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Levels of Acute Phase Protein and Some Biochemical Parameter in Cattle Infected with *Mycobacterium Bovis*

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Summary: In this study, it was aimed to determine the levels of acute phase proteins (APP) and some biochemical parameters in cattle infected with *Mycobacterium bovis.* Twenty-five *M. bovis* infected and twenty-five antibody-negative healthy bovine sera were used according to ELISA test results to investigate the biochemical parameters. Blood samples obtained from *Jugular veins* of animals were collected into plain tubes. Haptoglobin, serum amyloid A (SAA), ceruloplasmin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine and iron (Fe) levels were measured colorimetrically. Compared with the control group, cattle with tuberculosis were found have statistically significant increased levels of APP such as serum haptoglobin, SAA and ceruloplasmin and levels of biochemical parameters such as AST, ALP, GGT, urea and creatinine while decreased levels of serum Fe. It was determined that acute phase response occurred in cattle infected with *M. bovis* which led to increase APP and impairment in the normal functions of the liver.

Key words: Acute phase proteins, biochemical parameters, cattle, *Mycobacterium bovis*

***Mycobacterium bovis* ile Enfekte Sığırlarda Akut Faz Protein ve Bazı Biyokimyasal Parametre Düzeyleri**

Özet: Bu çalışmanın amacı *Mycobacterium bovis* ile enfekte sığırlarda akut faz proteinler (AFP) ve bazı biyokimyasal parametrelerin düzeyinin belirlenmesidir. Çalışmada biyokimyasal parametreleri araştırmak için ELISA test sonuçlarına göre 25 adet *M.bovis* ile enfekte ve 25 adet sağlıklı sığır kullanıldı. Hayvanların *V. jugularis*'inden kan örnekleri antikoagulansız tüplere alındı. Haptoglobin, serum amiloid A (SAA), seruloplazmin, aspartat amino transferaz (AST), alkalen fosfataz (ALP), gama-glutamil transferaz (GGT), üre, kreatinin ve demir (Fe) düzeyleri kolorimetrik olarak tayin edildi. Yapılan analizler sonucunda *M.bovis* ile enfekte sığırlar ile kontrol grubundaki hayvanlar karşılaştırıldığında AFP'lerden haptoglobin, SAA ve seruloplazmin; biyokimyasal parametrelerden ise AST, ALP, GGT, üre ve kreatinin düzeylerinin kontrol grubuna göre istatistiksel olarak anlamlı derecede yükseldiği, serum Fe düzeyinin ise düştüğü belirlendi. Sonuç olarak *M.bovis* ile enfekte sığırlarda akut faz yanıt oluştuğu ve bunun sonucu olarak da AFP'lerin arttığı, karaciğer fonksiyonlarının bozulduğu belirlenmiştir.

Anahtar kelimeler: Akut faz proteinler, biyokimyasal parametreler, *Mycobacterium bovis*, sığır

Introduction

Bovine tuberculosis is a chronic zoonotic disease that causes a loss in productivity in animals. The causative agent is an acid-fast, aerobic and sporeless bacterium, called *Mycobacterium bovis.* Although cattle are the main hosts, the disease has been able to infect a variety of animals including domestic animals such as sheep, goats, pigs, horses and wild animals, such as foxes, coyotes (23,28). Because it is a zoonosis, the disease poses risks for people in different professions, such as farmers, caregivers, butchers, veterinarians and veterinary technicians who are in contact with animals daily basis (26).

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As bovine tuberculosis is a chronic disease, the symptoms of the disease can take place for several weeks, and even months, later depending on the age and resistance of the host. Disease symptoms include emaciation, loss of appetite, undulating fever, enlarged lymph nodes, cough, and diarrhea or constipation in affected digestive system (2,3).

Proteins that emerge in response to stimuli such as inflammation, tissue damage and infection leading to acute phase response (APR) and that are synthesized by the liver are referred to as acute phase proteins (APP) (5,24,27). Although blood concentrations vary according to animal species, APPs that have some diagnostics importance in cattle and sheep are primarily haptoglobin and serum amyloid A (SAA) (36). In

several studies, APPs were reported to be utilized in discrimination between bacterial and viral infections, differential diagnosis of clinical and subclinical diseases, and determination of prognosis in sick animals (27,37).

Although there are many studies on changes in APP levels (10,11), the hematological and biochemical changes (21,29,31) in tuberculosis in human medicine, there is limited number of studies on hematology and biochemistry of the disease in veterinary medicine (18,20,22,33). No studies were encountered in the literature regarding APPs in animals with tuberculosis. In this study, it was aimed to determine the levels of APPs and some biochemical parameters in cattle infected with *M. bovis.* We believe that the obtained data will contribute to the elucidation of the pathogenesis and mechanism of the disease in cattle infected with *M. bovis*.

Materials and Methods

This study was initiated following ethics approval from Kafkas University Local Ethics Committee for Animal Experiments (KAU-HADYEK/2012-23). Study participants included 460 cattle (≥5 years) that were grown in family owned businesses around Kars and its neighboring districts. Animals used in the study were not vaccinated against *M. bovis*.

Blood samples obtained from *Jugular veins* of animals were collected into plain tubes, centrifuged at 3000 rpm for 15 minutes and obtained sera were stored at -20°C until analyzed. The presence of *M. bovis* antibodies in serum samples was investigated by an ELISA kit (Institut Pourquier, France). The test was performed according to manufacturer's instructions, and results were measured spectrophotometrically (Epoch, Biotek, USA) at a wavelength of 450nm. Samples with a mean sample-to-positive control (S/P) ratio of ≥ 0.30 were considered positive for *M. bovis* antibody. Twenty-five *M. bovis* infected and 25 antibody-negative healthy bovine sera according to ELISA test results were used to investigate the biochemical parameters.

Haptoglobin and ceruloplasmin analysis were performed spectrophotometrically (UV-1201, Shimadzu, Japan) according to the methods which has been previously reported by Skinner et al. (34) and Colombo and Ricterich (4), respectively. SAA levels were measured by an ELISA kit (Tridelta development limited, Ireland). Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine and iron (Fe) levels were measured colorimetrically by (Epoch, Biotek, USA) commercial test kit (DDS, Turkey).

Statistical analysis

SPSS (35) for Windows-20.0 was used in the analysis of the study data. Kolmogorov-Smirnov test was utilized for assessing the normality of distribution. As the groups were normally distributed, Student's t-test was used for the comparison of the groups.

Results

Compared with the control group, cattle with tuberculosis were found to have increased levels of serum haptoglobin, SAA and ceruloplasmin (P<0.01). While AST, ALP, GGT, urea and creatinine levels were increased (P<0.01) in cattle with tuberculosis, serum Fe (P<0.01) was decreased (Table 1).

Table 1. Levels of acute phase proteins and some biochemical parameters in clinically healthy and cattle infected with *Mycobacterium bovis* (Mean±SEM)

Parameters	Control	Infected with *Mycobacterium bovis*	P value
Haptoglobin (g/L)	0.098±0.005	0.124±0.008	P<0.01
SAA (µg/mL)	17.34±0.77	23.78±0.83	P<0.01
Ceruloplasmin (mg/dL)	14.51±0.46	19.33±0.60	P<0.01
AST (U/L)	53.46±2.02	72.73±3.37	P<0.01
ALP (U/L)	34.09±1.05	48.62±2.93	P<0.01
GGT (U/L)	29.45±1.37	44.83±2.42	P<0.01
Urea (mmol/L)	7.88±0.19	9.56±0.27	P<0.01
Creatinine (µmol/L)	90.54±1.42	148.93±3.96	P<0.01
Fe (µg/dL)	106.08±3.46	89.45±4.02	P<0.01

Discussion

Besides being a multisystemic, infectious and zoonotic disease, bovine tuberculosis is quite important disease because it causes a loss in productivity in animals and threatens public health (25,32).

APR happens in response to stimuli such as inflammation, tissue damage and infection, and alterations in the synthesis of APP in the liver occur as a result of APR. Several studies showed increased and decreased blood concentration of APPs, which are nonspecific markers of inflammation (13,27).

Although haptoglobin and SAA, which are important positive APPs in cattle, are fairly low in the serum of healthy cattle, their levels increase in case of inflammation (12,27). Haptoglobin is either not present or is present in small amounts (<0.1g/L) in the serum of healthy cattle (6). Levels of haptoglobin have been reported to significantly increase naturally or experimentally induced bacterial (15,34), parasitic (38), and viral (8,17) diseases.

The essential functions of multi-functional haptoglobin are immunomodulation are to prevent the use of free Fe by the harmful bacteria (27). Serum haptoglobin levels were reported to be used in determining the prognosis of the animal, and serum levels of 0.1-1g/L were referred to as “good prognosis” and levels of >1g/L is considered “poor prognosis” (6,7).

The other important APP in ruminants is SAA, and it has functions, such as induction of collagenase, increasing leukocyte adhesion to endothelial cells, and detoxification of endotoxins (24,27). Serum concentrations of SAA increase in aseptic inflammation, surgical trauma and natural infection. It was stated that SAA levels increased in 2-5 hours, reaching peak within 24 hours and that could be used in the earlier diagnosis of acute cases (27). Taken together, haptoglobin and SAA were reported to be important in differential diagnosis of acute and chronic cases (1,16). In their study in 81 acute and chronic sick cattle, Horadogada et al. (16) found 68% increase in the acute phase and 24% increase in the chronic phase in haptoglobin levels, and 100% increase in the acute phase and 54% increase in the chronic phase in SAA levels. Thus, these investigators were able to differentiate acute and chronic phases of the disease. In this present study, our findings were in line with Horadagoda et al. (16), increase in haptoglobin and SAA levels was detected, and this rise was found to be 26% and 37% in haptoglobin and SAA concentrations, respectively. The rate of increase in serum haptoglobin and SAA concentrations showed a chronic course and the prognosis was determined to be good as haptoglobin levels were between 0.1 and 1g/L. The possible reason for the increase in APP levels might be related to the extent of tissue damage.

Ceruloplasmin, which is another positive APP used in the evaluation of animal health, is an oxidoreductase and has an important role in APR. It has been proposed that increases phagocytic and antimicrobial potency of immune cells by regulating copper (14,36). The increase in ceruloplasmin levels in cattle infected with *M. bovis* is thought to be formed in parallel with the increase in the number of phagocytic cells that are important part of both innate and acquired immunity. The serum concentration of transaminases increases in erythrocytes, heart muscle, liver, bile duct and lung injury has been reported (19,30). In a study in cattle with tuberculosis, Shettar et al. (33) reported an increase in the levels of AST, ALT and ALP which are among these transaminases. In our study, elevated levels of serum AST, ALP and GGT concentrations were detected in *M. bovis*-positive animals and the likely cause of this elevation might be due to functional disorder resulting from inflammation of the liver that has a central role in the metabolism.

Serum urea and creatinine concentrations, which are used for assessment of renal functions (19), are reported to have an increase due to higher levels of protein catabolism in the case of infections, loss of appetite and high fever (9). Serum urea and creatinine levels have been found to increase in deer (20) and American bison (22) infected with tuberculosis. In this present study, similar to other studies (20,22), an increase in serum urea and creatinine level was detected in *M. bovis*-positive animals and this increase might stem from the increase in the protein catabolism associated with the disease. Serum Fe levels decreased in APR, malnutrition and chronic liver disease (13). In this present study, the reason for the decrease in serum Fe levels might be because of reduced Fe release and/or damaged liver due to APR.

In conclusion, it was determined that APR occurred in cattle infected with *M. bovis* and con-

sequently, the liver was damaged and had impaired functions. We believe that with the obtained data in this study will be useful in the elucidation of the pathogenesis of liver in bovine tuberculosis along with APR.

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