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Research Article

Determination of Some Oxidative Stress and Inflammation Markers in Serum, Blood and CSF in Cattle with Head-Eye Form of Malignant Catarrhal Fever

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Abstract

The aim of this study was to determine changes in total sialic acid (TSA), malondialdehyde (MDA), nitric oxide (NO), inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) levels on sera and CSF and reduced glutathione (GSH) levels on blood in cattle with Malignant Catarrhal Fever (MCF). For this purpose 17 cattle which clinically diagnosed "head-eye form" of MCF and clinically healthy 10 cattle were evaluated. Blood and cerebrospinal fluid (CSF) were taken from the animals on the MCF diagnosed group MDA, GSH, NO, eNOS, iNOS, and TSA values were 25.65±0.42 µmol/L, 37.21±1.12 mg/dL, 30.61±0.41 µmol/L, 4.05±0.09 U/L and 10.98±0.35 U/L, 88.33±1.03 mg/dL, on the control group 13.77±0.55 µmol/L, 60.06±1.73 mg/dL, 11.27±0.4 µmol/L, 3.12±0.18 U/L, 5.55±0.3 U/L and 63.60±1.86 mg/dL respectively, and all parameter changes between the groups were determined to be statistically significant (P<0.001). On the CSF, no statistically significant difference between taken from MCF diagnosed group and healthy group. NO value and iNOS activity obtained from control groups CSF were relatively higher than the same group's serum whereas eNOS activities were found to be low. The study group consisting of MCF diagnosed cattle's serum were found to have NO value, eNOS and iNOS activities relatively higher than the same group's CSF values. As a result, it was concluded that there is a need for more comprehensive studies for better understanding the reason of failure to obtain the significant changes of animals diagnosed MCF that determine in blood but not in CSF.

Keywords: *Malignant catarrhal fever, GSH, Nitric oxide, eNOS, iNOS, Cattle*

Koriza Gangrenosa Bovum Baş-Göz Formlu Sığırlarda Serum, Kan ve BOS'ta Bazı Oksidatif Stres ve İnflamasyon Belirteçlerinin Tespiti

Özet

Bu çalışmanın amacı Koriza Gangrenosa Bovum'lu sığırlarda serum ve beyin omurilik sıvısı (BOS), total sialik asit (TSA), malondialdehit (MDA), nitrik oksit (NO), indüklenebilir nitrik oksit sentetaz (iNOS) ve endotelyal nitrik oksit sentetaz (eNOS) ile kan redükte glutatyon (GSH), düzey değişikliklerinin belirlenmesidir. Bu amaçla klinik olarak Coryza Gangrenosa Bovum'un (CGB) "Baş-Göz Formu" teşhisi konulan 17 adet ve kontrol amacı ile klinik olarak sağlıklı sığırlardan oluşan 10 adet sığır değerlendirildi. Hasta ve kontrol gruplarındaki hayvanlardan kan ve BOS alındı. CGB teşhisi konulan grupta MDA, GSH, NO, eNOS, iNOS ve TSA değerlerinin sırası ile 25.65±0.42 µmol/L, 37.21±1.12 mg/dL, 30.61±0.41 µmol/L, 4.05±0.09 U/L ve 10.98±0.35 U/L, 88.33±1.03 mg/dL kontrol grubunda ise 13.77±0.55 µmol/L, 60.06±1.73 mg/dL, 11.27±0.4 µmol/L, 3.12±0.18 U/L, 5.55±0.3 U/L ve 63.60±1.86 mg/dL olduğu ve tüm parametre değişikliklerinin gruplar arasında istatistiksel olarak önemli (P<0.001) olduğu belirlendi. Sağlıklı ve CGB teşhisi konulan gurubun BOS'larından elde edilen değerler arasında istatistiksel olarak önemli bir farkın bulunmadığı tespit edildi. Kontrol grubunun BOS'undan elde edilen NO değeri ve iNOS aktivitesinin aynı grubun serumlarından elde edilen değere göre nispeten yüksek, eNOS aktivitesinin ise düşük olduğu, CGB'li sığırlardan oluşan çalışma grubunun serumlarından elde edilen NO değeri, eNOS ve iNOS aktivitelerinin ise aynı grubun BOS'larından elde edilen değerlerden nispeten yüksek olduğu belirlendi. Sonuç olarak CGB'li hayvanlarda yangısal süreç ve oksidatif strese bağlı olarak kanda ortaya çıkan anlamlı değişikliklerin BOS'ta elde edilememesinin daha iyi anlaşılabilmesi için yeni ve daha kapsamlı çalışmalara ihtiyaç olduğu kanısına varıldı.

Anahtar sözcükler: *Koriza Gangrenosa Bovum, GSH, Nitrik oksit, eNOS, iNOS, Sığır*

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INTRODUCTION

Malignant catarrhal fever (MCF) is usually fatal lympho-proliferative disorder of cattle and other hoofed animals [1-3]. Disease is caused by alcelaphine herpes virus-I (circumstances-I) and Ovine herpesvirus-II (OHV-II) carried by sheep as reservoir [4,5]. Agents located on *Macavirus* genus [6]. OHV-II leads to subclinical infection in sheep and sheep serves as the reservoir for the disease occurring in cattle [1]. Clinically fever, muco-purulent rhinorrhea, corneal opacity [7,8], erosive stomatitis (especially erosion of the buccal papillae ends) and gastroenteritis, tear flows, blepharospasm, upper respiratory tract erosion, encephalitis, cutaneous exanthema and enlarging in lymph nodes were observed. The reservoir sheeps infect environment with agent with nasal discharge. The maximum virus excretion to environment takes place when the sheeps are 6-9 months of age. Contact with sheeps should be prevented through the control of the disease [9].

Sialic acid (SA), increases quickly after the inflammation and injury process [10,11]. Until today, some researchers reported increased serum SA concentrations during the course of many diseases [12-19].

Malondialdehyde (MDA) content increases due to the induction of lipid peroxidation. This process activates antioxidant defense mechanisms [20]. Reduced glutathione (GSH) and MDA concentrations can be used as indicators of oxidative stress in some diseases but no studies determined the oxidative stress in MCF on sera or CSF previously [21,22]. In inflammations, via stimulation of inducible nitric oxide synthase (iNOS), nitric oxide (NO) production increases. This situation leads to NO mediated tissue injury [23].

On many physiological and pathological processes nitric oxide acts as a biologically active molecule with different effects [24]. Nitric oxide is a cytotoxic factor which is generated from the terminal guanidine nitrogen atom of L-arginine by NO synthase and released by a variety of cells [25-28]. Its plays a primary defence against some pathogens [29,30]. But it has also been reported to be immunosuppressive [31,32].

A large spectrum of pathologic events cause oxidative stress in farm animals [33]. Oxidative stress and lipid peroxidation may end up with cellular and tissue damage if the production of reactive oxygen species are excessive [34,35]. Both enzymatic and non-enzymatic anti-oxidative mechanisms used in the elimination of these reactive oxygen species [34,36,37].

This study was therefore designed to determine changes in TSA, MDA, NO, iNOS and eNOS levels on sera and CSF and GSH levels on blood in cattle with MCF.

MATERIAL and METHODS

Animals

A total of 27 animals, age 2-4 years used in the study, consisting of 17 patients with clinically diagnosed MCF, which were referred to the clinic of the Internal Medicine Department of Kafkas University, Faculty of Veterinary Medicine and 10 healthy cattles. In this study, control group were provided from the faculty farm animals which found to be healthy during routine examination. The study was approved from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2016-137).

Blood (n=27) and CSF (n=17) samples were taken from all the animals. Blood samples were taken from vena jugularis and CSF were taken from L6-S1 intervertebra with Tuohy epidural needle® (Perican epidural needle, 18G-80 mm. Braun, Germany). The blood samples were centrifuged 3.000 rpm x 10 min and serum was obtained. The whole blood samples with EDTA were taken for GSH analyses. The obtained samples stored at -20°C until analyses are done.

NO, MDA, iNOS, eNOS, TSA and GSH Analysis

Serum TSA levels were measured by the method of Sydow [38], serum MDA concentrations were determined by the method of Yoshoiko et al.[39], serum NO was determined according to the method of Miranda et al.[40]. Also levels of GSH was measured according to the method of Beutler et al.[41] iNOS and eNOS activities were determined commercial ELISA kits (MyBiosource®). Same procedures (excluding GSH) were performed during the measurement of the CSF and serum samples.

Statistical Analysis

The data obtained in this study were evaluated by SPSS® (SPSS 20, USA) programme using the t test.

RESULTS

The serum MDA, NO, eNOS, iNOS and TSA levels and whole blood GSH levels are shown in *Table 1*, CSF; MDA, NO, TSA levels and eNOS, iNOS activities are shown in *Table 2* which are obtained form study.

Clinical Examination Findings

In the clinical examination of the group consisting of diseased animal, typical clinical symptoms; keratitis on the cornea which starts from periphery and directs to center, keratoconjunctivitis and increased opacity, photophobia, mukoprulent lacrimation and nasal discharge, high fever (40-41°C), redness of the mouth and nasal mucosa, necrosis and erosive lesions in the mouth and buccal papillae, growth in all lymph nodes that are palpable, dysphagia

Table 1. *Analysis results of serum and blood in control and amimals with MCF*

Parameter	Control (mean±std error)	MCF (mean±std error)	P
MDA µmol/L	13.77±0.55	25.65±0.42	P<0.001
GSH mg/dL	60.06±1.73	37.21±1.12	P<0.001
NO µmol/L	11.27±0.4	30.61±0.41	P<0.001
eNOS U/L	3.12±0.18	4.05±0.09	P<0.001
iNOS U/L	5.55±0.3	10.98±0.35	P<0.001
TSA mg/dL	63.60±1.86	88.33±1.03	P<0.001

Table 2. *Analysis results of CSF in control and animals with MCF*

Parameter	Control (mean±std.error)	MCF (mean±std.error)	P
MDA µmol/L	19.38±0.43	19.48±0.69	P>0.05
NO µmol/L	21.22±0.58	20.84±0.69	P>0.05
eNOS U/L	1.95±0.07	1.93±0.07	P>0.05
iNOS U/L	7.71±0.26	8.07±0.26	P>0.05
TSA mg/dL	104.15±1.88	102.55±1.85	P>0.05

and the findings which are reported by researchers. Clinical symptoms associated with encephalitis such as somnolence, staying head put on a place, staggering gait, indifference to the environment were not evident in patients which were clinically diagnosed with head-eye form of MCF.

In addition, from anamnesis received from animal owners, it was determined as infected animals are being hosted along with sheep.

Biochemical Findings

The serum levels of MDA, NO, eNOS, iNOS and TSA were significantly higher (P<0.001) in animals with MCF than control group animals and whole blood GSH values were detected significantly lower (P<0.001) *(Table 1)*.

On the animals with MCF, levels of MDA and iNOS in CSF were detected high whereas levels of NO, eNOS ve TSA were detected low than control group animals. This changings were not statistically significant (P>0.05) *(Table 2)*.

DISCUSSION

This study aimed to demonstrate on sera, blood and CSF some indicators of oxidative stress and inflammation, which were determined different bacterial and viral diseases, in cases of MCF in cows that parameters are not fully investigated yet.

Clinical symptoms determined in this study like keratitis on the cornea which starts from periphery and directs to center, keratoconjunctivitis, corneal opacity, photophobia, high/acute fever (39.5-41.0ºC), disphagie, redness of the mouth and nasal mucosa, necrosis and erosive lesions in the mouth and buccal papillae, growth in the lymph nodes of infected animals with consequent excessive mucopurulent lacrimation and nasal discharge in agreement with those reported for MCF but clinical symptoms associated with encephalitis were not determined in patients [1-3,7-9]. Furthermore from anamnesis taken from animal owners, it was determined as infected animals are being hosted along with sheep which are involved in the occurrence of the disease in cattle as reservoir [1,4,5].

In this study, serum TSA level in MCF cases was found higher than the control animals as reported previously by some researchers in different diseases [15-19]. Increased sialic acid levels are reported in mammals during different pathological situations [11]. SA is present in all biological membranes. When the pathological situations mentioned above occur, SA is released from the cell membrane to circulation and rise in SA is observed [11,13,42,43]. TSA changes in CSF were not statistically significant. This is may be due to that symptoms of encephalitis do not occur sufficiently in patients.

Another indicator of cellular damage and lipid peroxidation during the course of MCF may be increased MDA, and decreased GSH. The MDA results of our study are similar to foot and mouth disease in recent years [19,44]. According the present study finding that MCF causes tissue damage in various organs and systems. Lower GSH levels on the animals with MCF than healthy animals is thought to be caused by oxidative stress on the animals due to disease, similar to the previous reports on oxidative stress situations [45,46].

NO is a free radical which is produced via inducible nitric oxide synthase (iNOS) from activated leukocytes by pro-inflamatuary cytokins [47-50]. It is known that NO plays an important role in the primary defense mechanism against several pathogens and NO production can be induced by various viruses which inhibit virus replication [44,51,52]. In present study, NO levels in MCF cases was determined higher than control animals in serum samples, therefore our study results indicate that herpesvirus can induce the production of NO.

NO level and iNOS activity obtained from the control group's CSF were relatively high compared to the serum levels of the same group and eNOS activity was low. NO level, eNOS and iNOS activities obtained from study group's serum with MCF detected relatively higher than the same group's CSF levels. Serum levels of NO, eNOS and iNOS were found to increase significantly in the cattles diagnosed MCF's head and eye form compared to healthy animals, this situation were found to be consistent with the studies reported for these markers in pathological events [53-56]. Besides all these on the MCF group, iNOS, induced by such situations like inflammation, and eNOS, known to locate in vascular endothelia, were not observed as the expected

increased level in CSF. At this point, it was concluded that there is a need for new studies for a better understanding that blood-brain barrier, or a different mechanism may be effective and why statistically significant changes on the blood of animals with MCF caused by inflammatory processes and oxidative stress were not seen on the CSF. Changes in the inflammatory and oxidative stress parameters observed in the serum, are not observed in CSF of animals diagnosed with head eye form may suggest that the encephalitis did not developed/occured.

This is the first study to our knowledge in which some oxidative and inflammation parameters was evaluated on sera, blood and CSF in the MCF. In this study some oxidative stress parameters can significantly increased in the serum, but not in the CSF, produced from diseases and oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of CSF 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.

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