Relationship between some biochemical parameters in healthy and sheep with infectious keratoconjunctivitis

Gülşah AKGÜL[*], M. Barış AKGÜL[**], Serpil Kahya DEMİRBİLEK[***], Kıvanç IRAK[****], Ö. Yaşar ÇELİK[*], Tekin ŞAHİN[*], Nihat ŞINDAK[**]

Abstract: The aim of the presented study was to compare sodium (Na), potassium (K), chlorine (Cl), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHOL), triglyceride (TG) values of healthy animals and the Romanov breed sheeps with infectious keratoconjunctivitis and find a correlation if existed. 10 Romanov breed sheep the ages of 1.5-2 years were selected for this study consisting of 5 healthy sheep and 5 sheep with infectious keratoconjunctivitis in a special dairy farm, in Siirt. Housing, feeding, and management conditions were same for all sheep. When the biochemical values of healthy animals and animals with infectious keratoconjunctivitis were compared, no statistically significant difference was determined. Results of this study can be expressed as no significant difference was determined in sheep having infectious keratoconjunctivitis compared to those in healthy animals.

Keywords: Aspartate aminotransferase. infectious keratoconjunctivitis, gamma glutamyl transferase,

Sağlıklı ve infeksiyon keratokonjunktivitisli koyunlarda bazı biyokimyasal parametreler arasındaki ilişkinin araştırılması

Öz: Sunulan çalışmada İnfeksiyöz keratokonjunktivitis'li ve sağlıklı Romanov ırkı koyunlar arasındaki sodyum (Na), potasyum (K), klor (Cl), gama-glutamil transferaz (GGT), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), kolesterol (CHOL), trigliserid (TG) değerlerinin karşılaştırılması amaçlanmıştır. Çalışmanın materyalini Siirt ilindeki özel bir işletmede aynı bakım ve besleme şartlarına sahip 5 adet sağlıklı ve 5 adet infeksiyöz keratokonjunktivitisli hayvanlar oluşturmaktadır. Sağlıklı hayvanlar ile infeksiyöz keratokonjunktivitisli hayvanların biyokimyasal değerleri karşılaştırıldığında bir fark tespit edilmemiştir. Sonuç olarak bu çalışma bulgularında infeksiyöz keratokonjunktivitis'li hayvanlarda biyokimyasal değerler incelendiğinde önemli bir fark bulunmamıştır.

Anahtar sözcükler: Aspartate aminotransferase, infeksiyöz keratokonjunktivitis, gamma glutamyl transferase,

Introduction

Infectious keratoconjunctivitis, also known as 'pink eye', in sheep is seen in many parts of the world

[*] Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey.
[**] Department of Surgery, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey.
[***] Department of Microbiology, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey.
[****] Department of Biochemistry, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey.

and is an economically important and contagious disease of small ruminants (7, 18) Clinical findings may begin unilaterally, however, bilateral insult has been observed in many cases (18). The first indication of the disease is conjunctival hyperemia, serous lacrimation, increased blinking, photophobia and blepharospasm (2). Subsequently, the conjunctival veins dilate and proceed over the cornea. On the surface of the cornea, especially in the peripheral parts, blackish and grayish formations are observed. Within 2-5 days, the serous discharge becomes purulent, due to bacterial infection. In the latter stages of the disease, keratitis and corneal ulcers may develop, leading to permanent loss of vision (10, 18).

The disease usually occurs during the winter, when the animals are kept in enclosed stalls, in some regions, and in dry and dusty environments during the summer. The main source of infection is infected ocular discharge. The infection spreads through susceptible animals in a short time via direct contact or via vectors (6). Although the disease is mostly seen in 5-10 day old lambs, it can be detected in all age groups (10, 15).

Although many different microorganisms are incriminated in the etiology of the disease, the causes and predisposing factors of the disease are still being investigated. *Branhamella ovis* (*B. ovis*), *Chlamydia psittaci* (*C. psittaci*) and *Mycoplasma conjunctiva* (M. *conjunctiva*) are reported as the major known pathogens of the disease (9, 10).

It was aimed to present the enlightening data about the diagnosis of infectious keratoconjunctivitis with the differences between the above mentioned biochemical parameters in sheep with infectious keratoconjunctivitis and healthy sheep.

Materials and Methods

Animals and sample collection: The study conducted with 10 Romanov breed sheep aged between 1.5-2, of which 5 were with infectious keratoconjunctivitis (G1, n=5) and 5 were in healthy conditions, in a single farm located at Siirt, Turkey. Housing, feeding, and management conditions were same for all sheep. The clinical examinations (body temperature, pulsation, number of respirations, lymph nodes, tracheal palpation, lung auscultation and percussion) of all animals in the study were extensively performed. Animals with conjunctival hyperemia, blepharospasm, photophobia, purulent lacrimation, and corneal opacity at various grades were evaluated as having infectious keratoconjunctivitis and were allocated in group G1. The animals that did not lose appetite and performance, and had normal clinical and ophthalmological examination results and were allocated in group G2.

Biochemical analyses: Blood samples were transferred from the vena jugularis to sterile gel-containing biochemical tubes. The samples were centrifuged at 1000 r.p.m. for 15 min and sera were immediately separated and stored at -20°C until analyses**.** Sodium (Na), potassium (K), chlorine (Cl), gamma-glutamyl transferase (GGT), alaninaminotransferaz (ALT), aspartate aminotransferase (AST), cholesterol (CHOL), triglyceride (TG) concentrations (advia 1800 chemistry system) were measured.

Microbiological analyses: Ocular specimens were collected from the right and left eyes of the control group and from the animals with

the disease with a moistened brush with sterile saline and applied to surfaces of the conjunctiva. Samples were taken into tubes containing Cary-Blair transport medium (Lab M, UK) and were sent to Uludag University Veterinary Faculty Microbiology Department with the cold chain in Cary Blair transport medium. Swabs were inoculated into blood agar (Merck, Germany), Mac Conkey agar (Merck, Germany), Eosine Methylene Blue agar (Merck, Germany), pathogenic fungi (Merck, Germany) and *Mycoplasma* agar (Merck, Germany) and incubated at 37 °C for 24-96 hours in both aerobic and microaerophilic conditions. After examination of the microscopic morphology of the colonies, biochemical tests were performed relevant to the suspected agents.

Statistical analyses: Statistical analyses were performed using the IBM SPSS Statistics 20 statistical software. Mann-Whitney U test was used to compare differences among groups. Level of significance was set at $p<0.05$.

Results

Biochemical analyses: The result of the serum biochemical analyzes is shown in Table 1. No statistically significant difference was found between the groups. There was no statistically significant difference between Na, K, Cl, GGT, ALT, AST, CHOL and TG values ($p>0.05$) (Table 1).

Microbiological analyses: *Staphylococcus aureus*, *Clostridium* spp, and *Penisillium* spp. were isolated from the conjunctiva of the sick animals when no growth was detected in the swaps of the control group.

Discussion and Conclusion

The first clinical findings are advanced lacrimation, photophobia, and vision impairment in the eye. Tear flow gets purulent in a short time. The first symptom of conjunctivitis is blepharospasm and local pain. Changes in the cornea occur within the first 3 days with the onset of the disease. The center of the cornea is cloudy with a diameter of about 3 mm. Conjunctival areas are hyperemic and with edema (16). In the presented study, similar findings were found in all sheep with keratoconjunctivitis

İnfectious keratoconjunctivitis can be associated with lots of bacteria. *Listeria monocytogenesis*, *Staphylococcus aureus*, *Corynebacterium* spp., *Escherichia coli* (2), *Mycoplasma* spp. and *Branhamella* spp. (11) and *Pseudomonas aeroginosa*, *Moraxella caprae* (1) are isolated bacteria from some literature. Also, *Clostridium* spp. and *Penisillum* spp. could be detected from both animal and human with eye diseases as a causes agent of primer and/or seconder infection (5).

The blood chemistry measurement has a great importance in the diagnosis and prognosis of various diseases and the evaluation of the progress of the treatment together with clinical findings (8). There is a great deal of parallelism between the values of Na, K, Cl, GGT, ALT, AST, CHOL and TG determined by the serum of healthy sheep and the values reported by various researchers for healthy sheep (3).

AST is a widely-distributed enzyme, which is found in many tissues and organs, with high activity in the liver (14). Increased AST activity in the serum is a sensitive marker of liver damage

(8). In primates, dog, cat, rabbit and rat, ALT is a specific cytosol liver enzyme, and its increase in the blood plasma is specific for changes in liver, but ALT activity in pigs, horses, goats, sheep and cattle is not specific for the liver, in order to have a diagnostic significance (13). ALT activity in the blood plasma is influenced by age and muscle activity (17). GGT is a membrane-bound enzyme that is found in cells with high rates of secretion or absorption. The high activity is found in the liver, kidneys, pancreas, intestine and the spleen (14). In the livers of cow, horse, sheep, and goat the GGT activity is relatively high and considerably lower in dog and cat. In the study conducted, AST, ALT and GGT were found to be within the normal physiological limits, and we can say in these findings that there is no cellular destruction in sheep with infectious keratoconjunctivitis.

Table 1: Median (Min-Max) serum biochemical values in infectious keratoconjunctivitis group (G1) and in control group (G2)

Tablo 1: İnfeksiyöz *keratokonjunktivitli grup (G1) ve kontrol (G2) grubundaki serum biyokimya parametrelerine ait Medyan (Min-Max) değerleri*

Parameter	G1	G2	P
Na (mEq/lt)	148.0 (146.0-150.0)	149.0 (148.0-150)	0.421
K (mEq/lt)	4.70 (4.20-4.90)	4.80 (4.60-4.50)	0.421
Cl (mEq/lt)	108.0 (106.0-110.0)	109.0 (105.0-111.0)	0.841
GGT (U/lt)	29.0 (17.0-41.0)	37.0 (28.0-42.0)	0.222
ALT (U/lt)	22.0 (10.0-29.0)	18.0 (17.0-24.0)	1.00
AST (U/lt)	78.0 (57.0-96.0)	75.0 (66.0-98.0)	0.841
CHOL (mg/dl)	55.0 (38.0-61.0)	49.0 (38.0-54.0)	0.310
TG (mg/dl)	31.0 (24.0-34.0)	27.0 (20.0-32.0)	0.222

(Na, Sodium; K, potassium; Cl, Chlorine; GGT, Gamma Glutamyl Transferase; ALT, Alaninaminotransferaz; AST, Aspartate Aminotransferase; Albumin; CHOL, Cholesterol; TG, triglyceride)

In nature, all the members of animal and plant kingdom require inorganic elements: minerals, as these are needed for their survival and efficient performance. Twenty two mineral elements are believed to be "essential" for the members of the animal kingdom. These comprise seven major or macromineral viz., Ca, P, Na, K, Cl, Mg, S and fifteen trace or micro minerals. The three important functions of minerals are, structural components of body, organ and tissues. Constituents of body fluid and tissues as electrolytes concern with the maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability. Catalysts in enzyme and hormonal system as an integral and specific component of structure of metallic-enzyme or as activators within those systems (12). In the presented study, the macrominerals like sodium, potassium and chloride in the blood of sheep (G2 and G2) are within normal physiological limits. Thus, we can say that some mineral deficiencies do not occur in sheep with keratoconjunctivitis.

Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components

in biomembranes and form insulation to allow nerve conduction and prevent heat loss (4). Triglycerides and cholesterol in the blood of sheep with infectious keratoconjunctivitis are within the normal physiological limits in the present study. So in this disease, no systemic lipid abnormalities might have been involved.

Infectious keratoconjunctivitis, which is still under investigation, maintains its update as it causes widespread outbreaks in our country. Considering to data of studies about the treatment of the disease, it is noteworthy that some complications related to the eye are permanent in the healing events. Therefore, further studies will contribute to the diagnosis and treatment of the disease.

In conclusion, the result of this study, when the biochemical values of healthy animals and animals with infectious keratoconjunctivitis were compared, no statistically significant difference was determined.

Animal Rights Statement: This study was approved by the Siirt University Animal Research Local Ethics Committee (No: 2016/19).

References

1. Abdullah FFJ, Radzuan NS, Abdulnasir Tijjani, Adamu L, Abba Y, Mohammed K, Osman AY, Roslim N, Awang DN, Saharee AA, Saad MZ, Haron AW (2014): *Stage II Keratoconjunctivitis in a goat: Acase report*. J Agric Vet Sci, **7**, 16-18.

2. Åkerstedt J, Hofshagen M (2004): *Bacteriological investigation of infectious keratoconjunctivitis in Norwegian sheep.* Acta Vet Scand, **45**, 1-3.

3. Batmaz H (2013): *Koyun ve keçilerin iç hastalıkları*. 1sth ed., Nobel Tıp Kitapevleri Ltd.Şti, Ankara, Türkiye.

4. Black S, Vanderweed V (1989): *Serum lipoproteins are required for multiplication of Trypanosoma brucei brucei under axenic culture conditions*. Mol Biochem Pathol, **37**, 65-72.

5. Chen Nancy, Lee YC (2011): *Mixed fungal keratitis of Penisillium species and cremonium species*. Tzu Chi Med J, **23**, 26-27.

6. Egwu GO, Faull WB, Bradbury JM, Clarkson MJ (1989): *Ovine infectious keratoconjunctivitis: a microbiological study of clinically unaffected and affected sheep's eyes with special reference to Mycoplasma conjunctivae*. Vet Rec, **125**, 253-256.

7. Egwu GO (1991): *Ovine infectious keratoconjunctivitis: an update*. Vet Bulletin, **61**, 547-559.

8. Fiore E, Barberio A, Morgante M, Rizzo M, Giudice E, Piccione G, Lora M, Gianesella M (2015): *Glucose infusion response to some biochemical parameters in dairy cows during the transition period*. Anim Sci Pap Rep, **33**, 129–136.

9. Giacometti M, Nicolet J, Johansson KE, Naglić T, Degiorgis MP, Frey J (1999): *Detection and identification of Mycoplasma conjunctivae in infectious keratoconjunctivitis by PCR based on the 16S rRNA gene*. J Vet Med B, **46**, 173-180.

10. Greig A (1989): *Ovine keratokonjunctivitis*. In Practice, **11,** 110-113.

11. Jansen BD, Heffelfinger JR, Noon TH, Krausman PR, Vos JC (2006): *Infectious keratoconjunctivitis in Bigborn Sheep, silver bell mountains, Arizona, USA*. J wildlife Dis, **42**, 407-411.

12. Jittakhot S, Schonewille JTH, Wouterse HS, Yuangklang C, Beynen AC (2004a): *Apparent magnesium absorption in dry cows fed at 3 levels of potassium and two levels of magnesium intake*. J Dairy Sci, **87**, 379–385.

13. Kaneko JJ, Harvey JW, Bruss ML (2008): *Porphyrins and the porphyrias.*237-245. In: Clinical biochemistry of domestic animals. Academic Press, London, England.

14. Mortensen B, Huseby N (1997): *Clearance of circulating G-glutamyltransferase by the asialoglycoprotein receptor. Enzyme forms with different sialic acid content are eliminated at different clearance rates and without apparent desialyation*. Clin Chim Acta, **258**, 47-58.

15. Naglić T, Hajsig D, Frey J, Šeol **B, Busch K, Lojkić M** (2000): *Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep*. Vet Rec, **147**, 72-75.

16. Samsar E, Akın F, Bilir B, Gökçe P, Güvenç T, Köküuslu C, Sulu N (1993): *Sığırların enfeksiyöz keratokonjunktivislerinde subkonjunktival antibiyotik ve alfakimotripsin enzimi uygulamaları.* Ankara. Üniv Vet Fak Derg, **40**, 453-474.

17. Valentine BA, Blue JT, Shelley SM, Cooper BJ (1990): *Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog*. J Vet Intern Med, **4** , 140-143.

18. Van HA, Van RWJJ, Geyer A, Vorster JH (1994): *The identification of Mycoplasma conjunctivae as an aetiological agent of infectious keratoconjunctivitis of sheep in South Africa.* Onderstepoort J Vet, **61**, 231-237.

Received: 25.10.2017 / Accepted: 28.12.2017

Corresponding Author:
Assist. Prof. Dr. Gülşah AKGÜL
Siirt University,
Faculty of Veterinary Medicine,
Department of Internal Medicine,
Siirt, Turkey.
e-mail: gulsahvet@gmail.com