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SERUM SIALIC ACID, LIPID-BOUND SIALIC ACID LEVELS IN SHEEP NATURALLY CHRONIC INFECTED WITH FASCIOLA HEPATICA

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Abstract: The aim of this study to show the importance of sialic acid and lipid-bound sialic acid levels besides some hematological and biochemical parameters with respect to prognosis in detecting the degeneration and functional disorders in the livers of sheep with chronic fascioliasis. This study was carried out on twenty-two female Akkaraman sheep consisting of 10 healthy sheep and 12 sheep naturally chronic infected with Fasciola hepatica.

In the statistical analyses, in the beginning of the study (day 0.), levels of sheep with chronic fascioliasis were compared with the control group levels, a significant decrease was observed in the Hct ($p<0.001$), Hb ($p<0.001$), RBC ($p<0.05$), lymphocyte ($p<0.05$), total protein ($p<0.001$), albumin ($p<0.001$), globulin ($p<0.01$), and glucose ($p<0.01$) levels and a significant increase in the eosinophil ($p<0.001$), AST ($p<0.05$), GLDH ($p<0.001$), GGT ($p<0.001$), sialic acid ($p<0.001$), and lipid-bound sialic acid ($p<0.001$) levels.

In conclusion, serum sialic acid and lipid-bound sialic acid levels were increased in parallel with the AST, GLDH and GGT levels in liver degeneration and disorders in sheep with chronic fascioliasis. Sialic acid and lipid-bound sialic acid levels of sheep with fascioliasis are parameters that should be taken into consideration in prognosis after appropriate and effective treatment of the disease.

Key Words: Sheep, Fasciola hepatica, sialic acid; lipid-bound sialic acid

DOĞAL KRONİK *FASCIOLA HEPATICA*'LI KOYUNLARDA SERUM SIALIK ASIT SERUM LIPID-BAĞLI SIALIK ASIT DÜZEYLERİ

Özet: Kronik Fasciolazis'li koyunlarda karaciğerde meydana gelen dejenerasyon ve fonksiyonel bozuklukların tespitinde prognoz bakımından hematolojik ve bazı serum biyokimyasal parametrelerinin yanında sialic asit ve lipid-bağlı sialik asit düzeylerinin önemini ortaya koymak için yapıldı. Çalışmada 12'si kronik doğal Fasciola hepatica' lı ve 10 sağlıklı olmak üzere toplam 22 koyun kullanıldı.

Yapılan istatistiksel analizlerde, çalışmanın başlanıcın (0.günde) kronik Fasciolazis'li koyunların değerleri kontrol grubun değerleri karşılaştırıldığında Hct ($p<0.001$), Hb ($p<0.001$), RBC ($p<0.05$), lenfosit ($p<0.05$), total protein ($p<0.001$), albumin ($p<0.001$), globulin ($p<0.01$), ve glikoz ($p<0.01$) düzeyleri önemli düzeyde düşük ve eosinophil ($p<0.001$), AST ($p<0.05$), GLDH ($p<0.001$), GGT ($p<0.001$), sialic asit ($p<0.001$), ve lipid-bağlı sialik asit ($p<0.001$) düzeyleri önemli derecede yüksek bulundu.

Sonuç olarak; kronik Fasciolazis'li koyunların karaciğer bozukluğu ve dejenerasyonunda, artan AST, GLDH ve GGT düzeylerine paralel olarak, serum sialik asit ve lipid-bound sialik asit düzeyleri de yükselmektedir. Kronik Fasciolazis'li koyunlarda sialik asit ve lipidbağlı sialik asit düzeylerinin, hastalığın tedavisinde ve prognozu sırasında alınması gerekli olduðu göz önünde bulundurulmalýdýr.

Anahtar Kelimeler: Koyun, Fasciola hepatica, sialik asit, lipid-bound sialik asit

INTRODUCTION

Fascioliasis is a zoonotic disease caused by *Fasciola hepatica*, a liver fluke, that may be common found in ruminants. Fascioliosis is an economically important disease in cattle and sheep where the parasite induces production waste and economic loss. Many diagnostic methods for the hepatic disease caused by *Fasciola hepatica* are needed. There are so many researches which were studied especially serum activities of specific enzymes on the diagnosis of liver damage caused by *Fasciola hepatica*. Glutamate dehydrogenase (GLDH) and gamma-glutamyl transpeptidase (GGT) were the most sensitive indicators of liver cell damage in fascioliasis (1). Sykes et al.(2) reported plasma GLDH and GGT activities are more sensitive indicators of liver cell damage in chronic subclinical fascioliasis than aspartate aminotransferase (AST) activity and gamma glutamyltransferase (GGT) may be more suitable as a diagnostic aid on account of its greater stability.

Sialic acid, a class of important ketoses that contain negatively charged nine carbon atoms, is an acetylated derivative of neuraminic acid (2-keto-5-amino-3, 5-dideoxy-D-nonulosonic acid) (3). The negative charge present in sialic acid means that the compound takes part in binding and transport of positively charged molecules, and in the attraction and repulsion of cells and molecules (4) Total sialic acid (TSA) consists of glycoprotein and glycolipid bound sialic acid whereas lipid bound sialic acid (LSA) comprises of glycolipid bound sialic acid (5).

Sialic acid may have a variety of biological functions in vivo, such as stabilization of the conformation of glycoproteins and cellular membranes, cell-to-cell interactions, membrane transport, membrane receptor functions (6).An increase in TSA was previously found in various malignancies (7, 8) and in severe infectious diseases(9, 10) and some autoimmune disorders (11), coronary heart disease (12), and many pathologic conditions including recurrent abortion (13) However, there are few papers found on the relationship between fascioliasis and serum sialic acid levels in animals.

In this research, it was aimed to find out the possibility of diagnosis of liver damage by determining sialic acid levels and to detect the importance of sialic acid for the prognosis of disease as a biochemical marker and also find out the levels of some enzymes activities and hematological changes.

MATERIALS AND METHODS

Animals

This study was conducted on 22 female Akkaraman sheep consisting of 10 healthy sheep aged between 3 and 5 years old, and 12 sheep with chronic fascioliasis aged between 3 and 6 years old. All sheep has been controlled for 60 day during the study.

Parasitological examination

Feces samples were taken from all sheep for the parasitological examination and they were analyzed through native, flotation and Modified Benedek Sedimentation Method (14) at the beginning of study. No nematode and cestode factor were detected in the feces analysis in all sheep (14). The number of trematode egg per gram feces of the sheep infected with *Fasciola hepatica* at the beginning of treatment was specified through the mentioned method (15). The sheep diagnosed with fascioliasis upon parasitological analysis were categorized as test group, and the healthy sheep as control group.

Study design and treatment

Control group was subcutaneously injected with 200 µg/kg moxidectine against probable nematodes infections (16) and was orally given a commercial preparation of 7.5 mg/kg rafoxanide + 100 mg/kg thiabendazole (17, 18) against probable trematodes and cestodes infections before the study (day 0).

Test group was only subcutaneously injected with moxidectin 200 µg/kg against probable nematodes infections. Then, sheep infected with natural chronic *Fasciola hepatica* were treated with a commercial preparation of rafoxanide (7.5 mg/kg) + thiabendazole (100 mg/kg) (17, 18) at the beginning of treatment (day 0). Blood samples were taken from the vena jugularis of both groups into tubes with lithium heparine for hematological parameters and into tubes without heparine for serum parameters. Fecal samples were taken from test group on the 30th day after treatment and no trematode factor was detected in the feces analysis (14).

Hematological and Biochemical Analysis
Hematological parameters analysis

Blood samples were taken into lithium heparine tubes from sheep at the beginning of treatment (day 0.) and on day 30 after of treatment for the hemoglobin (Hb) and hematocrit (Hct) estimate as described by Jain (19) Red blood counts (RBC), leucocyte blood count (WBC), and formulated leucocyte were counted through Thoma microscope slide (19).

Biochemical parameters analysis

Blood samples were taken into tubes without heparin for biochemical parameters at the beginning of treatment (day 0.) and on day 30 after of treatment were centrifuged in 3000 rpm for 10 minutes. Then sera were put to the tubes for biochemical parameters. Serum total protein, albumin, globulin AST, GLDH, GGT, ALT, and glucose levels were measured with Roche-Cobas Integra 800 autoanalyzer. The colorimetric analysis of the serum sialic acid and serum lipid-bound sialic acid levels of both groups was made in PerkinElmer IA spectrophotometer (20, 21).

Statistical analysis

All statistical calculations were carried out with unpaired *t*-test.

RESULTS

In the clinical examination, weight loss, appetite depression, decreased productivity, paleness in mucosa and conjunctivas and edema under chin (10 sheep) was observed in the test group. While the average number of *F. hepatica* eggs in per gram feces of the sheep with naturally fascioliasis was found to be 4120±5 at the beginning of treatment (day 0), no trematode egg was found on day 30 after treatment.

The hematological and biochemical parameters of test and control groups were given in Tables 1-2. While the Hct, Hb, RBC and lymphocyte values of test group were found to be less than those of control group ($p<0.001$, $p<0.001$, $p<0.05$ and $p<0.05$, respectively) before treatment (day 0), eosinophil value was found higher in test group at the level of $p<0.001$.

The serum total protein, albumin, globulin and glucose values of test group were significantly lower than control group ($p<0.001$, $p<0.001$, $p<0.01$, $p<0.01$ respectively). In addition, in the beginning of the study(day 0.),the serum AST, GLDH, GGT, sialic acid and lipid-bound sialic acid values of test group were compared with those of control group. They were found significantly higher ($p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$ and $p<0.001$), whereas the ALT and ALP values were not found significant ($p>0.05$). When both the hematological and biochemical parameters of test group were compared with those of control group on day 30 after treatment, they were not statistically significant ($p>0.05$).

Table 1: Hematological Parameters of Healthy Sheep (Control) and Sheep with Fasciolosis (Test)

Parameters	**Control Group n=10** $\overline{X} \pm SE$	**Test Group n=12**	
		Before Treatment 0. Day $\overline{X} \pm SE$	After Treatment 30. Day $\overline{X} \pm SE$
Hct (%)	36.2 ± 2.61^{a}	21.3 ± 0.66^{Ab}	32.7 ± 1.96^{B}
Hb (g/dl)	12.1 ± 0.87^{a}	7.11 ± 0.22^{Ab}	10.9 ± 0.65^{B}
RBC (10^3/mm^3)	10169 ± 606.8^{a}	7710 ± 224.7^{d}	9142 ± 482.7
WBC (mm^3)	10339 ± 612	10249 ± 391.2	10191 ± 524.5
MCV (fl)	35.6 ± 1.96^{a}	27.7 ± 0.79^{c}	37.4 ± 1.89
Lymphocyte(%)	55.9 ± 1.46^{a}	48 ± 2.14^{d}	53.6 ± 2.08
Neutrophil (%)	31.9 ± 1.59	33.2 ± 1.64	37.4 ± 1.46
Monocyte (%)	6.10 ± 0.56	5.33 ± 0.45	5.42 ± 0.51
Eosinofil (%)	4.50 ± 0.34^{a}	12.7 ± 1.09^{Ab}	3.17 ± 0.44^{B}
Basophil (%)	0.70 ± 0.15	0.75 ± 0.13	0.58 ± 0.15

a. b. c. d.: The significance of difference between groups has been shown in small letters

ab: $p<0.001$, **ac**: $p<0.01$, **ad**: $p<0.05$.

A. B. The significance of difference within groups with different capital letters within columns. **AB**: $p<0.001$

Table 2: Biochemical Parameters of Healthy Sheep and Sheep with Fasciolosis

Parameters	Control Group n=10 $\overline{X} \pm SE$	Test Group n=12 Before Treatment 0. Day $\overline{X} \pm SE$	Test Group n=12 After Treatment 30.Day $\overline{X} \pm SE$
Total Protein (g/dl)	7.7 ± 0.34^{a}	5.4 ± 0.32^{Ab}	7.3 ± 0.16^{B}
Albumin (g/dl)	3.1 ± 0.20^{a}	2.1 ± 0.10^{Ab}	2.9 ± 0.09^{B}
Globulin (g/dl)	4.6 ± 0.20^{a}	3.4 ± 0.33^{Ac}	4.3 ± 0.18^{D}
Glucose (mg/dl)	57.7 ± 2.91^{a}	46.1 ± 2.30^{Ac}	56.2 ± 1.74^{C}
ALP (U/L)	273.9 ± 31.81	282.1 ± 16.9	250.5 ± 17.29
AST (U/L)	63.30 ± 2.08^{a}	90.67 ± 8.12^{Ad}	68.58 ± 3.15^{D}
ALT (U/L)	12.2 ± 1.58	13.9 ± 1.38	13.0 ± 1.33
GLDH (U/L)	1.78 ± 0.13^{a}	10.7 ± 1.17^{Ab}	2.2 ± 0.16^{B}
GGT (U/L)	22.2 ± 1.83^{a}	44.4 ± 1.72^{Ab}	25.1 ± 1.02^{B}
SA(mg/dl)	39.42 ± 2.10^{a}	51.68 ± 1.75^{Ab}	43.38 ± 2.33^{B}
LSA (mg/dl)	8.56 ± 0.38^{a}	10.65 ± 0.39^{b}	9.27 ± 0.26

a. b. c. d.: The significance of difference between groups has been shown in small letters

ab: $p<0.001$, **ac**: $p<0.01$, **ad**: $p<0.05$.

A. B. C. D.: The significance of difference within groups with different capital letters within columns. **AB**: $p<0.001$, **AC**: $p<0.01$, **AD**: $p<0.05$

DISCUSSION

Fascioliasis is of great economic importance in areas dedicated to the rearing of ruminants all over the world, producing considerable economic losses in agriculturally based countries (22). This disease causes significant economic losses, great expenses with antihelmintics, in addition to liver condemnation, production loss due to mortality, lower production of meat, milk and wool; reduced weight gain, and impaired fertility, also impeding the selection of animals (23, 24).

There are so many information available concerning hematological values and the hepatic enzymes activity which escapes from damaged hepatic cells in sheep infected with *F. hepatica*. Diagnosis has been aided by measuring the serum activities of hepatic enzyme levels of AST, GLDH or GGT may be used as markers of the different stages of Fasciola hepatica infection. Determination of plasma liver enzyme levels is also valuable for assessing the anthelmintic activity of drugs (rafoxanide) and the efficacy of treatment in sheep infected with Fasciola hepatica. (25).

A significant increase in plasma GLDH activity from 20 days post-infection and in GGT activity from 40 days post-infection was found (26).

Galtier et al. (27) and Ferre et al. (28) were reported that the determination of AST, LDH and GLDH provides information on the passage of young flukes through the liver parenchyma (parenchymal phase), whereas a GGT increase indicates penetration into the bile ducts (ductular phase). In this study, the serum GLDH and GGT activities of test group were found significantly higher than that of control group ($p<0.001$). The high serum GLDH and GGT activities (day 0) in test group could be developed by the degeneration caused by parasites in the liver. The relatively decreased GLDH and GGT activities in group 2 on day 30 after treatment may be associated with the partial recovery of the liver.

Kouider and Kolb (29) have reported that increased ALT and AST activities in sheep infested with liver fluke (*Fasciola hepatica*). In this study the serum AST value of the sheep with fascioliasis was slightly higher ($p<0.05$) than the serum AST values of control group and on the 30th day of therapy (68.58±3.15 U/ L), but it was not found statistically significant ($p>0.05$). However, ALT activity was compared among two groups and no significant differences were observed.

Increase serum ALP activity in animals commonly is the result of the hepatic isoenzyme of alkaline phosphatase because its production by biliary epithelium and hepatocytes

is induced during obstructive cholestasis (30, 31). Timoteo et al.(32) reported that the parasite reached the liver by the 4 weeks in Alpacas and produced damage in the liver parenchyma elevating both liver enzymes ALT and AST but no change in bilirubin and ALP suggested that the infection did not produce biliary obstruction. In this study in spite of slight increase of ALP activity in test group, there was no significant difference between test and control group (p0.05)

In order to detect the hematological disorders caused by liver trematode infections, Hct, Hb, erythrocyte, total leukocyte count and formulated leukocyte parameters are used. Of the hematological parameters, Hct, Hb, WBC and formulated leukocyte normal values vary depending on the physical characteristics of the animal such as race, age, sex, birth and lactation as well as the analysis method, maintenance, nutrition and altitude (33).

The cellular immune response to *F. hepatica* infection in different animal species has been widely studied. A significant transient proliferation in vitro of peripheral blood mononuclear cells stimulated by parasite antigens appears during the first weeks of infection in *F. hepatica*-infected sheep (34).

Conboy and Stromberg (35) found that an eosinophilia present in calves infested with *Fascioloides magna* extending from weeks 2 to 26 post-infection. Jain (19) has been reported that eosinophil-mediated destruction of parasites is an important mechanism of protective parasitic immunity.

Hawkins (36) found a decrease in Hb and PCV in infected sheep with *F. Hepatica* and reported that death occurred when haemoglobin fell below 5 g 100 ml^{-1}, and packed-cell volume to 15%. Presidente et al. (37) found mild macrocytic normochromic anemia characterized the fascioliasis in lambs. Crook et al. (38) reported that serum total sialic acid was an inverse correlation with haemoglobin concentration ($r = -0.62$, $P < 0.0001$) and erythrocyte count ($r = -0.42$, $P < 0.01$). In this study, while the Hct, Hb, RBC and lymphocyte values of test group were found to be less than those of control group ($p<0.001$, $p<0.001$, $p<0.05$ and $p<0.05$, respectively) before treatment (day 0), eosinophil value was found higher at the level of $p<0.001$.

Hypoalbuminemia in acute and chronic fascioliasis cases has been reported (1, 39) the effect of *F. gigantica* infection on liver function of sheep was studied (40) and hypoproteinemia with decrease in albumin and increase in total globulin was found. In this study, the pre-therapy total protein value of test group was lower than that of the control group ($p<0.001$). In parallel to the decrease total protein value of the sheep with fascioliasis, a decrease was also observed in the albumin and globulin levels as well.

We found lower glucose concentration value in infected animals; it is well known that glucose concentration usually drops in animals with liver disease. Swarup et al. (41) reported that infected goats had significantly lower levels of serum glucose (47.6 ±1.8 mg dl-1).

Sialic acid level in blood plasma and circulating glycoproteins is considered to be a marker for a number of pathologic conditions, including atherosclerosis, cancer, and excessive alcohol consumption, etc. Elevated levels of serum TSA have been reported in patients diagnosed with various cancers such as lymphoma, malignant melanoma, lung cancer and gastrointestinal cancers (42, 43). Recent studies have further shown that SA concentration in serum may be increased in alcoholics (44). Lindberg et al. (45) found that serum sialic acid concentration was a strong predictor of cardiovascular disease mortality. Other studies showed that serum sialic acid was elevated in diabetes mellitus (46).

Kongtawelert et al. (47) determined that the mean serum TSA concentration in cholangiocarcinoma that constitutes a common primary liver cancer in Southeast Asia where the liver fluke, (2.41±0.70 mmol/L) was significantly higher than those of hepatocellular carcinoma, cirrhosis, chronic hepatitis and healthy blood donors (1.41±0.37 mmol/L, 1.13±0.31 mmol/L, 1.16±0.26 mmol/L, and 1.10±0.14 mmol/L, respectively; $P<0.001$) and emphasized serum TSA would be a useful marker for the differential diagnosis of cholangiocarcinoma from hepatocellular carcinoma. In this study, while the serum sialic acid levels in test group were found as 51.68±1.75 mg/dl on day 0 and as 43.38±2.33 mg/dl on day 30 after treatment, that of control group was found as 39.42±2.10 mg/dl. When the serum sialic acid level of sheep with chronic fascioliasis are statistically compared with that of the control group, on day 0 and with that on day 30 after treatment, the lowest SA levels were found in control group. Before treatment 0 day (test group), SA levels were increased significantly $p<0.001$ but after treatment it was decreased to 43.38±2.33 mg/dl ($p>0.05$). This increase in serum SA concentration may occur via changes in the biosynthesis and post-translational glycosylation processing of the acute-phase proteins in the liver (6, 48, 49), many of the acute-phase proteins are glycoproteins with sialic acide residues at the terminal positions of their oligosaccharide side chains (50) or the phenomenon may be related to the intensified cell metabolism and increased serum sialyltransferase activity, an enzyme that is responsible for the synthesis of sialoglycoconjugates (51) may also be responsible for increased serum sialic acid concentrations.

LSA has been suggested to be the sialic acid bound to sialic acid containing glycolipids,i.e. gangliosides. An elevation in the levels of serum LSA has been found in various malignancies including breast cancer (52), ovarian carcinoma (53) head and neck carcinoma (54). In this study, control group had lowest LSA levels, an increase was found in before treatment (0 day) ($p<0.001$) and a decrease was estimated after treatment.

In conclusion, serum sialic acid and lipid-bound sialic acid levels were increased in parallel with the AST, GLDH and GGT levels in liver degeneration and disorders in sheep with chronic fascioliasis. Sialic acid and lipid-bound sialic acid levels of sheep with fascioliasis are parameters that should be taken into consideration in prognosis after appropriate and effective treatment of the disease.

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