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Research Article

Serum and Saliva Sialic Acid and Oxidative Stress Parameters Changes in Bulls with Foot and Mouth Disease [1]

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Abstract

The study comprised of 12 bulls, aged between 18-36 months, determined severe symptoms of Foot-and-mouth disease (FMD) and 10 clinically healthy bulls of similar age. Serum and saliva total sialic acid (SA), malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels were measured. In this study were determined acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity as clinical signs. Mean serum SA, MDA, GSH and NO levels were 503.96±21.43 mg/L, 31.82±3.43 µmol/L, 63.43±2.92 mg/dL, and 6.49±0.36 nmol/L in healthy bulls and 862.01±17.35 mg/L, 82.49±9.90 µmol/L, 24.96±2.32 mg/dL, and 13.89±0.53 nmol/L in FMD cases, respectively. Mean saliva SA, MDA, GSH and NO levels were 75.98±10.25 mg/L, 1.06±0.17 µmol/L, 0.67±0.05 mg/dL, and 1.44±0.14 nmol/L in healthy bulls and 156.49±14.07 mg/L, 1.81±0.15 µmol/L, 0.34±0.03 mg/dL, and 2.44±0.16 nmol/L in FMD cases, respectively. The differences between the two groups were statistically significant (P<0.001 and P<0.01). Mean serum and saliva GSH level was lower in FMD while all other parameters were considerable high. As a result, showing signs of foot and mouth disease in bulls, serum and saliva in sialic acid and oxidative stress parameters are affected very significantly.

Keywords: *Bull, Malondialdehyde, Foot-and-mouth disease, Total sialic acid,* Glutathione, Nitric oxide

Şap Hastalıklı Boğalarda Serum ve Salya Sialik Asit ve Oksidatif Stres Parametrelerindeki Değişiklikler

Özet

Bu çalışmada 18-36 ay yaşları arasında klinik açıdan şiddetli şap belirtileri tespit edilen 12 ve aynı yaş aralığındaki sağlıklı 10 adet boğa değerlendirildi. Serum ve salyada total sialik asit (SA), malondialdehit (MDA), glutathione (GSH) ve nitrik oksit (NO) düzeyleri ölçüldü. Çalışmada klinik belirtiler olarak; akut ateş, iştahsızlık, ağızda çok yaygın veziküler lezyonlar ve buna bağlı aşırı salivasyon, şiddetli topallık ve verim düşüklüğü belirlendi. Sağlıklı boğalarda ortalama serum SA, MDA, GSH ve NO düzeyleri sırasıyla 503.96±21.43 mg/L, 31.82±3.43 µmol/L, 63.43±2.92 mg/dL ve 6.49±0.36 nmol/L, şaplı olarak değerlendirilen boğalarda ise 862.01±17.35 mg/L, 82.49±9.90 µmol/L, 24.96±2.32 mg/dL ve 13.89±0.53 nmol/L olarak belirlendi. Sağlıklı boğalardan elde edilen salyalarda ortalama SA, MDA, GSH ve NO düzeyleri sırasıyla 75.98±10.25 mg/L, 1.06±0.17 µmol/L, 0.67±0.05 mg/dL ve 1.44±0.14 nmol/L iken bu değerler şaplı olarak değerlendirilen boğalarda 156.49±14.07 mg/L, 1.81±0.15 µmol/L 0.34±0.03 mg/dL ve 2.44±0.16 nmol/L, olarak tespit edildi. İki grup arasındaki farkın istatistiksel olarak anlamlı olduğu belirlendi (P<0.001 ve P<0.01). Şaplı olarak değerlendirilen hayvanlarda serum ve salya GSH değerleri düşük, diğer parametreler ise yüksek olarak tespit edildi. Sonuç olarak şap belirtileri tespit edilen boğalardan elde edilen serum ve salyada sialik asit ve oksidatif stres parametrelerinin önemli derecede etkilendiği belirlendi.

Anahtar sözcükler: *Boğa, Şap hastalığı, Total sialik asit, Malondialdehit, Glutathione, Nitrik oksit*

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and economically important disease caused by foot-and-mouth disease virus (FMDV). Animals that can be affected include cattle, buffaloes, sheep, goats, pigs and wild ruminants [1-3]. FMDV is a positive sense, single-stranded RNA virus (genus *Aphthovirus*, family *Picornaviridae*) occurring in seven serotypes, O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3, each with a wide spectrum of antigenic and epidemiological distinct subtypes. The wide diversity is considered a consequence of the high mutation rate, quasi-species dynamics and recombination [4,5]. The disease spreads by contact between infected and domestic animals, by animal products (milk, meat and semen), by mechanical transfer on people, wild animals and birds, by vehicles and fomites and by the airborne route [2,6,7].

The clinical severity of FMD varies with the strain of virus, as well as the infecting dose, the species and individual susceptibility of the host. It is clinically most apparent in high-yielding dairy cattle and intensively reared pigs, in which the lesions can be severe and debilitating. In adult sheep and goats, FMD is frequently only a mild disease, with transitory clinical signs which can easily be missed by the stockman or veterinarian, or confused with other diseases presenting similar lesions [8,9]. However, even in some breeds of cattle, FMD can also be clinically difficult to recognize because of the mild appearance of the disease [10]. The disease is typically characterized by acute fever and the development of vesicular lesions in the mouth and feet of infected cloven-hoofed animals (principally cattle, pigs, sheep and goats) with consequent excessive salivation, anorexia, lameness, mortality of young animals and reduced productivity [10-13]. Foot-and-mouth disease usually has a high morbidity and low mortality, with mortality occurring mostly in young animals [6].

Sialic acid (SA), an acetylated derivative of neuroaminic acid, increases rapidly following the inflammatory and injury process [14,15]. Therefore the detection of SA particularly lipid bound sialic acid (LBSA) concentrations may be a valuable indicator of inflammatory diseases [16]. Previous studies already indicated increased serum SA concentrations during the course of many diseases including bovine leptospirosis [17-23].

The induction of lipid peroxidation gives rise to an increase in malondialdehyde (MDA) content. This procedure activates cell-protective antioxidant defense mechanisms such as glutathione, uric acid (UA) [24]. The measurement of UA, albumin, reduced glutathione (GSH) and MDA concentrations can therefore be used as indicators of oxidative stress in some diseases but not enough studies previously determined the oxidative stress in FMD [25,26]. In inflammatory conditions, nitric oxide (NO) production increases through stimulation of inducible nitric oxide synthase (iNOS) via activation of pro-inflammatory cytokines and causes NO mediated tissue injury by reacting with superoxide to generate peroxynitrite, a powerful [27].

This study was therefore designed to determine changes in SA, MDA, GSH and NO levels on plasma and saliva in cattle with FMD. And to evaluate usability of these markers obtained from body fluids by a non-invasive simple method for the first time.

MATERIAL and METHODS

The study comprised of 12 bulls, aged between 18-36 months, in all clinical symptoms of the disease is detected and concluded that clinically FMD and 10 clinically healthy bulls of similar age. All animals were from Kars district, Turkey and were subjected to similar management conditions. A complete physical examination was performed on each animal. Blood samples were collected from all animals via jugular vein into plain tubes and carried to laboratory immediately. Sera were collected by centrifugation at 3.000 g for 10 min at room temperature and kept frozen (-25°C) until analysis. All serum samples were analyzed within 15 days. Saliva samples directly taken from oral flowing clear saliva into sterile Eppendorf tubes which are closed after putting the samples. The samples are stored until the analysis phase on -25°C.

Serum and saliva SA levels were measured calorimetrically according to the method detailed by Sydow [28]. Serum and saliva MDA concentrations were determined by the Thiobarbituric acid (TBA) reactivity method [29]. NO was determined according to the method of Miranda *et al.*[30]. The GSH content was measured according to the method of Beutler *et al.*[31]. Same procedures was performed during the measurement of the saliva and serum samples.

Statistical Analyses

Statistical analysis was performed using the SPSS statistical program. Normal distribution of the data was determined using Anderson-Darling Normality test. Values were expressed as mean ± standard error (SE). Independent t test was used to compare the parameters between the groups. Significant level was set at $P<0.05$.

RESULTS

Clinical Findings

In this study were determined acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity as clinical signs.

Serum and Saliva Biochemical Findings

The results of serum biochemical parameters examined for diseased and healthy animals are shown in *Table 1*. Mean SA, MDA, GSH and NO levels were 503.96±21.43 mg/L, 31.82±3.43 µmol/L, 63.43±2.92 mg/L, and 6.49±0.36 nmol/L in healthy bulls and 862.01±17.35 mg/L, 82.49±9.90 µmol/L, 24.96±2.32 mg/dL, and 13.89±0.53 nmol/L in FMD cases, respectively. The results of saliva biochemical parameters examined for diseased and healthy animals are shown in *Table 2*. Mean SA, MDA, GSH and NO levels were 75.98±10.25 mg/L, 1.06±.17 µmol/L, 0.67±0.05 mg/dL, and 1.44±0.14 nmol/L in healthy bulls and 156.49±14.07 mg/L, 1.81±0.15 µmol/L, 0.34±0.03 mg/dL, and 2.44±0.16 nmol/L in FMD cases, respectively. The differences between the two groups were statistically significant (P<0.001 and P<0.01). Mean GSH level was lower in FMD while all other parameters were considerable high *(Table 1, Table 2)*.

DISCUSSION

This study tried to disclose some indicators of oxidative stress and inflammation in natural cases of FMD in bulls. Although the pathogenesis of FMD is of complex nature and the underlying factors are not yet fully understood, several mechanisms have been studied; role of toxins released by the organism, viral attachment, inflammation and/or immune mediated organ dysfunction.

Clinical signs (acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity) determined in this study were in agreement with those reported for FMD [6,11-13].

Our study revealed a marked increase in SA in FMD cases as reported previously by some researchers in RPT, IBK and Leptospirosis [20-23]. Sialic acid is reported to increase in human and animals during a number of pathological situations where the contributory event is either of tissue damage, tissue proliferation or inflammation [15]. In these circumstances, rise in SA is attributed to liberation of sialic acid from cell membrane into circulation as SA is abundantly present in all biological membranes [15,18,32,33].

Another indicator of cellular damage during the course of FMD may be increased MDA, and decreased GSH pool, an indicator of lipid peroxidation. The MDA results of our study are similar to foot and mouth disease in recent years [34]. These findings may suggest the production of free radicals and of lipid peroxidation. This might have been the case in our study as FMD causes tissue damage in various organs via different mechanisms.

In our study, an important increase of NO levels was determined in serum and saliva samples obtained from FMD group therefore our study results indicate that picornavirus can induce the production of NO *in vivo*. It is known that NO plays an important role in the primary defense mechanism against several pathogens; bacteria, viruses and parasites and NO production to be induced by various viruses which inhibit virus replication *in vivo* and

Table 1. Changes in oxidative and biochemical parameters in serum, in bulls with FMD an control. Values are expressed as mean±SE

Tablo1. Şaplı ve sağlıklı boğaların serumlarındaki biyokimyasal ve oksidatif parametrelerdeki değişimler. Değerler ort.±standart hata olarak gösterilmiştir

Parameters	Control (n=10)	FMD (n=12)	P Values
SA mg/L	503.96±21.43 a	862.01±17.35 b	P<0.001
MDA µmol/L	31.82±3.43 a	82.49±9.90 b	P<0.001
GSH mg/dL	63.43±2.92 a	24.96±2.32 b	P<0.001
NO nmol/L	6.49±0.36 a	13.89±0.53 b	P<0.001

a, b *refers to statistical significance between the groups (P<0.001)*
SA: *Total sialic acid,* ***MDA:*** *Malondialdehyde,* ***GSH:*** *Glutathione,* ***NO:*** *Nitric oxide*

Table 2. Changes in oxidative and biochemical parameters in saliva, in bulls with FMD. Values are expressed as mean±SE

Tablo2. Şaplı ve sağlıklı boğaların salyalarındaki biyokimyasal ve oksidatif parametrelerdeki değişimler. Değerler ort. ± standart hata olarak gösterilmiştir

Parameters	Control (n=10)	FMD (n=12)	P Values
SA mg/L	75.98±10.25 a	156.49±14.07 b	P<0.001
MDA µmol/L	1.06±0.17 a	1.81±0.15 b	P<0.01
GSH mg/dL	0.67±0.05 a	0.34±0.03 b	P<0.001
NO nmol/L	1.44±0.14 a	2.44±0.16 b	P<0.001

a, b *refers to statistical significance between the groups (P<0.01 and P<0.001)*
SA: *Total sialic acid,* ***MDA:*** *Malondialdehyde,* ***GSH:*** *Glutathione,* ***NO:*** *Nitric oxide*

in vitro [35-37]. Increased NO, a gaseous free radical, in this study is in agreement with the study of cellular elements such as lipopolysaccharide and glycolipoprotein, have been reported to activate leukocytes and stimulate the production of pro-inflammatory cytokines which induces production of NO through activation of inducible nitric oxide synthase (iNOS) [38-42]. This finding may add credence to that NO may play role in the pathogenesis of FMD [37]. On the other hand, the protective or harmful effect of NO is suggested to be associated with the NO concentration [37].

According to the results obtained in this study some oxidative stress parameters can significantly increased in the saliva, as well as sera produced from diseases and oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of FMD and further detailed studies at cellular level are needed to fully understand the pathogenesis and clinical expression of the disease in cattle, an important source of infection. It concluded that saliva could provide an appropriate quality of material for researchers in similar studies and the amount of these markers in other body fluids should be reviewed and evaluated in different diseases because saliva can be obtained by noninvasive method and, blood and saliva provide similar statistically significant results on the markers used to evaluate oxidative stress.

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