase response is a very fast response, developing with increased and/or decreased concentrations of APP within a few hours, which remain altered as long as the inflammatory stimulus persists. For this reason, they can be used as early identification of inflammation or injury, diagnosis and prognosis of diseases. These proteins can also be used as indicators in the monitoring of animal health [9,10,12,23,24]. In this study, haptoglobin and ceruloplasmin concentration were increased and albumin concentration was decreased, indicating the development of APR in sheeppox infected sheep.

The main lesions occur in the skin in poxvirus disease, and that many organ or system functions such as liver and respiratory system are impaired as a result of systemic inflammatory response [4]. Acute phase proteins, a non-specific marker of tissue damage, are synthesized in the liver as a result of the systemic inflammatory response developed in the organism. Haptoglobin, a marker of acute inflammation and tissue damage in ruminants, is a glycoprotein synthesized in the liver by pro-inflammatory cytokines [9,11,12,24]. Haptoglobin has been reported to be present in very low concentrations (<0.1 mg/mL) in healthy animals. But its concentrations increase and remain high in many bacterial, viral, parasitic and inflammatory diseases as long as the stimulus persists [7,25-28]. It is a nonspecific marker of tissue damage, and suggested to be used to determine prognosis and severity of the diseases. Its levels between 0.1-1 g/L, and >1 g/L suggested to indicate good and poor prognosis of the diseases, respectively [20,29]. In addition, haptoglobin concentrations of 0.2-0.4 g/L were defined as mild and 1-2 g/L as severe infection [20]. In a study in cattle with FMD, Merhan et al.[30] detected increased APP levels and they reported that APP concentrations might be a relationship between the severity of disease. In our study, serum haptoglobin levels were approximately 0.7 g/L in naturally infected sheep, and the disease prognosis was good, severity was moderate in according to reported studies [20,29]. In addition to these, all the infected sheep fully recovered and no cases of death were observed in the study, supporting to the good prognostic value of haptoglobin. Ceruloplasmin is considered as an indicator of the presence of infection [27,30,31]. It protects the cells against oxidative damage by reducing the effects of oxygen radicals, as well as increasing the phagocytic and antimicrobial efficiency of immune system cells [32]. Increased in haptoglobin and ceruloplasmin levels determined in the study were presumably due to increased activity in phagocytic cells and/or tissue damage and inflammation reported in poxvirus infection [4,14].

Albumin, a negative APP, is a indicator of liver function and, its blood concentrations decrease in case of anorexia, liver disorders and intestinal malabsorptions [27,33]. Furthermore, its concentration has been shown to decrease in tissue damage and inflammatory conditions, in association with increased albumin catabolism [9,11]. In this study, albumin concentration was also decreased and it was thought that this might be caused by an increase in albumin catabolism due to liver cell damage, tissue damage and inflammation or anorexia, observed in poxvirus infected sheep.

Aminotransferases found in hepatocytes are considered to be an important indicator of hepatotoxicity in patients with elevated blood levels in liver injury. The relative activity of AST and ALP indicates the severity of liver damage [33,34]. Aspartate aminotransferase activity is increased in passive congestion, tension in proventriculus, muscle destruction and liver damage. Alkaline phosphatase activity is known to increase during the cholestasis, bone formation/repair, impaired hepatobiliary circulation and stress [4,33,34]. In addition, poxvirus has been reported to cause liver cell damage and cause changes in enzyme activities [4]. In the study, increased AST and ALP activities are most probably occurred due to liver cell damage and glucocorticoid activity and/or disease-induced stress [4,33]. Serum Fe concentration is reduced in malnutrition, APR, acute or chronic infections and chronic liver disease [33,35]. It was thought that, reduced Fe levels obtained in the study might be due to APR, liver disorders and anorexia, occurs in poxvirus infection [4,14]. Serum urea and creatinine are used to evaluate renal function [33]. However, its concentrations also increase during the infection, anorexia, high fever and increased protein catabolism [36]. In addition to these, systemic inflammation that develops in the infections, disturb kidney perfusion and impair its filtration capability, resulting with increase in blood urea and creatinine [37]. In this study, serum urea and creatinine levels were also increased and it was thought that this might be caused by an increase in protein catabolism or impaired renal function due to systemic inflammation.

Oxidative stress develops when the balance between oxidants and antioxidants shift in favour of oxidants [13,15,38]. Viral infections have been reported to cause damage to the cells and tissues via increased free radicals in host cells [13,38]. However, it is also stated that this increase is an important factor in body defence against viral infections and that pathogens are destroyed in this way [39]. Kirmizigul et al.[16] were reported that the increase in nitric oxide, MDA, total sialic acid, total oxidant capacities and total antioxidant capacity decreased in the study of natural infected sheep with poxvirus. In addition, Issi et al.[14] were reported that the increase in blood catalase activities, MDA and antioxidants (vit E, vit C and GSH) decreased in the study of natural infected sheep with poxvirus. In this study, MDA level was increased while GSH level was decreased, which was thought to be caused by free radicals formed by phagocytes, which are part of the stress and/or defence system in animals. In the present study, supported by the other reports [14,16], MDA levels were increased, while GSH levels were decreased, indicating the development of oxidative stress in sheep infected with poxvirus.

In conclusion, it is suggestive that oxidative stress and APR develop in sheep infected with poxvirus. Furthermore, both liver and kidney functions are also affected in the infected animals. We consider that the data obtained from this study might contribute to the understanding of the pathogenesis and diagnosis of the disease.

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