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Research Article

Tumour Necrosis Factor-alpha, Haptoglobin, Serum Amyloid A and Neopterin Levels in Cattle with Lumpy Skin Disease [1][2]

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Abstract

Lumpy skin disease (LSD) is a viral disease of cattle, characterised by the formation of nodules in different parts of the body. In this study, it was conducted to assess the pattern of changes of albumin, tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels in cattle with LSD, to assess the clinical course of the disease, and to the demonstration of inflammatory process in cattle with LSD. This study was carried out in 30 cattle, including 20 animals naturally infected with LSD and 10 healthy animals. It was determined that, in the cattle infected with LSD, while albumin concentrations had significantly decreased (P=0.004) in comparison to the control group, Hp (P<0.001), SAA (P<0.001) and neopterin (P<0.001) concentrations had significantly increased. Receiver operating characteristic (ROC) curve was used to calculate the sensitivity and specificity of Hp, SAA and neopterin. The cut-off values of the healthy and infected cattle for Hp, SAA and neopterin were determined to be 0.196 mg/mL, 41.38 µg/mL and 23.93 nmol/mL, respectively. At these cut-off values, high levels of sensitivity (85% for Hp, 95% for SAA and 70% for neopterin) and specificity (90%) were detected. It was determined that SAA levels were of higher sensitivity and specificity compared to Hp and neopterin levels with respect to the demonstration of inflammation associated with LSD. Furthermore, the clinical picture of the disease was found to be significantly correlated with the Hp, SAA and neopterin levels.

Keywords: *Lumpy Skin Disease, Acute phase, Neopterin, Cattle*

Lumpy Skin Disease'li Sığırlarda Tümör Nekroz Faktör-alfa, Haptoglobin, Serum Amiloid A ve Neopterin Düzeyleri

Özet

Lumpy Skin Disease (LSD), vücudun çeşitli bölgelerinde nodül oluşumu ile karekterize sığırların viral bir hastalığıdır. Bu çalışmada; LSD'li sığırlarda albümin, tümör nekroz faktör-α (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterinin, hastalığın klinik seyrini değerlendirme, inflamatuar sürecin gösterilmesi ve bu testlerin öneminin belirlenmesi amaçlanmıştır. Bu çalışma, doğal olarak LSD'li 20 hasta sığır ile aynı bölgeden 10 sağlıklı sığır olmak üzere toplam 30 hayvan üzerinde yürütüldü. LSD'li sığırların albümin konsantrasyonun önemli düzeyde (P=0.004) düşük olduğu; serum Hp (P<0.001), SAA (P<0.001) ve neopterin (P<0.001) konsantrasyonunun kontrol grubuna göre anlamlı düzeyde yüksek olduğu belirlendi. Hp, SAA ve neopterin değerlerinin sensitivite ve spesifiteyi hesaplamada receiver operating characteristics (ROC) eğrisi kullanıldı. Sağlıklı ve LSD'li sığırların cut off değerleri Hp 0.196 mg/mL, SAA 41.38 µg/mL ve neopterin ise 23.93 nmol/mL olarak belirlendi. Bu değerler baz alındığında yüksek bir sensitivite (Hp %85, SAA %95 ve neopterin %70) ve spesifite (%90) olduğu anlaşıldı. LSD'deki inflamasyonu göstermede SAA düzeylerinin Hp ve neopterin düzeylerine göre daha yüksek sensitivite ve spesifiteye sahip olduğu belirlendi. Ayrıca hastalığın klinik görünümü ile Hp, SAA ve neopterin arasında önemli korelasyonlar bulundu.

Anahtar sözcükler: *Lumpy Skin Disease, Akut faz, Neopterin, Sığır*

INTRODUCTION

In recent years, several studies have been conducted on the establishment of diagnostic and prognostic markers that can be used for infectious diseases [1-5]. Thus, several researchers have reported that biological markers,

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including haptoglobin (Hp), serum amyloid A (SAA) and neopterin, which demonstrate non-specific immune responses that develop during the course of infectious diseases, can be used for the diagnosis of infections [6-9].

Hp and SAA, which are produced during the acute phase reaction, are glycoproteins of hepatic origin. Veterinary medicine research has shown that, of species-specific acute-phase proteins, serum Hp and SAA provide valuable insight into the clinical picture of ruminant diseases [1,3,10,11]. Hp and SAA levels aid in the clinical diagnosis of the inflammatory diseases of cattle as well as in the differentiation of acute and chronic infections [1,12].

Changes in the levels of acute-phase proteins are induced by proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and gamma interferon (IFN-γ) [10,13]. The release of these cytokines also causes fever, anorexia, leukocytosis, activation of the coagulation system, and changes in trace elements and the endocrine system [10].

Due to the fact that it can significantly contribute to diagnosis and prognosis and can also be readily measured, neopterin, which is synthesized by monocytes and macrophages stimulated by IFN-γ released from activated T lymphocytes, is commonly used in medical research on human infectious diseases [14,15]. Medical studies have demonstrated that, during the course of acute viral infections, including among others human immunodeficiency virus (HIV), influenza and hepatitis, neopterin levels start to rise before the development of a specific antibody response against the viral agent and display correlation with the clinical course of the disease [16,17].

Lumpy skin disease (LSD) is an infectious disease of cattle, which is characterised by the formation of nodules on the skin and in various parts of the body, and which may be fatal [18,19]. Outbreaks have been reported the morbidity rate of the disease to range between 3-100% and the mortality rate of the disease to range from 0% to 26% [18,20]. The aetiological agent of the disease, the lumpy skin disease virus (LSDV), belongs to the family *Poxviridae* and genus *Capripoxvirus* [21]. The incubation period of LSD is reported to vary between 1-4 weeks. The disease may follow an acute or subclinical course in cattle [19,22]. The clinical symptoms of LSD include fever, reduced food intake, nodules on the skin and mucous membranes as well as in the internal organs, enlargement of the lymph nodes, salivation, lacrimation, nasal discharge, oedema in various regions of the body, and in the case of severe infections, ulcerative lesions of the mucous membranes [18,20,22,23]. LSD causes significant economic losses as a result of reduced milk yield, weight loss, damaged hides due to skin nodules, abortion, infertility and mortality [18,20,24]. Reports indicate that the polymerase chain reaction (PCR) produces more reliable diagnostic results in comparison to other immunological tests [20,25,26].

To the authors' knowledge, no literature report has been published on serum Hp, SAA and neopterin levels in cattle infected with LSD. In this study, the inflammatory potential of Hp, SAA and neopterin levels was investigated in cattle infected with LSD.

MATERIAL and METHODS

Animals and Samples Collection

This study was performed in the years 2014-2015, Sivas province of Turkey. This study was carried out in 30 cattle, including 20 animals naturally infected with LSD and 10 healthy animals, all which were raised in the same region. The cattle included in the infected (LSD) group were of different breed (8 Holstein, 8 Brown Swiss, and 4 Simmental cattle), age (1-4 years) and sex (16 females, 4 males). Similarly, the cattle included in the control group were also of different breed (4 Holstein, 4 Brown Swiss, and 2 Simmental cattle), age (1-4 years) and sex (8 females, 2 males). The cattle in the control group was identified as Infectious Bovine Rhinotracheitis (IBR), Brucellosis, Mucosal Disease-negative. The study protocol was approved by the Ethics Committee of Cumhuriyet University, Turkey (Approval No: 2014/50).

On the basis of the clinical examination of the cattle infected with LSD, clinical scoring was performed with respect to body temperature (>39.4°C, 1 point, >40.0°C, 2 point), the frequency of skin lesions (very few-1 point, moderate-2 points, diffuse-3 points), the presence of oedema (1 point), lacrimation and nasal discharge (1 point), and the enlargement of the lymph nodes (1 point) *(Table 1)*.

Following the clinical examination of the animals, blood samples were taken from the jugular vein for laboratory analyses. For Real-Time PCR and haematological analyses, the blood samples were drawn into tubes coated with tripotassium ethylenediamine tetra-acetate (K_3EDTA), and for biochemical analyses and ELISA, the blood samples were collected into sterile plastic tubes. Haematological analyses were performed within an hour after the collection of the blood samples. For biochemical analyses and ELISA, the blood samples drawn into sterile plastic tubes were centrifuged (10 min; 3.000 × g) for serum extraction.

Table 1. *Clinical scoring of cattle infected with LSD*
Tablo 1. *Lumpy skin disease (LSD)'li sığırların klinik skorlaması*

Parameters	1 point	2 points	3 points
Rectal temperature	>39.4°C	>40.0°C	-
Presence of nodules	few	moderate	diffuse
Enlargement of lymph nodes	+	-	-
Lacrimation	+	-	-
Nasal discharge	+	-	-
Oedema	+	-	-

Until being analysed for TNF-α, Hp, SAA, neopterin and biochemical, the serum samples were stored at -80°C.

DNA Isolation

DNA isolation from whole blood samples was performed using a DNA isolation kit (MagNA Pure Compact Nucleic Acid Isolation Kit I, Roche Cat No: 03730964001) and the samples were stored at -80°C.

Real Time PCR

Virus detection by Real Time PCR was performed using the forward primer 5'-AAA ACG GTA TAT GGA ATA GAG TTG GAA-3, the reverse primers 5'-AAA TGA AAC CAA TGG ATG GGA TA-3' and 5'-6FAM-TGG CTC ATA GAT TTC CT-MGB/NFQ-3' probe [27] and a Real Time PCR device (Light Cycler 480, Roche Diagnostic, GmBh, Germany). The master mix solution was prepared as described below: 9.0 µL of PCR-purity ddH_2O, 4.0 µL of TaqMAN Probe Master Kit (Roche Cat No: 04535286001), 0.5 µL of Primer F, 0.5 µL of Primer R, 1.0 µL of Probe, and 5.0 µL of viral DNA were mixed to obtain a total volume of 20 µL. The initial denaturation cycle was performed at 95°C for 600 sec, and was followed by a quantification protocol of 45 denaturation cycles at 95°C for 3 sec, annealing at 60°C for 30 sec, extension at 72°C for 1 sec (single), and a cooling cycle at 40°C for 30 sec.

Haematological and Biochemical Analyses

Haematological analyses (total leukocyte, erythrocyte, thrombocyte, haemoglobin and haematocrit measurements) were performed using an automated hematology cell counter (Mindray BC-2800Vet, PRC). Serum glucose, creatinine, total bilirubin, total protein, albumin, aspartate aminotransferase (AST), ɣ-glutamyl transferase (GGT), alkaline phosphatase (ALP), urea and creatine kinase levels were measured using an auto-analyser (Mindray BS 200, PRC).

Analyses for haptoglobin, SAA (Tridelta LTD, Ireland), TNF-α (SunRed, PRC) and neopterin (Yehua, PRC) were performed with commercial kits. The optical density the samples was measured by use of a micro plate reader (Thermo Multiskan GO, Microplate Spectrophotometer, USA).

Measurement of TNF-α Concentrations

Serum TNF-α concentrations were measured using the sandwich ELISA method, in accordance with the recommendations of the manufacturer (Sunred Biological Technology Co. Ltd., Shanghai, PRC). It has been reported that the measurement limit and sensitivity of the test are 15-4.000 ng/L and 14.155 ng/L, respectively, and the intra- and inter-assay precision (reproducibility) (coefficient of variation, CV, %) are <9% and <11%, respectively.

Hp Measurements

The inhibition of haemoglobin peroxidase activity and the level of Hp in the samples are directly proportional. Therefore, serum Hp levels were measured using commercial kits (Tridelta Development Ltd., Maynooth, County Kildare, Ireland) on the basis of the inhibition of haemoglobin peroxidase activity. It has been reported that the analytic sensitivity, and intra- and inter-assay precision (reproducibility) (CV, %) of this test are 0.005 mg/mL, 5.3-12.1% and 4.1-5.7%, respectively.

SAA Measurements

Serum SAA concentrations were measured using the solid-phase sandwich ELISA method (Tridelta Development Ltd., Maynooth, County Kildare, Ireland). The samples were analysed after being diluted at a proportion of 1:500. The manufacturer has reported the bovine serum or plasma analytic sensitivity of this test as 1.5 ug/mL. The intra- and inter-assay precision (reproducibility) (CV, %) of the test have been indicated as 7.5% and 12.1%, respectively, in cattle.

Neopterin Measurements

Serum neopterin concentrations were measured using commercial kits (Yehua Biological Technology Co., Ltd. Shanghai, China) and by the sandwich ELISA method. The measurement limit, sensitivity and intra- and inter-assay precision (reproducibility) (CV, %) of this test have been reported as 0.2-60 nmol/mL, 0.11 nmol/mL, and <10%, respectively.

Statistical Analysis

Statistical analyses were performed using the 15.0 SPSS package programme (Statistical Package for Social Sciences, Chicago, IL). The variables were tested for normal distribution with the Kolmogorov-Smirnov test. Comparisons between groups were made by use of Student's t test for continuous variables with a normal distribution, whilst variables with non-normal distribution were analysed with the Mann-Whitney U test. Furthermore, correlations between serum albumin, TNF-α, Hp, SAA and neopterin levels were assessed with Pearson's correlation coefficients.

Receiver operating characteristic (ROC) curves and the area under these curves were used to assess the diagnostic potential of serum albumin, TNF-α, Hp, SAA and neopterin levels. ROC analyses were performed for both the cattle infected with LSD and the healthy control animals. To assess the diagnostic potential of serum albumin, TNF-α, Hp, SAA and neopterin levels in the diagnosis of LSD, the area under curve (AUC) and some cut-off values were analysed. The results were assessed at a 95% confidence interval and at a significance level of $P<0.05$.

RESULTS

Clinical examination *(Table 2)* revealed increased body temperature (n=14), excessive salivation and lacrimation

Table 2. *Clinical scores of cattle infected with LSD*
Tablo 2. *Lumpy skin disease (LSD)'li sığırların klinik skorları*

Samples No	Rectal Temperature (°C)	Presence of Nodules	The Enlarged Lymph Nodes	Lacrimation	Nasal Discharge	Oedema
LSD 1	++	++		+	+	
LSD 2	+	+	+			+
LSD 3	++	++				
LSD 4	++	+		+		
LSD 5	++	+	+			+
LSD 6	+	+				
LSD 7	++	+				+
LSD 8	++	++	+			+
LSD 9	++	+++		+	+	
LSD 10	+	+				+
LSD 11	+	+++	+	+	+	
LSD 12		+++		+	+	
LSD 13		+		+	+	
LSD 14	+	++				
LSD 15	++	++			+	
LSD 16		++	+			
LSD 17		++		+	+	
LSD 18	++	+++		+	+	
LSD 19		++	+			+
LSD 20		+		+	+	

Table 3. *Haematological and biochemical parameters of cattle control and with Lumpy Skin Disease (LSD)*
Tablo 3. *Lumpy Skin Disease'li ve kontrol sığırlarının hematolojik ve biyokimyasal parametreleri*

Parameters	Control Group X ± Sx	LSD Group X ± Sx	P value
WBC ($X10^9L$)	9.05±0.70	11.38±8.59	.302
RBC ($X10^{12}L$)	6.42±0.21	6.1±0.21	.309
HGB (g/dL)	9.65±0.57	8.87±0.38	.272
HCT (%)	29.65±1.24	27.85±0.83	.245
PLT ($X10^9L$)	428.10±26.13	543.65±54.91	.071
Total protein (g/dL)	6.80±0.17	6.97±0.15	.694
Albumin (g/dL)	3.60±0.06	3.30±0.08	.004**
Creatine kinase (mg/dL)	197.78±31.63	645.00±359.27	.699
Creatinine (mg/dL)	1.04±0.04	0.97±0.027	.164
BUN (mg/dL)	16.50±0.69	15.40±1.01	.376
Total bilirubin (mg/dL)	0.08±0.02	0.29±0.14	.104
AST (u/L)	92.60±5.83	95.20±9.76	.821
GGT (u/L)	27.90±2.60	25.75±1.60	.491
ALP (u/L)	77.56±9.29	101.16±15.93	.212

WBC, *White blood cell count;* ***RBC,*** *Red blood cell count;* ***HGB,*** *Hemoglobin;* ***PCV,*** *Packed cell volume;* ***PLT,*** *Platelet;* ***BUN,*** *Blood urea nitrogen;* ***AST,*** *Aspartate aminotransferase;* ***GGT,*** *Gamma-glutamyl transferase;* ***ALP,*** *Alkaline phosphatase; ** Correlation is significant at the 0.01 level*

Table 4. *Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels of cattle control and with Lumpy skin disease (LSD)*
Tablo 4. *Lumpy skin disease'li (LSD) ve kontrol sığırların tümör nekroz faktör-α (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterin düzeyleri*

Parameters	Control Group X ± Sx	LSD Group X ± Sx	P value
TNF-α (ng/L)	1079.41±311.72	1502.19±254.13	.307
Hp (mg/mL)	0.13±0.015	1.54±0.25	.001
SAA (µg/mL)	19.80±4.19	275.98±17.13	.001
Neopterin (nmol/mL)	18.55±1.60	31.54±2.43	.001

(n=9), enlargement of the lymph nodes (n=6), oedema in various regions of the body (n=6), and multifocal skin nodules ranging from 1 cm to 5 cm in size (n=20). Although these skin nodules were particularly diffuse in the head, neck, genital region, perineum and legs, they were distributed throughout the body.

The results of the haematological and biochemical analyses for each group are presented in *Table 3*. TNF-α, Hp, SAA and neopterin levels are shown in *Table 4*. It was determined that, when compared to the control group, the albumin concentrations of the cattle infected with LSD were significantly lower (P=0.004), and the serum Hp (P<0.001), SAA (P<0.001) and neopterin (P<0.001) levels were significantly higher *(Table 3, Table 4)*.

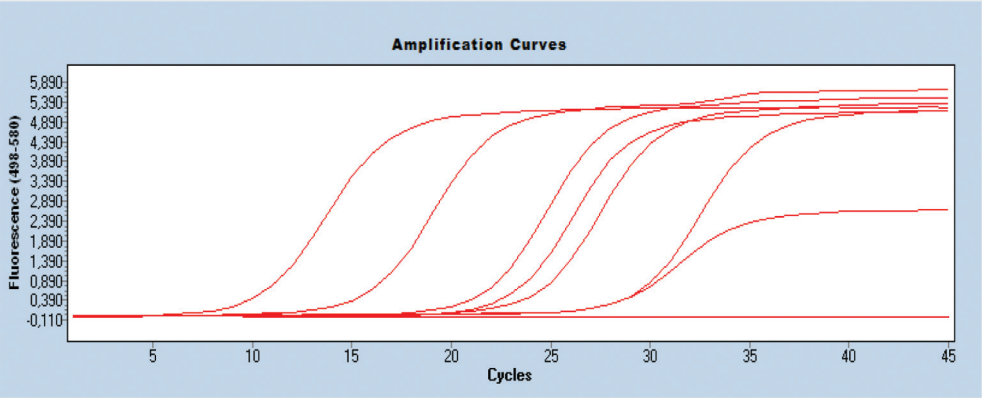

Fig 1. Amplification graph of cattle determined to be LSD-positive by RealTime PCR

Şekil 1. Real-Time PCR ile Lumpy skin disease (LSD) pozitif belirlenen sığırların amplifikasyon grafiği

Table 5. *The areas under the ROC curves (AUC), cut-off values, sensitivity and specificity of Albumin (Alb), Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels*

Tablo 5. *Albümin (Alb), Tümör nekroz faktör-alfa (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterin düzeylerinin ROC eğrisi altında kalan alan (AUC), cut off değeri, sensitivitesi ve spesifikliği*

Parameters	Alb (g/dL)	TNF-α (ng/L)	Hp (mg/mL)	SAA (µg/mL)	Neopterin (nmol/mL)
AUC	0.808	0.781	0.950	0.975	0.865
Cut off	3.39	814.03	0.196	41.38	23.93
Sensitivity	60.0	70.0	85.0	95.0	70.0
Specificity	90.0	87.5	90.0	90.0	90.0

Table 6. *Correlations amongs albumin (Alb), Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA), neopterin and clinical score*

Tablo 6. *Albümin (Alb), Tümör nekroz faktör-alfa (TNF-α), haptoglobin (Hp), serum amyloid A (SAA), neopterin ile klinik skorlama arasındaki korelasyonlar*

Parameters	Alb (g/dL)	TNF (ng/L)	Hp (mg/mL)	SAA (µg/mL)	Neopterin (nmoL/mL)	Clinical Scoring
Alb (g/dL)		-.251	-.563(**)	-.476(**)	-.599(**)	-.617(**)
TNF (ng/L)	-.251		.430(*)	.409(*)	.776(**)	.310
Hp (mg/mL)	-.563(**)	.430(*)		.636(**)	.745(**)	.587(**)
SAA (µg/mL)	-.476(**)	.409(*)	.636(**)		.640(**)	.862(**)
Neopterin (nmol/mL)	-.599(**)	.776(**)	.745(**)	.640(**)		.601(**)
Clinical scoring	-.617(**)	.310	.587(**)	.862(**)	.601(**)	

** Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level*

ROC analysis was performed to ascertain albumin, TNF-α, Hp, SAA and neopterin activity in the control animals and the cattle infected with LSD. The ROC curves, used to assess the diagnostic potential of albumin, TNF-α, Hp, SAA and neopterin levels, are presented in *Fig. 1* and the areas under the curves (AUC) are shown in *Table 5*. The ROC analysis was used to establish the cut-off, sensitivity and specificity values.

When the control animals and cattle infected with LSD were differentiated on the basis of Hp, SAA and neopterin cut-off values of 0.196 mg/mL, 41.38 µg/mL and 23.93 nmol/mL, respectively, the sensitivity rates for serum Hp, SAA and neopterin levels were determined as 85%, 95% and 70%, respectively, and the specificity rate was ascertained as 90% *(Table 5)*.

Correlations between albumin, TNF-α, Hp, SAA and neopterin levels were analysed in both the control group and the cattle infected with LSD. TNF-α, Hp, SAA and neopterin levels were found to be positively correlated with each other. While albumin levels were found to be negative correlate with TNF-α, Hp, SAA and neopterin levels *(Table 6)*.

DISCUSSION

Tests, which demonstrate the diagnostic and prognostic factors in diseases, are of particular significance in both veterinary and human medicine. Reports indicate that the levels of Hp and SAA, which are acute-phase proteins that have important functions in the various phases of inflammation, provide valuable information throughout

the clinical course of infectious diseases [1,4,28,29]. Research carried out in animals infected either experimentally or naturally with viral diseases has yielded results demonstrating the correlation of acute-phase protein levels with the clinical picture. Höfner et al.[30] reported that, in cattle exposed to the foot and mouth disease (FMD) virus, as from the onset of viraemia on the 8. day, clinical symptoms were observed and increased haptoglobin levels were detected.

Heegaard et al.[5] reported that, in calves experimentally infected with bovine respiratory syncytial virus, serum Hp concentrations exceeded 10 mg/mL and SAA levels exceeded 80 µg/mL. These researchers suggested that these increased levels and local pathological changes associated with the disease induced the acute phase response, and indicated that the acute phase response was correlated with the clinical course of the disease.

In the present study, it was determined that the serum Hp and SAA levels were 1.54±0.25 mg/mL and 275±17.3 µg/mL, respectively, in the cattle infected with LSD, and it was ascertained that, when compared to the control group, both markers had significantly increased (P<0.001). A statistically significant positive correlation (P<0.001) was detected between the clinical scoring, Hp and SAA levels (r=588 and r=862, respectively). This showed that the disease was associated with a strong acute phase response and that the pathological changes were correlated with the Hp and SAA levels. Also, increase in SAA levels may be a indicator of inflammatory process associated with LSD as evident of high test sensitivity and specificity.

Neopterin is produced in monocytes and macrophages by the guanosine triphosphate (GTP) cyclohydrogenase-I enzyme via γ-interferon (INF-γ), following the activation of Th1 lymphocytes [31,32]. As its secretion depends on the level of activation of T cells, neopterin has been suggested to be a non-specific indicator of the activation of the cellular immune system. Reports indicate that changes resulting in the activation of T lymphocytes during the pathogenesis of viral infections also affect neopterin levels [8,31,32]. Kaleli et al.[17] reported that, in carriers of the hepatitis B virus, macrophage activity, which increases with virus replication, elevates neopterin levels. Plata-Nazar et al.[8] reported to have observed significantly elevated neopterin levels in pediatric gastroenteritis cases caused by adenovirus and rotavirus infections. In rotavirus and adenovirus infections, at a neopterin cut-off value of 11.0 nmol/L, these researchers determined sensitivity and specificity rates of 86.6% and 94.3%, respectively. In their study on the assessment of the potential of serum neopterin levels in the detection of the severity of disease and causative agent in pneumonia patients, Prat et al.[9] observed that serum neopterin levels varied with the aetiology and severity of pneumonia. Furthermore, these researchers detected that the serum neopterin levels of bacteraemic patients with pneumococcal pneumonia were higher than those of non- bacteraemic pneumonia patients. Ercan et al.[33] indicated that in healthy cattle, neopterin levels were significantly higher particularly during the neonatal period, and suggested that this could be related to monocyte activation in new-born animals.

In the present study, the neopterin levels of the animals infected with LSD were determined to within the range of 31.54±2.43 nmol/mL, and it was ascertained that the mean values of the control group and the animals infected with LSD differed significantly (P<0.001). A statistically significant positive correlation (r=640) was determined to exist between clinical scoring and serum neopterin levels (P<0.001). Furthermore, in cattle infected with LSD, at a cut-off value of 23.93 nmol/mL, the sensitivity and specificity rates were determined as 70% and 90%, respectively. The high serum neopterin levels detected in the present study were attributed to the activation of the cellular immune system. Thus, in view of the data obtained, it is suggested that serum neopterin levels can be used as a biochemical parameter indicative of infectious activity in LSD cases.

TNF-α is a cytokine that can be determined in blood at an early stage following the activation of macrophages and other proinflammatory cells. It plays an important role in the regulation of the immune system [34]. Sordillo and Peel [35], reported that, in experimental *Escherichia coli* infections, increased TNF-α levels were observed up to 12-24 h, and suggested that the elevated TNF-α levels could aid in the assessment of the clinical course of the disease. In viral infections, the *in vitro* antiviral effect of TNF-α is observed as the inhibition of virus replication [35]. Indicated that the blood levels of cytokines, including TNF-α increased in cattle infected with the FMDV, and suggested that these increased levels could be involved in the inhibition of the development and growth of the virus in T cells, and thereby, in the prevention of the establishment of the virus in the body [13].

In the present study, the TNF-α levels detected in the cattle infected with LSD (1502.19±254.13 ng/L) were observed to have insignificantly increased in comparison to the levels determined in the control group (1079.41±311.72 ng/L) (P<0.307). This increase was attributed to the response of the host immune system to the virus. Clinical scoring and TNF-α levels were determined to be insignificantly correlated (r=310).

In study, no statistically significant differences in haematological and biochemical values except of albumin were determined in the animals with LSD and control. Most of the mean of the haematological and biochemical values found in the reference range [36].

Reports indicate that the level of albumin, which is considered to be the most significant negative acute-

phase protein in cattle, decreases in the event of infectious diseases [10]. Furthermore, it has been suggested that albumin levels could be used to assess the clinical picture of patients in human medicine [37]. In the present study, the serum albumin levels of the cattle infected with LSD ranged between 3.30±0.08 g/dL, and were found to significantly differ from the levels detected in the control group (P<0.001). Clinical scoring and albumin levels were ascertained to be negatively correlated with each other (r=-617). When evaluated together with Hp and SAA levels with which they have been determined to be correlated, decreased albumin levels can be interpreted as an indicator of inflammation.

In conclusion, a positive correlation was determined to exist between clinical findings and Hp, SAA and neopterin levels in cattle infected with LSD. Furthermore, it was ascertained that, when compared to Hp and neopterin, SAA showed a higher sensitivity in the detection of the severity of inflammation.

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