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Evaluation of Blood and Cerebrospinal Fluid Biochemistry, Cytology and Haematological Parameters in Head-and-Eye Form of Malignant Catarrhal Fever in Cattle

Erdoğan UZLU[1,a*], Ekin Emre ERKILIÇ[2,b], Yasemen ADALI[3,c], Metin ÖĞÜN[4,d], Kezban CAN ŞAHNA[5,e], Nilhan ERYEĞEN[6,f], Hüseyin Avni EROĞLU[7,g], Hasan ABAYLI[5,h], Celal Şahin ERMUTLU[8,j], Ali Haydar KIRMIZIGÜL[2,k]

[1]Balıkesir University, Faculty of Veterinary Medicine, Department of Internal Medicine, Balıkesir-TÜRKİYE
[2]Kafkas University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kars-TÜRKİYE
[3]İzmir Ekonomi University, Faculty of Medicine, Departmet of Pathology, İzmir-TÜRKİYE
[4]Kafkas University, Faculty of Medicine, Department of Biochemistry, Kars-TÜRKİYE
[5]Fırat University, Faculty of Veterinary Medicine, Department of Virology, Elazığ-TÜRKİYE
[6]Balıkesir Atatürk Şehir Hastanesi, Department of Pathology, Balıkesir-TÜRKİYE
[7]Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Physiology, Çanakkale-TÜRKİYE
[8]Kafkas University, Faculty of Veterinary Medicine, Department of Surgery, Kars-TÜRKİYE
ORCID: [a]0000-0002-3064-6633; [b]0000-0003-2461-5598; [c]0000-0002-8004-7364; [d]0000-0002-2599-8589; [e]0000-0001-9211-5419; [f]0000-0001-8509-3442; [g]0000-0002-1040-3255; [h]0000-0003-2116-105X; [j]0000-0002-8923-7682; [k]0000-0002-9283-1391

***Corresponding author;** Erdoğan UZLU; E-mail: euzlu@hotmail.com
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Abstract: In this study, it was aimed to examine the biochemical changes, hematological changes and cerebrospinal fluid (CSF) cytology and blood serum of cattle with head-eye form of Malignant Catarrhal Fever (MCF). For this purpose, 22 cattle diagnosed with "head-eye form" of MCF and clinically healthy 10 cattle were evaluated. Blood and cerebrospinal fluid (CSF) were collected from all cattle. In sera, AST, urea, glucose, CK (P<0.05), LDH levels (P<0.01) were found be high, ALT, ALP, cholesterol (P<0.05), Ca, total protein (P<0.01) and Mg, albumine and Fe levels (P<0.001) were found to be low in MCF group when compared to the control group. In CSF, Ca (P<0.01) and total protein levels (P<0.001) were found high glucose level (P<0.05) was found low in MCF group when compared to the control group. In haematology, some parameters were determined to be different between the groups. In cytological results of CSF in MCF group, polymorphonuclear leucocytes, lymphocytes, erytrocytes, macrophages and plasma cells were determined. In conclusion, since there were a limited number of studies examining biochemical, cytologic and hematological results of MCF especially in CSF, the results from our study were thought to be important for future studies in which viral diseases affects the nervous system of cattles.
Keywords: Biochemistry, cattle, CSF, cytology, haematology, malignant catarrhal fever

Coryza Gangrenosa Bovum'un Baş-Göz Formu Belirlenen Sığırlarda Kan ve Beyin Omurilik Sıvısı Biyokimyası, Sitolojisi ve Hematolojik Parametrelerin Değerlendirilmesi

Öz: Bu çalışmada, coryza gangrenosa bovum'un (CGB) baş-göz formu belirlenen sığırların beyin omurilik sıvısı (BOS) ve kan serumlardaki biyokimyasal değişiklikler, hematolojik değişiklikler ve BOS'un sitolojik olarak incelenmesi amaçlanmıştır. Bu amaçla CGB'nin baş-göz formu belirlenen 22 adet sığır ve klinik olarak sağlıklı 10 adet sığırdan kan ve beyin omurilik sıvısı (BOS) alınmıştır. CGB grubunun kan serumlardaki AST, üre, glukoz, CK (P<0.05), LDH düzeyleri (P<0.01), ALT, ALP, kolesterol (P<0.05), Ca, total protein (P<0.01) ve Mg değerleri kontrol grubuna göre yüksek, albümin ve Fe düzeyleri (P<0.001) ise düşük bulunmuştur. CGB gurubundaki sığırların BOS'larında Ca (P<0.01) ve total protein düzeyleri (P<0.001) yüksek, glukoz düzeyleri (P<0.05) ise kontrol grubuna göre düşük olarak belirlenmiştir. Hematolojik bazı parametrelerde de gruplar arasında farklılık görülmüştür. CGB gurubundaki sığırların BOS'larında yapılan sitolojik incelemelerde, sitolojik lamlarda polimorfonükleer lökositler, lenfositler, eritrositler, makrofajlar ve plazma hücreleri belirlenmiştir.Sonuç olarakCGB'da, özellikle BOS'ta sitolojik ve biyokimyasal, ayrıca kanda biyokimyasal ve hematolojik sonuçları bir arada inceleyen sınırlı sayıda çalışma olduğundan, çalışmamızdan elde edilen sonuçların sığırların sinir sistemini etkileyebilenviral hastalıklarda gelecekte yapılacak çalışmalar için önemli bir veri oluşturabileceği düşünülmüştür.
Anahtar kelimeler: Biyokimya, BOS, coryza gangrenosa bovum, hematoloji, sığır, sitoloji

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Introduction

Malignant Catarrhal Fever (MCF) is a disease in which two factors may be involved in the etiology ofclinically distinct disease. "*Alcelaphine herpesvirus* (AHV1-2)/*Bovid-Bovine herpesvirüs 3*" plays a role in cases in wild ruminants in Africa (wildebeest-associated) *ovine herpesvirus-2* (OvHV-2) plays a role in cases in Europe, North America and Asia (sheep-associated). It is known that in the cases seen in Africa, theagent is transmitted to cattle by an antelope of *Connochaetes taurinus* breed (Blue wildebeest), so domestic ruminants may be at environmental risk worldwide. In other continents, sheepare commonly responsible for transportation of the agent and therefore the disease. Sheep have been reported to be able to infect cattle at short-to-medium distancesviaaerosols and oculer or nasal fluids. MCF can also be caused by ingestion of contaminated food, direct contact with caretakers and even from birds (Crawford et al., 1999; Andrews, 2004; Radostits et al., 2007). MCF has a very high mortality rate but cattle, one of the last hosts, do not spread the virus when they die because their secretions do not contain virus (Metzler, 1991; Smith, 1996; Andrews, 2004; Radostits et al., 2007).

The disease occurs several forms in cattle: peracute, digestive system and the"*Head-Eye*" form which is the most common. Typical signs of the head-eye form of MCF are weakness, loss of appetite, fever up to 41°C, increased pulsation (100-120/bpm), redness of the buccal mucosa, erosion and necrosis in the mouth, labial papillae and anterior nasal mucosa, congestion of the scleral vessels, edema oft he eyelids, photophobia, blepharospasm, centripetal corneal opacity starting at the edge of the sclera and discharge causing narrowing of the nasal cavity. Centripetaly corneal opacity can be considered as pathognomonic and is almost always present invarying degrees. Sick animals often have neurological manifestations especially in the terminal phase (Metzler, 1991; Smith, 1996; Radostits et al., 2007). A significant or moderate leucopenia associated with agranulocytosis is observed in the "early stages of infection" of the disease but this can easily be missed (Liggit and DeMartini, 1980a; Liggit and DeMartini, 1980b; Dewals and Vanderplasschen, 2011).

The aim of this study was to assess possible changes in routine hematological and biochemical values, as well as to determine biochemical and cytological findings obtained from cerebrospinal fluid (CSF) and to evaluate their clinical significance in the head-eye form of MCF in cattle. Although CSF is a body fluid that can be directly affected by many brain inflammations and diseases, the changes that can occur in this fluid in many large or small animal diseases, including MCF disease, have not been adequately studied to date.

Material and Methods

Animals

The study involved a total of 32 cattle, including 22 cattle with head-eye form of MCF diagnosed at the Teaching Hospital of the Faculty of Veterinary Medicine on admission by the owners, 10 healthy cattle on routine health checks in Research Farm of the Faculty.

A routine full physical examination was performed on all animals. Blood samples (n=32) were properly collected from juguler vein from all animals into plain tubes and transported immediately to laboratory. Sera were obtained by centrifugation at 3000 rpm for 10 minutes at room temperature and were kept frozen (-20°C) until the analysis were performed.

CSF samples (n=17) were collected properly in sterile micro tubes after the animal was sacrified, and routine biochemical analyzes were performed on fresh material. Cytological evaluation of CSF was performed in the Department of Pathology of the Faculty of Medicine.

Hematologic and biochemical analysis

Blood samples from MCF and healthy cattle were analyzed on (VG-Ms4e) automated hematology analyzer and serum and CSF samples on (Mindray BS 120) fully automated biochemistry analyzer.

Cytological analysis

Cytological examinations were carried out on specimen prepared by direct smear and cytospin methods from CSF samples obtained in the study. Hematoxylin and Eosin (H&E), May-Grünwald-Giemsa (MGG) and Papanicolaou (PAP) stains were used in the preparations. Cases were scanned at 200x magnification and detailed cellular assessments were made at 400x magnification in cellular areas.

PCR analysis

Total DNA was extracted from whole blood using phenol-chloroform method (Sambrook et al., 1989). Atwo-step PCR amplification was performed as described previously (Dabak and Bulut, 2003). PCR conditions were as follows: a preliminary denaturation at 99°C/5 min followed by 39 cycles at 94°C/20 sec, 60°C/30sec and 72°C/30 sec and a final extension at 72°C/1 min with primers set 556 (5'-AGTCTGGGTATATGAATCCAGATGGCTCTC-3') and 775 (5'-AAGATAAGCACCAGTTATGCATCT-GATAAA-3') to obtain a PCR product of 422 bp. A nested PCR was also conducted, with exactly the same conditions as detailed above, the primers were 556 and 555 (5'-TTCTGGGGTAGTGGCGAGC-GAAGGCTTC-3') to amplify a sequence of 238 bp.

Products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide. We used SA-MCF positive samples of previous study in our department (Erkılıç et al., 2017) as a positive control and a SA-MCF negative cattle whole blood sample as a negative control.

Biochemical results

The serum level of ALT, AST, GGT, ALP, creatine, urea, calcium, magnesium, glucose, phosphor, total protein, albumin, lipase, creatine kinase (CK) and creatine kinase-myocardial band are shown in Table 1, and the same parameters in the CSF are shown in Table 2 without cholesterol, Fe and LDH.

Table 1. Biochemical results obtained from the sera of control and MCF groups

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
ALT (U/L)	35.7±1.53	22.06±3.42	P<0.05*
AST (U/L)	82.89±4.21	117.69±10.32	P<0.05*
GGT (U/L)	20.56±1.31	24.08±1.70	P>0.05
ALP (U/L)	64.79±3.77	46.06±4.82	P<0.05*
Crea (mg/dL)	1.96±0.08	1.82±0.13	P>0.05
Urea(mg/dL)	8.01±0.67	11.06±0.72	P<0.05*
Ca (mg/dL)	9.15±0.11	7.55±0.32	P<0.01**
Mg (mEq/L)	2.73±0.12	1.85±0.11	P<0.001***
Glu (mg/dL)	59.04±2.86	70.12±2.67	P<0.05*
P (mg/dL)	5.42±0.34	4.62±0.26	P>0.05
TP (g/dL)	7.06±0.15	5.75±0.23	P<0.01**
Alb (g/dL)	3.08±0.13	2.42±0.7	P<0.001***
Lipaz (U/L)	2.79±0.19	2.57±0.19	P>0.05
CK (U/L)	231.88±39	781.22±143.84	P<0.05*
CK-MB (U/L)	84.03±10.01	62.43±6.84	P>0.05
Chol (mg/dL)	84.89±3.88	67.47±4.99	P<0.05*
LDH (U/L)	433.16±20.66	730.32±51.51	P<0.01**
Fe (µmol/L)	19.45±1.26	9.04±0.77	P<0.001***

Statistical analysis

Related with the assumptions of central limit theory, by increasing sample size (over 30) the distribution approaches to normal. Because of the sample size in this study is over 30, to compare the differences between the control and the study group, independent samples t test is applied. All the statistical analyses are done at 95% confidence level. P values of tests are given in relevant tables. The data obtained in this study were evaluated by SPSS®software.

Results

Clinical examination results

Typical clinical symptoms of the head-eye form of MCF have been identified in clinical examinations of infected cattle which were housed with sheep as stated by the owners. These typical symptoms consist of high fever (39.5-41ºC), keratoconjunctivitis, resulting in excessive mucopurulent lacrimation and photophobia, centripetal keratitis, corneal opacity, disphagie, redness of the mouth and nasal mucosa, nasal discharge, necrotic and erosive lesions in the mouth and buccal papillae, enlargement of lymph nodes. In addition, findings such as a tendency to sleep, indifference to the environment, a tendency to tilt the head to one side and changes in gait were observed, which were identified by researchers and thought to be caused by encephalitis.

Biochemical evaluations of the sera from both groups revealed statistically significant higher levels of AST, urea, glucose, CK (P<0.05) and LDH (P<0.01) levels, and statistically significant lower levels of ALT, ALP, cholesterol (P<0.05), Ca, TP (P<0.01), Mg, Albumin and Fe (P<0.001) levels in the MCF-diagnosed group of cattle than the healthy group.

Table 2. Biochemical results obtained from control and MCF groups CSF's

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
ALT (U/L)	5.45±0.45	6.01±0.78	P>0.05
AST (U/L)	16.75±0.59	22.82±2.52	P>0.05
GGT (U/L)	0.18±0.07	0.55±0.15	P>0.05
ALP (U/L)	3.93±0.31	4.66±0.52	P>0.05
Crea (mg/dL)	0.33±0.06	0.36±0.06	P>0.05
Urea (mg/dL)	16.09±3.6	20.23±3.09	P>0.05
Ca(mg/dL)	1.7±0.6	2.8±0.22	P<0.01**
Mg (mEq/L)	2.07±0.22	2.32±0.3	P>0.05
Glu(mg/dL)	49.21±2.10	34.60±3.63	P<0.05*
P (mg/dL)	4.24±0.88	4.61±0.89	P>0.05
TP (g/dL)	0.16±0.02	0.39±0.04	P<0.001*
Alb(g/dL)	0.1±0.01	0.11±0.01	P>0.05
Lipaz(U/L)	4.78±0.18	4.98±0.24	P>0.05
CK(U/L)	7.23±0.35	7.32±0.27	P>0.05
CK-MB(U/L)	2.01±0.59	2.66±0.30	P>0.05

Biochemical evaluations of CSF obtained from both groups resulted statistically significant lower levels of glucose (P<0.05) and statistically significant higher levels of Ca (P<0.01) and total protein levels (P<0.001) in the MCF-diagnosed group than the healthy group.

Hematological results

Results of hematological parameter levels are shown in Table 3.

Table 3. Hematological results obtained from Control and MCF groups

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
WBC	11.5±0.7	15.04±2.52	P<0.05*
LYM %	51.06±2.15	55.33±1 .94	P<0.05*
MON%	3.96±0.3	4.01±0.59	P>0.05
GRA%	44.98±3.34	45.62±4.10	P>0.05
LYM	5.45±0.21	7.16±1.2	P<0.01**
MON	0.42±0.02	0.36±0.05	P>0.05
GRA	4.18±0.7	4.71±0.4	P>0.05
RBC	5.68±0.43	8.43±0.43	P<0.001***
MCV	50.15±1.33	45.32±2.05	P>0.05
MCH	18.63±0.5	12.48±0.8	P<0.001***
MCHC	37.16±0.55	27.72±1.53	P<0.001***
Hct	28.21±1.89	37.05±1.35	P<0.001***
RDW	13.22±0.23	13.71±0.36	P>0.05
Hb	10.43±0.56	10.17±0.60	P>0.05
MPV	6.83±0.16	7.18±0.18	P>0.05
PCT	0.18±0.04	0.37±0.06	P<0.05*
PLT	229,2±36.57	467.26±62.63	P<0.01**
PDW	5.05±0.85	7.61±0.45	P<0.01**

Hematological evaluations of whole blood obtained from both groups showed statistically significant higher levels of WBC, LYM%, PCT (P<0.05), LYM, PDW, PLT (P<0.01), RBC and Hct (P<0.001) in the MCF-diagnosed group than the healthy group while statistically significant lower levels of MCH and MCHC (P<0.001) were observed.

Cytological results

The results of direct and cytospin slides of CSF are shown in Table 4.

Table 4. Cytologic slide results obtained from MCF group CSFs (n=10)

Case No	Results
CSF--0	A small number of lymphocytes and polymorphonuclear leukocytes (PNL)
CSF--1	A small number of lymphocytes and a small number of macrophages
CSF--2	Squamous epithelial cells compatible with contamination and a small number of macrophages
CSF--3	Blood components (erythrocytes and PNL), a small number of lymphocytes and plasma cells
CSF--4	A small number of PNL, lymphocytes and macrophages ***(Fig. 1)***
CSF--5	A large number of PNL and lymphocytes
CSF--6	Squamous epithelial cells compatible with contamination and PNL's, lymphocytes and macrophages
CSF--7	Hypocellular slides and a small number of PNL and lymphocytes
CSF--8	Acellular slides
CSF--9	Hypocellular slides and lymphocytes

Direct smear and cytospin preparations were scanned at 200x magnification and evaluated at 400x magnification in cellular areas (Figure 1). The presence of epithelial cells was not observed in any of the preparations examined to assess cytopathic effect. The blood elements detected in the slides were thought to be secondary to the traumatization caused by the process, whereas the squamous epithelium was thought to be due to contamination during passage through the skin.

In general evaluation of the preparations, it was considered that the existing numbers and proportions of inflammatory cells present were not too high for CSF according to the "human meningoencephalitis assessment". As the central nervous system findings in cattle in the study group were limited or mild, the cytological findings were evaluated according to the cases investigated.

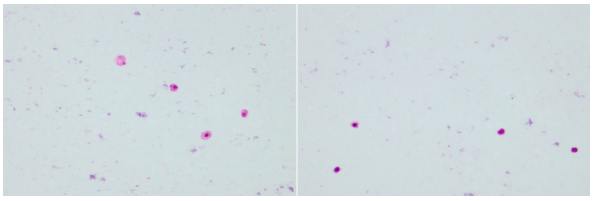

Figure 1. Degenerated macrophages and lymphocytes in CSF in MCF group (H&E, 400x).

PCR results

All of the 22 samples were detected as positive by nested-PCR (Figure 2) in the MCF group. A targeted 238 bp of PCR product was visualized from the positive control DNA extract.

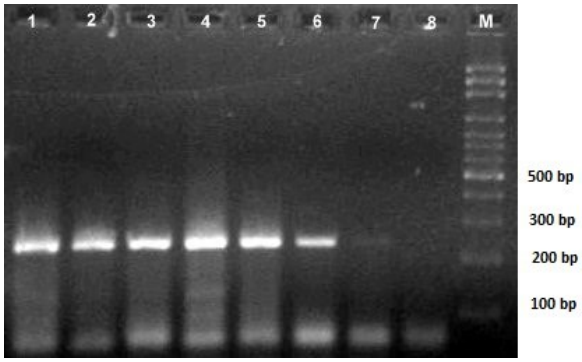

Figure 2. Nested-PCR product samplified from whole blood samples.
Lane 1**:** positive control sample**,** ***Lane 2-7*****:**nested-PCR products (238 bp), ***Lane 8*****:** negative control sample, M: 100 bp molecular weight marker (SolisBiodyne).

Discussion and Conclusion

In the aim of this study was to evaluate some physiological and biochemical parameters of blood, serum and CSF, as well as to investigate cytological changes that may occur in CSF and to evaluatetheir clinical significance in cattle clinically diagnosed with the head-eye form of MCF. To understand the pathogenesis of MCF, investigations based mainly on inflammation, immune mediated organ failure, toxins and effects of virus are frequently evaluated in natural cases. However, it is very important to reveal all the organs, tissues and related markers influenced by the forms investigated in the researches in order to reveal the pathogenesis and effective mechanisms that are quite complicated due to the affected organs and systems in different forms of the disease.

We determined specific clinical symptoms such as; high fever (39.5-41ºC), keratoconjunctivitis, resulting in excessive mucopurulent lacrimation and photophobia, centripedal keratitis, corneal opacity, disphagia, redness of the mouth and nasal mucosa, nasal discharge, necrotic and erosive lesions in the mouth and

buccal papillae, enlargement in lymph nodes in all infected animals in this study. We have also detected the finding that can be seen secondary to encephalitis such as; somnolence, keeping the head on one side, anxious and shaky gait, indifference to the environment etc. which are compatible with the reports for MCF (Masters et al., 2003; Radostits et al., 2007; Russel et al., 2009; Cunha et al., 2012; Headley et al., 2015; Lankester et al., 2016). Furthermore, the case history revealed that all the infected cattle werekept with sheep, which is a well-known source of infection in cattle (Roizman et al., 1992; Muller-Doblies et al., 1998; Masters et al., 2003; Erkılıç et al., 2017).

In the study, biochemical evaluations of the sera revealed statistically higher levels of AST, urea, glucose, CK ($P<0.05$) and LDH ($P<0.01$), and statistically lower levels of ALT, ALP, cholesterol ($P<0.05$), Ca, TP ($P<0.01$), Mg, albumin and Fe ($P<0.001$) in the group diagnosed with MCF-compared to healthy cattle. These findings were found to be consistent with those previously reported (Hill et al., 1993; Dettwiler et al., 2011; Dabak et al., 2012). As albumin is a negative acute phase protein, it is considered normal for it to decrease during inflammatory events. It is known that this decrease is also common for Fe. The decrease in albumin and Fe obtained in our study was considered as compatible with the disease. In a study on ruminants with septicemia, it is suggested that low values of Ca, Mg, and P are due to anorexia and malabsorption (Çitil et al., 2004). Similar values obtained in our study are probably due to the same clinical symptoms.

Biochemical evaluation of CSF from cattle diagnosed with MCF revealed that Ca ($P<0.01$) and total protein levels ($P<0.001$) were higher than in healthy cattle in the control group, and glucose ($P<0.05$) level was lower. The normal CSF protein level is less than 30 mg/dL. This value was found by some investigators to be 36-98 mg/dL in MCF. Damage to the blood-brain barrier causes the amount of protein in CSFto increase. Although this is usually explained by the amount of albumin in the blood, in some diseases it may also be caused by γ-globulin, which is over expressed by B lymphocytes in the nervous system. The CSF protein value obtained in our study is consistent with those reported by researchers (Abate et al., 1998; Di Terlizzi and Platt, 2006; Stokol et al., 2009; Scott, 2010; Pandey et al., 2015). Normal Ca values in CSF have been reported by researchers to be 1-1.5 mmol / L. Calcium is released from the choroid plexus and its level is provided by an active system. Plasma Ca level has no significant effect on Ca level in CSF. Some investigators have considered increased Ca in CSF as a marker of blood-brain barrier damage, which in various studies is thought to reflect the increased CSF protein concentrations (Rutter and Smales, 1976; Di Terlizzi and Platt, 2006; Stokol et al., 2009; Scott, 2010; Pandey and al., 2015).

In our study, CSF glucose levels are lower in healthy animals. Normally, CSF glucose levels are directly related to blood glucose levels. The fact that the CSF glucose is 60-80% of the blood glucose levels indicates that the functions of the central nervous system are normal. In our study, the values obtained from the CSF of MCF animals are 50% of the glucose values obtained from the sera of these animals. The low glucose level in CSF is also an important parameter for distinguishing bacterial/supportive meningitis from aseptic meningitis. Researchers have also reported that the low glucose level obtained from CSF is due to changes in the physiological function of the choroidal epithelium and the consumption of pathogenic elements or leukocytes in the system (Abate et al., 1998; Di Terlizzi and Platt, 2006; Pandey et al., 2015; Sri Rekha et al., 2015).

Increased CK is usually a sign of muscle damage, but may also be elevated in the CSF in diseases of the nervous system. Although there is no direct relationship between serum level and CSF levels, increases in CSF are considered to be indicative of poor prognosis in neurological diseases. In our study, serum CK levels were determined to be statistically significantly increased in MCF cases, but this increase was not at levels that would make a statistical difference in the CSF of the same group. It has also been reported that increases in AST and CK in CSF may be an important indicator of myelin degeneration (Hill et al., 1993; Di Terlizzi and Platt, 2006). Although the CSF AST and CK values in our study were not statistically significant, the relative increases were present.

In the present study, hematological evaluations of whole blood obtained from both groups showed higher LYM%, PCT ($P<0.05$), LYM, PDW, PLT ($P<0.01$), RBC and Hct ($P<0.001$) and lower MCH and MCHC ($P<0.001$) in the MCF group than in the healthy group. Although it was determined that the LYM, LYM%, RBC and Hct values obtained in the study were high in statistically different ratios compared to the healthy group, it was determined that these increases obtained from the MCF group were within the reference values and that the relative increase in the WBC value was not statistically significant. It was found that the low values obtained at the MCH and MCHC levels are compatible with the values found by the researchers (Hill et al., 1993; Detwiller et al., 2011; Kırbaş et al., 2013).

In our study, the presence of epithelial cell was not observed in the cytological evaluation of CSF. Blood elements and squamous epithelium, which were rarely detected in the slides, were thought to be related to traumatization during the procedure and contamina-

tion through the skin. A total of nine cases were examined; in four of the cases macrophages, six of the cases polymorphonuclear leukocytes (PNL) and eight of the cases lymphocytes were detected cytologically. Normal CSF consists of smaller number than 10 cells/µL of predominantly lymphocytes and neutrophils. In animals with encephalopathy, pleostosis is an important sign. In general, the lymphocytic mononuclear response is more prominent in viral infections, whereas PNLs predominate in acute bacterial CNS disease. In a study of calves infected by bovine herpes infested calves, the increase in CSF mononuclear cells was also found to be more pronounced after day 21. It has been stated that the presence of macrophages in CSF may occur after destruction of cerebral tissue or cerebral hemorrhage and/or may be due to protein-energy malnutrition (Stokol et al., 2009; Scott, 2010; Insemhagen et al., 2011).

In this study, it was aimed to reveal the changes in cattle diagnosed with the head-eye form of MCF by physical examination and laboratory tests, which were brought to Teaching Hospital of Faculty of Veterinary Medicine, as a whole by making CSF and blood biochemical, hematological and CSF cytological examinations and it was considered that these values, which have been studied in this disease very limited other viral diseases effecting the nervous system of cattle, would be the reference for future studies. As a result, in this study, although there were statistically significant differences in many biochemical parameters in blood serum, it was determined that especially ALB, Fe and Mg values showed greater differences, while TP, Glu and Ca values in CSF were statistically different. Many haematological parameters were found to be statistically different.

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