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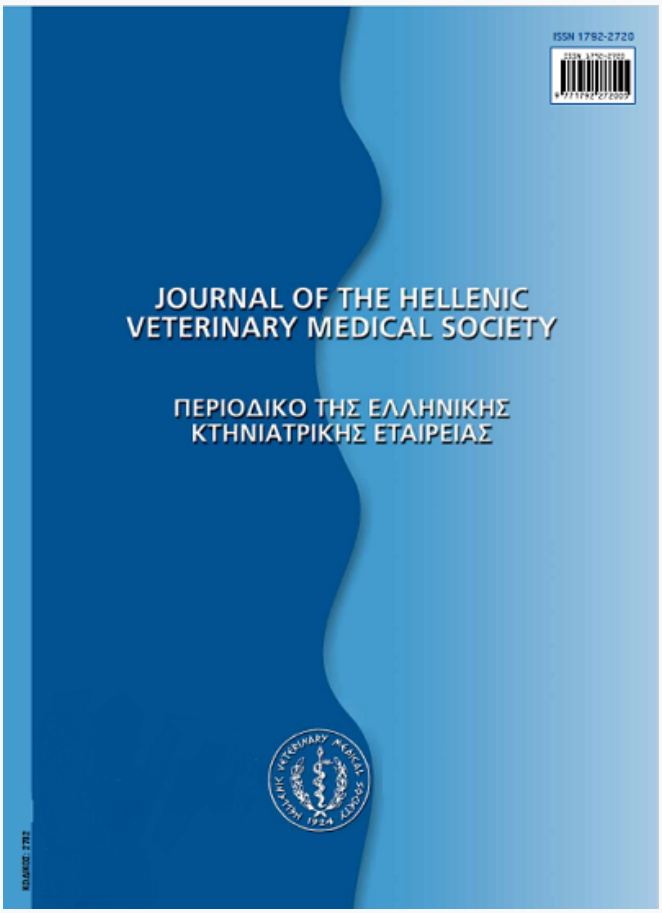

Levels of certain biochemical and oxidative stress parameters in cattle with Brucellosis

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Research article
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Levels of certain biochemical and oxidative stress parameters in cattle with Brucellosis

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ABSTRACT. The purpose of the present study was to determine concentrations of some biochemical parameters and oxidative stress levels in cattle with brucellosis. For this purpose, a study group included with 20 cattle with brucellosis and a control group with 10 clinically healthy cattle were used. Blood samples were collected into the tubes (with and without anticoagulant agent) from the Jugular vein of animals in each group. The reduced glutathione (GSH) in whole blood and levels of malondialdehyde (MDA) and nitric oxide (NO) were determined spectrophotometrically. Additionally, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and iron (Fe) levels in serum samples were colorimetrically determined. Compared with the animals in the control group, it was determined that cattle with brucellosis had significantly ($P<0.01$) higher levels of AST, ALT, creatinine and NO and lower level of Fe. The increases of MDA and GSH levels were moderate and significantly important ($P<0.05$) while serum urea manner was not significantly altered. It was determined that significant alterations occurred in various biochemical parameters and antioxidant activity decreased in cattle with brucellosis.

Keywords: biochemical parameters, Brucella abortus, cattle, oxidative stress

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INTRODUCTION

Brucellosis is an infectious disease characterized by chronic inflammation in various organs such as liver, kidney, spleen and heart of human and leads contagious abortion in animals as a consequence of reproductive disease. The causative agent of the disease in cattle is a Gram-negative, facultative intracellular bacteria called as *Brucella abortus*. Because its zoonotic aspect, professional groups such as veterinarians, farmers, butchers and shepherds are at high risk (Otlu et al., 2008; Nicoletti, 2010). Following entrance through the digestive system, conjunctiva, respiratory system or skin, microorganisms are transported to regional lymph nodes and then spread to the spleen, liver, bone marrow, central nervous system and reproductive organs via lymphatic and blood circulation (Baldwin and Winter, 1994). Brucellosis is a multisystemic disease and the most common symptoms in cattle are abortion, metritis, decreased milk production, weak and/or dead offspring birth and infertility (Neta et al., 2010).

Free oxygen radicals caused by oxidative stress prevent normal functioning of the cells and lead to an increase in malondialdehyde (MDA), the end product of lipid peroxidation, by causing damage in biological molecules (lipids, proteins and DNA) (Halliwell, 1994). MDA is used to determine the severity of cellular damage (Pekmezci et al., 2012). The defense system acting for preventing damage caused by free radicals in the organism and/or lipid peroxidation is called antioxidant defense (e.g. reduced glutathione (GSH)) system (Değer et al., 2008). Nitric oxide (NO), which is synthesized from L-arginine by nitric oxide synthase, is a cytotoxic, free molecule with various functions such as immunoregulation (Marletta, 1988). Besides having inflammation-inducing features, NO transforms into peroxynitrite, which is quite harmful to many organs and tissues (Petermann et al., 1999). NO has been reported to be very important in the defense against many microorganisms (De Groote and Fang, 1995; Kreil and Eibl, 1996). An important role of NO was reported in the pathogenesis of brucellosis. It inhibits intracellular proliferation of the pathogen and is an important molecule in innate immunity against this disease (Jiang et al., 1993). Iron (Fe) plays an important role in biosynthesis of heme which is essential for the intracellular replication and virulence of *B. abortus*. Serum Fe concentration decreases in cases of malnutrition, acute phase response (APR) and chronic liver disease (Gruys et al., 1994).

Brucella agents cause damages in various organs and impairments in their functions. Although there are many studies on oxidative stress and biochemical parameters in brucellosis in human medicine (Ali, 2009; Serefhanoglu et al., 2009), limited number of studies are available on biochemical parameters (Kushwaha et al., 2014; Elazab, 2015) and oxidative stress (Nisbet et al., 2007; Kataria et al., 2010) in cattle with brucellosis in veterinary medicine. The purpose of the present study was to determine concentrations of some biochemical parameters and oxidative stress levels in cattle with brucellosis. The data that we obtained by this study will further elucidate the pathogenesis of brucellosis in cattle and will provide with a better understanding of the disease by veterinarians.

MATERIALS AND METHODS

Ethical approval and study design

Ethics approval was obtained from Local Ethics Committee for Animal Experiments of Kafkas University (KAU-HADYEK-2015/59). In a total of 30 animals were used in the study including both study and control groups. The study group is composed of twenty cross-breed female cattle refered clinical history of brucellosis-like symptoms such as late-term abortion and subsequent faults as endometritis, retained placenta and infertility were sampled from family owned businesses around Kars. The control group is formed from 10 clinically healthy cross-breed cattle that were farming in hypothetically brucellosis free region of Kars. All animals were in the same age range (3-5 years) and were selected randomly from ten different farms including animals with similar care and feeding conditions as all were subjected to extensively farming. General clinical examination of the animals in study and control groups was performed and clinical examination findings (body temperature, respiration, pulse rate, etc.) were within normal values. Animals in both groups did not have any vaccination history against brucellosis.

Sampling

Blood samples obtained from jugular vein's of animals were collected into both anticoagulant (EDTA) included and plain tubes. Whole blood was used for measurement on the same day. The samples in plain tubes were centrifuged at 3000 rpm for 15 minutes and sera were obtained.

Detection of *Brucella* antibodies

Antibodies against *Brucella* species in serum samples were investigated by Rose Bengal Plate Test (RBPT) and serum tube agglutination test (SAT). Test antigens are derived from *B. abortus* strain S99, which were produced by Pendik Veterinary Control and Research Institute, Ministry of Food, Agriculture and Livestock, could detect entire *Brucella* species with smooth structure. RBPT and SAT were performed according to the method reported by Alton et al. (1988). For RBPT, 20 µl of RBPT antigen was mixed with an equal volume of serum on a clean slide and agglutination (clustering) occurring within 3-4 minutes was evaluated as positive. For SAT, serum samples prepared with addition of tube agglutination antigen with a final concentration of 1:10 to 1:320 were left to an overnight incubation at 37 °C and samples with lace-style agglutination were evaluated as positive. Both tests were performed in the presence of Brucella positive and negative control sera. Reactions of two positive and above in RBPT and positive dilutions of 1:40 and above in SAT were considered as diagnostic titers (Otlu et al., 2008; Öztürk and Büyük, 2015).

Biochemical analysis

Whole blood GSH levels were measured on the same day and sera were stored at -20 °C until analyzed. Whole blood GSH, serum MDA and serum NO were measured spectrophotometrically (UV-1201, Shimadzu, Japan) according to the methods reported by Beutler et al. (1963), Yoshioko et al. (1979) and Miranda et al. (2001), respectively. Glutathione GSH is a method based on the development of a stable yellow color when a 2-nitrobenzoic acid is added to sulfhydryl compounds. The products were read at 412 nm. MDA as an end product of lipid peroxidation concentrations were measured by the method based on the reaction between thiobarbituric acid and MDA. The end products were read at 535 nm. In NO method nitrate is reduced to nitrite by vanadium chloride ($VaCl_3$), and then in acidic environment nitrite was reacted with sulphanilamide to produce colored diazonium compound, which was read at 540 nm.

The blood serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the blood serum concentrations of urea, creatinine and Fe were measured colorimetrically by commercially available diagnostic kits (Erba Diasis Diagnostic Systems Inc, Turkey).

Statistical analysis

SPSS version 20.0 for Windows was used for the statistical analysis. The distribution of the data obtained from the groups was shown as normal distribution according to the Kolmogorov-Smirnov test. Therefore, Student's t-test was then used to compare the differences between the values determined in two groups of animals. A significance level of $P \leq 0.05$ was used in all comparisons.

RESULTS

In all 20 serum samples obtained from cattle with brucellosis, high (++++) positivity with RBPT and Brucella antibody at 1:320 (10 samples) and 1:640 (10 samples) titers were observed. All the serum from 10 cattle in the control group were found negative as the result of double run with RBPT and SAT.

Compared to the animals in the control group, it was determined that cattle with brucellosis had significantly ($P < 0.05$) higher activities of AST and ALT and significantly higher concentrations of creatinine, MDA and NO, whereas the concentrations of Fe and GSH were significantly lower ($P<0.01$ for Fe, $P<0.05$ for GSH) Table 1. Although blood serum concentration of urea was higher in cattle with brucellosis than the controls, the difference was not significant ($P>0.05$; Table 1). The differences of the parameters tested were also insignificant ($P>0.01$) among the animals with mild (1:320) or high (1:640) antibody titers.

Table 1. Mean values and standard errors of biochemical and oxidative stress parameters in clinically healthy and in cattle with brucellosis (X±SEM)

Parameters	Control	Infected	P
AST (U/L)	59.30±1.50	84.25±3.07	P<0.01
ALT (U/L)	62.59±3.24	101.29±4.65	P<0.01
Creatinine (µmol/L)	87.48±1.54	182.26±4.59	P<0.01
Urea (mmol/L)	7.02±0.43	7.76±0.34	NS
Fe (µg/dL)	112.12±7.27	59.18±3.14	P<0.01
MDA (nmol/mL)	2.03±0.23	2.90±0.29	P<0.05
GSH (mg/dL)	76.30±3.61	67.68±1.58	P<0.05
NO (µmol/L)	32.06±3.25	54.62±2.35	P<0.01

NS: Non Significant

DISCUSSION

Besides being a chronic infectious disease that affects many organs and systems and threatens public health, brucellosis negatively affects livestock economy especially in developing countries by leading to abortion, decrease in milk production and infertility (Nicoletti, 2010).

The liver, which is the largest organ of the reticuloendothelial system, is affected by infectious and parasitic diseases because of its central role in immunity (Albayrak and Albayrak, 2011). Brucellosis with multisystemic features is able to settle in liver and thus liver plays an important role in protection and causes a slight increase in transaminase levels and moderate hepatosplenomegaly. (Ariza et al., 2001; Albayrak and Albayrak, 2011).

In studies on brucellosis in cattle, Kushawa et al. (2014) reported decreased sorbitol dehydrogenase (SD), AST and ALT activity and Elazab et al. (2015) reported increased AST and ALT activity. In another study conducted in camels, El-Boshy et al. (2009) reported increased levels of SD, AST and ALT. In this study, consistent with other studies (Ariza et al., 2001; Albayrak and Albayrak, 2011), AST and ALT activities might have increased significantly due to functional disorders associated with inflammation in the liver.

Alterations in serum urea and creatinine levels are generally used for the evaluation of renal function (Kaneko et al., 2008). However, protein catabolism increases in cases of infection, anorexia and pyrexia, and as a result, serum urea and creatinine levels increase (Gokce and Woldehiwet, 1999). In addition, brucellosis was also reported to rarely affect the kidneys (İbrahim et al., 1988). In the present study, statistically insignificant increase in urea concentration and significant increase in creatinine levels were determined. These increases were probably due to impairment in kidneys and/or increase in protein catabolism due to infection.

Serum Fe concentration decreases in cases of malnutrition, acute phase response (APR) and chronic liver disease (Gruys et al., 1994). In this study, Fe concentration was determined to be low in animals with brucellosis. The reason of the decrease may be due to liver dysfunction or a huge bacterial uptake of Fe which has a core role in biosynthesis of heme which is essential for the intracellular replication and virulence of *B. abortus*.

MDA, which is the most widely known form of lipid peroxides, is used to determine the severity

of cellular damage (Cighetti et al., 2002). In a study in cattle infected with Brucella spp, Nisbet et al. (2007) reported a significant increase in MDA and NO levels. In addition, oxidative stress has been reported in bacterial diseases such as traumatic reticuloperitonitis (Atakişi et al., 2010), osteoarthritis (Surapaneni and Venkataramana, 2007) and tuberculosis (Madebo et al., 2003). Oxidative pathway has an important role in the destruction of intracellular bacteria by macrophages and polymorphonuclear leukocytes (Serefhanoglu et al., 2009). In this study, in parallel with other studies (Madebo et al., 2003; Nisbet et al., 2007; Surapaneni and Venkataramana, 2007; Atakişi et al., 2010), it was found that oxidant-antioxidant balance had deteriorated and as a result, MDA concentration was increased and GSH levels were decreased in the brucellosis group.

Nitric oxide is an important molecule in defense against many microorganisms (De Groote and Fang, 1995; Kreil and Eibl, 1996). It acts on pathogens and tumor cells by inhibiting the enzymes that are responsible for the production of ATP (Taylor-Robinson et al., 1996). NO, which is produced by macrophages in bacterial infections, shows antibacterial properties against bacteria and host defense depends on the concentration of NO (Akaike et al., 1998). In a study, it has been reported to increase in brucellosis (Nisbet et al., 2007) in cattle. In the present study, NO levels were increased in cattle with brucellosis and it was thought to be due to induced NO synthesis by macrophages defending the organism against infection.

CONCLUDING REMARKS

It was determined that significant alterations occurred in biochemical parameters and antioxidant activity decreased in cattle with brucellosis. We concluded that obtained parameters would contribute to the pathogenesis and diagnosis of the diseases.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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