

Total and Lipid-bound Serum Sialic Acid and Hematological-Biochemical-Blood Gas Changes in Dogs with *Dirofilariosis*

H. Kesici¹, Y. Değer¹, B. Oğuz^{2*}, U. Özdek³

¹ Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemical, Van, Turkey

² Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Parasitology, Van, Turkey

³ Van Yuzuncu Yil University, Vocational School of Health Services, Van, Turkey

ABSTRACT: The measurement of serum sialic acid concentration is of importance to indicate damage in cells or tissues. *Dirofilaria immitis* causes cardiovascular, pulmonary, hepatic and kidney lesions. Therefore, present study was planned to investigate the potential changes in the levels of serum total sialic acid (TSA), lipid-bound sialic acid (LSA) and hematological-biochemical parameters in the dogs naturally infected by *Dirofilaria immitis*. The patient group included the 7 dogs clinically and parasitologically (Modified Knott and PCR) diagnosed with *Dirofilaria immitis* while 7 healthy dogs were assigned as the control group. The biochemical parameters, blood gas parameters and hematological parameters were tested in the blood samples taken properly according to the guidelines from all the study animals using automated biochemistry analyzer, blood gas analyser and automated hematology analyzers, respectively. Serum TSA and LSA levels were measured spectrophotometrically using Sydow and Katapodis methods, respectively. Compared with the healthy group, the dogs diagnosed with dirofilariosis were found to have significantly increased levels of serum cardiactroponin, triglycerides, VLDL, LDL, BUN, urea and creatinine and enzymatic activities of CK-MB, ALT, AST, ALP and LDH, and statistically significantly decreased levels of cholesterol and HDL ($p<0.05$). A significantly decreased level of RBC and a significantly increased level of WBC was determined in the dogs with dirofilariosis ($p<0.05$). The differential leucocyte count test of the dogs with dirofilariosis indicated statistically significantly increased eosinophil count ($p<0.05$). In the dogs with dirofilariosis, the decreases in the levels of pCO_2 , HCO_3 and BE in venous blood were statistically significant ($p<0.05$). The levels of serum TSA and LSA of the dogs with dirofilariosis were found statistically significantly higher than the healthy group ($p<0.05$). As a conclusion, statistically significant differences were identified between the dogs with dirofilariosis and healthy dogs in terms of sialic acid levels and certain biochemical, venous blood gas and hematological parameters. On the other hand, the present study is the first to investigate the serum sialic acid levels in the dogs with dirofilariosis.

Keywords: Dog, *Dirofilariosis*, Lipid bound sialic acid, Sialic acid, PCR

Corresponding Author:

B. Oğuz, Faculty of Veterinary Medicine, Department of Parasitology, Van Yuzuncu Yil University, 65040, Van, Turkey
E-mail address: bekiroguz@yyu.edu.tr

Date of initial submission: 09-07-2020
Date of revised submission: 06-09-2021
Date of acceptance: 04-12-2021

INTRODUCTION

Dirofilariosis is a nematode disease caused by *Dirofilaria immitis* or *D. repens* that leads to serious systemic disorders in the carnivores, particularly dogs. *D. immitis* adults localize in the right ventricle, pulmonary arteries, right atrium and vena cava in the heart of the host. Their microfilariae are found in the peripheral bloodstream. The excessive work load on the right ventricle leads to congestive heart failure, organ dysfunctions such as particularly heart and kidneys, and various grades of pulmonary embolism and pulmonary hypertension in the dogs with dirofilariosis (Atkins, 2005; Sevimli et al., 2007).

Sialic acid (SA) is the general name of the acyl derivatives of neuraminic acid. N-acetylneuraminic acid (NANA) is the most common form of the sialic acids. Sialic acids are the terminal components of glycoproteins, glycolipids and oligosaccharide units of the proteoglycans. Total sialic acid (TSA) consists of two fragments as lipid-bound (lipid-bound sialic acid, LSA) and protein-bound (protein-bound sialic acid, PSA). The essential lipoproteins that contain LSA are VLDL, LDL, HDL and lipoprotein (a). The changes in the levels of serum SA indicate the damage in the cells or the tissues. The monitoring of these changes provides useful information in diagnosis, differential diagnosis and prognosis of many diseases (Sydow et al., 1988; Taşkın, 2017). For instance; increase of LSA and TSA levels have been demonstrated on babesiosis (Esmailnejad et al., 2014), theileriosis, anaplasmosis (Ertekin et al., 2000), leptospirosis (Erdoğan et al., 2008), dermatophytosis (Karapehlivan et al., 2007) and pneumonia (Karapehlivan et al., 2007).

Since this cardiopulmonary disease of the dogs is a zoonosis, the detection, elimination and treatment of the dogs is of great importance for both dog and human health. Besides, increased dog adopting in the recent years elevates risk for transmitting dirofilariosis in the humans. No study that aimed at the levels of TSA and LSA in the dogs with dirofilariosis was found in the literature review. The present study was planned to investigate the potential changes in the levels of serum TSA, LSA and hematological-biochemical parameters in the dogs naturally infected by *Dirofilaria immitis*.

MATERIALS AND METHODS

Parasitological analysis

The study was conducted on 96 male stray dogs between 4 and 5 years old brought to the Animal

Care and Rehabilitation Center for castration from Van Central and surrounding districts between May and October 2019. Before being castrated, the animals were fasted for one day and only drank water as the last 12 hours. Negative control samples were obtained from PCR negative dogs with no previous history of dirofilariosis. The approval of the Ethics Committee for this research was obtained from the Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University (2019/01). Blood samples were drawn from vena cephalica antebrachium of the dogs with 2 mL of blood to heparinized injectors for blood gas analyses, 3 mL of blood to EDTA tubes for hematological analyses and to 5 mL gel polyethylene tubes without anticoagulants for biochemical analyses. Blood samples taken into the EDTA tubes were analyzed by performing modified Knott's (Gioia et al., 2010) and PCR (T100 BioRad, USA) techniques using PCR kit (NorgenBiotek, EP44500). PCR positivity was detected in only 7 of 96 dogs. This study included totally 14 dogs aged 4-5 years old including 7 dirofilariosis-positive and 7 healthy dogs.

Biochemical analysis

Blood samples in serum separator tubes were centrifuged at 3000 rpm for 10 min within 2 h of collection, and the obtained sera were separated, stored at -20 °C. Cardiac troponin I (cTnI), total creatine kinase (CK), creatine kinase-myocardial isoenzyme (CK-MB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, total bilirubin triglyceride, cholesterol, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), urea, blood urea nitrogen (BUN) and creatinine in the sera were analysed with commercially available kits using an automated biochemistry analyser (Cobas Integra 800, Roche).

Serum TSA and LSA analyses were carried out colorimetrically on a spectrophotometer (Boeco, Germany) using the methods reported by Sydow et al. (1988) and Katopodis et al. (1982) respectively. Briefly, a mixture of 0.2 mL of serum and 1.5 mL of 5% perchloric acid (HClO₄) was incubated at 100 °C for 5 minutes, cooled and centrifuged at 500g for 4 minutes. Then 0.2 mL of Ehrlich reagent was added to 1.0 mL of clear supernatant and heated at 100 °C for 15 minutes. After cooling, 1.0 mL of distilled water was added to this mixture and the optical density was measured at 525 nm in a spectrophotometer. The amount of TSA was determined using a standard

curve developed from a standard sample of N-acetyl neuraminic acid (Sydow et al. 1988).

For LSA, 44.7 µL of serum was transferred with a capillary pipette to 150 µL of distilled water. Contents were vortexed for 5 seconds. The tube was transferred to crushed ice. Three milliliters of cold (4-5 °C) 2: 1 (v / v) chloroform: methanol were added to the tube and the mixture was vortexed for 30 seconds. 0.5 mL of cold distilled water was added to this mixture and the tube was closed. The tube was then inverted repeatedly for 30 seconds to mix the contents. After the tube was centrifuged at 500g for 5 minutes at room temperature, 1 mL of the upper layer was transferred to another tube. Fifty microliters of phosphotungstic acid solution (1 g / mL) was added and, after mixing, the tube was left at room temperature for 5 minutes. The tube was centrifuged at 500g for 5 minutes and the supernatant was suction removed. Continuing the researchers's method, the final color obtained was read at 580 nm. The amount of LSA was determined using a standard curve developed from a standard sample of N-acetyl neuraminic acid (Katopodis et al. 1982).

Blood samples collected heparinized injector were delivered to the laboratory with ice within 10 minutes. Blood samples were used for determination of venous blood gases parameters. Acidity (pH), carbon dioxide pressure (pCO₂), partial oxygen pressure (pO₂), bicarbonate (HCO₃⁻), base excess (BE) were measured using a blood gas analyzer (ABL90 FLEX).

Haematological analysis

The blood samples containing heparin were refrigerated and analysed within 48 h. Blood samples were used for determination of haematological parameters. Red blood cell (RBC), haemoglobin (Hb) hematocrit (Hct), white blood cell (WBC), neutrophil, lymphocyte, monocyte, eosinophil and basophil were measured using a automated haematology analyser (Vet. Wasson MC-1200).

Statistical analysis

SPSS Version 22 Software Package was used for the statistical analysis of the study data. First of all, distribution normality test was performed to assess whether the groups showed normal distribution. The groups were found to show normal distribution since significance value of Shapiro-Wilk test was over statistical significance level of 0.05. Therefore, the statistical differences between the groups were evaluat-

ed using Independent Sample T-Test. The obtained results were expressed as X±SE.

RESULTS

The analysis of 96 blood samples taken from the stray dogs by Modified Knott's technique revealed that 5 (5.2%) dogs were dirofilariosis-positive. However, PCR method was applied to all the samples and *D. immitis* was detected in totally 7 (7.2%) dogs (Figure 1). The clinical examination of the infected animals indicated coughing and respiratory distress in 3 and 2 dogs, respectively, whereas other 2 infected animals were asymptomatic. It was determined that TSA, LSA, cTnI, CK-MB, triglycerides, VLDL, LDL, BUN, urea, creatinine levels and AST, ALT, ALP, LDH enzyme activities statistically significantly increased; however, cholesterol, HDL, pCO₂, HCO₃⁻, BE levels statistically significantly decreased in dogs naturally infected with *D. immitis* compared to the healthy group (P< 0.05). The changes in the total protein and total bilirubin levels with total CK enzyme activity in dirofilariosis group were not statistically significant compared to the healthy group (p>0.05) (Table 1). It was detected that the RBC level significantly decreased while WBC level and eosinophils count significantly increased in dogs naturally infected with *D. immitis* compared to the healthy group (P<0.05). The changes in Hb and Hct levels with lymphocytes, monocytes, eosinophils and basophils counts were not statistically significant among dirofilariosis and healthy groups (P>0.05) (Table 2).

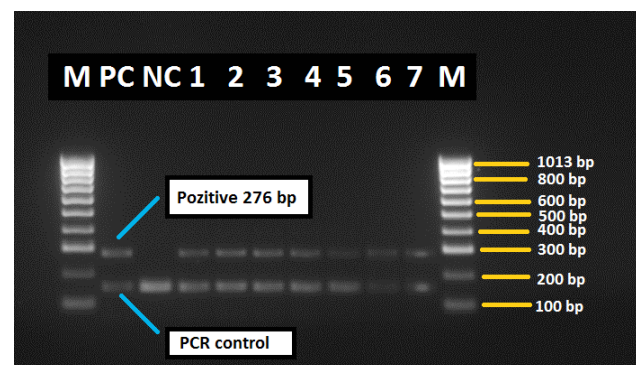


Figure 1. Analysis of PCR products by agarose gel electrophoresis M: 100 bp Marker; 1- 7: Positive samples; PC: Positive control; NC: Negative control.

Table 1. The biochemical parameter levels of healthy dogs and dogs naturally infected with *D. immitis*

Parameters	Healthy group (n=7) (X ± SE)	Dirofilariosis group (n=7) (X ± SE)	Reference values ^{a, b, c, d}
TSA (mg/dl)	8.25±0.92	11.23±2.65*	-
LSA (mg/dl)	2.00±0.90	4.02±1.13*	-
cTnI (µg/l)	0.09±0.04	1.49±0.49*	0.03-0.11b
CK-MB (µg/l)	0.60±0.14*	2.07±0.53*	0-0.64b
Total CK (u/l)	15.42±62.30	22.84±96.65	5-25a
AST (u/l)	43.59±9.76	80.39±16.70*	23-66a
ALT (u/l)	48.39±16.52	94.92±26.31*	21-102a
ALP (u/l)	60.86±21.84	118.18±34.96*	20-156a
LDH (u/l)	136.71±66.18	279.29±146.70*	45-233a
Total protein (g/l)	63.43±6.40	65.43±4.35	53-73a
Total bilirubin (µmol/l)	0.11±0.03	0.12±0.04	0-6.84a
Triglycerides (mg/dl)	61.85±9.73	97±12.16*	50-100a
Cholesterol (mg/dl)	202.85±28.35	140.14±17.78*	125-250a
VLDL (mg/dl)	11.97±2.2	20.31±3.08*	-
LDL (mg/dl)	61.91±5.05	133.57±28.54*	5-86c
HDL (mg /dl)	79.77±7.18	56.54±4.03*	49-165c
BUN (mg/dl)	26.14±12.60	60.00±31.12*	12-25a
Urea (g/dl)	31.10±11.97	51.89±5.19*	42.8-59.9a
Creatinine (mg/dl)	0.86±0.18	1.47±0.35*	0.5-1.5a
pH (-log H ⁺)	7.31±0.05	7.29±0.01	7.35-7.42d
pCO ₂ (mm/Hg)	41.84±4.00	39.49±3.65*	29.0-42.0d
pO ₂ (mm/Hg)	48.87±8.32	44.83±3.99	49.9-54.2d
HCO ₃ ⁻ (mm/L)	22.55±2.12	19.16±1.90*	22.2-22.4d
BE (mm/L)	0.73±1.70	-2.61±2.80*	-

*p<0.05 shows the significance between the parameters on the same row. a (Karagül et al., 2000), b (Ok et al., 2010), c (Loria et al., 2020), d. (Anonymous, 2020). Cardiac troponin I (cTnI), creatine kinase (CK), creatine kinase-myocardial isoenzyme (CK-MB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), carbon dioxide pressure (pCO₂), partial oxygen pressure (pO₂), bicarbonate (HCO₃⁻), base deficit (BE)

Table 2. The hematological parameter levels of healthy dogs and dogs naturally infected with *D. immitis*

Parameters	Healthy group (n=7) (X ± SE)	Dirofilariosis group (n=7) (X ± SE)	Reference values ^e
RBC (106/mm ³)	6.93±1.11	5.62±0.96*	6.0-9.0
Hb (g/dl)	18.11±2.74	17.72±2.45	10.5-20
Hct (%)	49.41±5.47	43.71±5.96	37-54
WBC (10 ³ /mm ³)	10.98±3.45	18.77±3.24*	6.0-17.0
Lymphocytes (%)	24.95±12.99	19.26±8.97	12-30
Monocytes (%)	10.33±6.92	6.44±4.53	3-10
Neutrophils (%)	56.65±24.31	66.04±13.76	60-75
Basophils (%)	0.13±1.34	0.23±1.12	0-1
Eosinophils (%)	2.75±1.34	7.34±2.42*	2-10

*p<0.05 shows the significance between the parameters on the same row. Reference, (Yılmaz, 2000), red blood cell (RBC), haemoglobin (Hb) hematocrit (Hct), white blood cell (WBC).

DISCUSSION

Age and gender of dogs are also important as well as environmental circumstances such as presence of mosquitoes and temperature in development of dirofilariosis caused by *D. immitis*. It has been reported

that elderly, male and stray dogs carry a higher risk for infection than younger, female and house dogs (Firat et al., 2005; Taylor et al., 2007). The male stray dogs aged 4-5 years old analyzed in the present study are consistent with the reports in the literature. High-

er infection risk in the elderly stray dogs can be explained by the higher risk of exposure to mosquitoes and longer prepatent period of parasites in these dogs (Balıkçı, 2005). The presence of the findings such as coughing and respiratory distress in 3 and 2 dogs, respectively, as well as 2 asymptomatic dogs in the clinical examination of the infected dogs in our study were consistent with clinical findings reported for that disease (Börkü et al., 1996; Taylor et al., 2007).

The excessive work load on the right ventricle leads to congestive heart failure, organ dysfunctions such as particularly heart and kidneys, and various grades of pulmonary embolism and pulmonary hypertension. Microfilarial diseases involve several findings such as changes in the blood gases, increased enzymatic activities in the liver, reduced hepatic function, proteinuria and uremia accompanied with the lesions in the lungs, heart, kidneys and liver (Atkins, 2005).

The heart is affected both functionally and morphologically in the dogs with dirofilariosis. *Dirofilaria immitis* adults leads to endothelial damage, myocardial infarction and arteriosclerosis by locating in the heart of the dogs (Pasca et al., 2012). The serum levels of cTnI (Gazyagci et al., 2011; Carretón et al., 2011; 2012; 2013; Yoon et al., 2017) and the enzymatic activities of CK (Voyvoda et al., 1996; Balıkçı, 2005; Meral et al., 2007; Sevimli et al., 2007; Kına, 2009) and CK-MB (Kına, 2009; Carretón et al., 2013) were detected to be significantly increased suggesting myocardial damage in the dogs with dirofilariosis. It was determined also in this study that serum cTnI level and enzymatic activity of CK-MB significantly increased in the dogs with dirofilariosis compared with the healthy group and that this increase was higher than the reference value. Differently from the mentioned reports, an insignificant increase (Sri-bhen et al., 1999) was determined in the enzymatic activity of total CK in present study. The increased levels of cTnI and CK-MB enzymatic activity may be a consequence of myocardial damage caused by adult forms of *D. immitis* that localized in the right ventricle of the dog heart.

Increased activities of the liver enzymes are resulted from the ascites and passive congestion of the liver developing secondary to right-sided heart failure in the dogs with dirofilariosis (Tabrizi, 2012). Consistently with the studies that have reported increased liver enzymes in the dogs with dirofilariosis (Goggin et al., 1997; Balıkçı, 2005; Niwetpathomwat et al., 2006; 2007; Sevimli et al., 2007; Kına, 2009; Aslan et

al., 2010; Ranjbar-Bahadori et al. 2010; Borthakur et al., 2011; Tabrizi, 2012; Atyha and Alani, 2017), we have encountered in the present study that enzymatic activities of the several enzymes such as AST, ALT, ALP and LDH significantly increased in the group of infected dogs compared with the healthy dogs. Additionally, the increased enzymatic activity levels of AST and LDH were over the reference values. That increased activity levels of these liver enzymes may be caused by the microfilariae detected in the blood samples or the adult parasites that localize in the liver and cause liver injury. Beside that, increased enzymatic activities of AST and LDH may support the presence of cardiovascular injury caused by *D. immitis* in the infected dogs.

In the present study, insignificant elevations were encountered in the levels of total protein (Balıkçı, 2005; Aslan et al., 2010; Ranjbar-Bahadori et al., 2010; Borthakur et al., 2011) and total bilirubin (Sevimli et al., 2007; Borthakur et al., 2011) in the dogs with dirofilariosis supporting the reports of the previous studies. In contrast to that result, significantly increased levels of total protein (Sevimli et al., 2007) and total bilirubin (Goggin et al., 1997; Tabrizi, 2012) were also found.

The development of atherosclerotic cardiovascular disease depends on the state of cholesterol as LDL and HDL in the bloodstream. The high levels of VLDL and LDL and low level of HDL create predisposition for the cardiovascular diseases (Karagül et al., 2000). Jacobs et al. (1992) have reported elevated levels of triglycerides and cholesterol besides the decreased level of HDL in the dogs with dirofilariosis. Another study has reported that the levels of triglycerides, VLDL and LDL increased whereas the levels of cholesterol and HDL decreased (Kına, 2009). That study has also detected that serum triglyceride, VLDL and LDL levels significantly increased whereas HDL and cholesterol levels significantly decreased in the group with dirofilariosis. Additionally, the increased level of LDL was higher than the reference value. The high levels of triglycerides and VLDL may be resulting from lipolysis that stimulates the production of triglycerides and VLDL cholesterol depending on glycolysis. The low level of the cholesterol may be a consequence of impaired cholesterol synthesis in the hepatocytes due to the liver damage caused by the parasite. On the other hand, Kına (2009) has suggested that the high level of LDL may be resulting from the reduced entry of LDL into the cells from the plasma due to the suppression of the LDL receptors caused by

D. immitis. The low level of HDL may be depending on the reduction in the cholesterol synthesis during the disease. Besides, high levels of VLDL and LDL beside low level of HDL may have contributed to the development of the cardiopulmonary diseases due to dirofilariosis. Differently from our results, no difference was encountered in terms of cholesterol level (Aslan et al., 2010; Ranjbar-Bahadori et al., 2010).

The blood levels of urea, creatinine, BUN and uric acid are usually tested as the indicators of the renal function. Blood urea nitrogen (BUN) and serum creatinine determinations are related to the amount of nitrogenous residues removed by the kidney. Serum creatinine and urea analysis are used as a kidney function test. BUN may increase in cases such as bleeding, fever, corticosteroid administration, burns, hunger, infection, tetracycline administration, decrease in protein intake, severe liver failure (Karagül et al., 2000). Kidney injuries such as glomerulonephropathy, glomerulosclerosis, chronic interstitial nephritis and amyloidosis have been identified in the dogs infected by *D. immitis* (Niwetpathomwat et al., 2007). It has been stated that formation of immune complexes by accumulation of the microfilarial antigens on the glomerular basal membrane causes glomerulonephropathy (Balıkçı, 2005). It was determined in this study supporting the findings of many researchers that the levels of serum urea (Şahal et al., 1997; Balıkçı, 2005; Atyha and Alani, 2017), creatinine (Börkü et al., 1996; Niwetpathomwat et al., 2006; 2007) and BUN (Niwetpathomwat et al., 2006; 2007; Sevimli et al., 2007; Tabrizi, 2012) significantly increased in the dogs with dirofilariosis compared with the healthy dogs. At the same time, the increase in the level of BUN was found to be higher than the reference value. Differently from the results of our study, some studies demonstrated that no difference was present in terms of urea, creatinine and BUN levels (Ranjbar-Bahadori et al., 2010; Atyha and Alani, 2017).

Parasitic agents leads to anemia in the host by causing decreased circulatory erythrocyte count or decreased erythrocyte count per unit volume of blood or reduced hemoglobin concentrations (Atyha and Alani, 2017). The dogs with dirofilariosis have various grades of anemia as a consequence of the changes in the RBC, Hb and Hct levels (Niwetpathomwat et al., 2007; Sevimli et al., 2007). Although, its etiological mechanism is not exactly clear yet, anemia was reported to be resulting from the haemolysis of the circulatory erythrocytes with increased fragility due to

the obstruction of bloodstream depending on the presence of numerous adult infectious agents in the veins during the infection period (Atyha and Alani, 2017). The related studies have encountered significant (Kitagawa et al., 1993; Borthakur et al., 2011) and insignificant (Şahal et al., 1997) declines in the RBC, Hct and Hb levels of the infected dogs. In this study, a significant decline in the RBC level (Balıkçı, 2005; Sarıtaş et al., 2005; Ranjbar-Bahadori et al., 2010; Atyha and Alani, 2017) and insignificant declines in the Hct and Hb levels (Ranjbar-Bahadori et al., 2010; Atyha and Alani, 2017) in the dogs with dirofilariosis compared with the healthy group (Ranjbar-Bahadori et al., 2010; Atyha and Alani, 2017).

It was determined in this study that WBC level significantly increased in the dogs with dirofilariosis compared with the healthy dogs (Şahal et al., 1997; Balıkçı, 2005; Sarıtaş et al., 2005; Niwetpathomwat et al., 2007; Sevimli et al., 2007) and this increase was over the reference value. The development of leukocytosis in the infected dogs may be resulting from the reduced resistance against the other infectious diseases and secondary infections such as pneumonia and nephritis. In contrast to this result, Ranjbar-Bahadori et al. (2010) and Atyha and Alani (2017) have determined that no difference was encountered in the level of WBC.

Although, increased eosinophil and basophil counts are considered as the general characteristic findings for dirofilariosis according to the differential blood count, nevertheless, it has been reported that these findings may be obtained also in the infections caused by other intestinal parasites and were not specific for dirofilariosis (Şahal et al., 1997). In contrast to the studies which suggested that no difference was encountered in the eosinophil counts of the dogs with dirofilariosis (Ranjbar-Bahadori et al., 2010; Atyha and Alani, 2017), a significant increase was found in the eosinophil count in this study. The detection of the increased eosinophil count confirms the conclusion that eosinophil may have a supportive diagnostic value in dirofilariosis. In our study, the changes in the neutrophil, basophil, lymphocyte and monocyte counts were found insignificant. Differently from this result, significant increases and/or decreases were determined in the neutrophil, basophil, lymphocyte and monocyte counts in the other studies (Şahal et al., 1997; Balıkçı, 2005; Niwetpathomwat et al., 2007; Sevimli et al., 2007).

In the severely infected dogs with dirofilariosis; thoracic radiography displays right ventricular enlargement, prominent major pulmonary arteries,

edema in the caudal lung lobes, haemorrhage and parenchymal impairments (Atkins, 2005; Meral et al., 2007). The pathological impairments in the lungs lead to respiratory distress and coughing in the infected dogs (Börkür et al., 1996). Respiratory distress causes impairments in the pulmonary gas exchange functions (Saritaş et al., 2005). The significantly low levels of arterial and venous pO_2 and pCO_2 levels in the blood gas analysis are the sensitive criteria in the diagnosis of pulmonary embolism in the dogs infected with dirofilariosis (Kitagawa et al., 1993). In this study, significant declines were detected in the levels of pCO_2 , HCO_3 and BE in the dogs with dirofilariosis compared with the healthy dogs. Besides, reduction in the level of HCO_3 was found lower than the reference value. An insignificant decline was encountered in the levels of pH and pO_2 . Hypoxemia develops in the clinical picture of the cardiopulmonary disorders in the dogs, the body, under this circumstance, produces buffering by accelerating respiration to increase the alveolar O_2 and excreting HCO_3 to balance alkalosis caused by the decreased levels of CO_2 in the blood (Balıkçı, 2005). In this study, significantly low levels of pCO_2 , HCO_3 and BE in the infected dogs indicate the buffering capacity of the organism. In the infected dogs, the low levels of HCO_3 and BE despite absence of a difference in blood pH compared with the healthy group points out the development of metabolic acidosis in dirofilariosis.

Sialic acids are the terminal components of the oligosaccharide units of the glycoconjugates (glycoproteins, glycolipids, proteoglycans). Sialic acids have an important impact with respect to host recognition by the pathogens, intercellular interactions, hormone-receptor relationships and protection of the cell from proteolysis due to carrying negative charge. Sialic acids are diffusely found on the outer surface of the biological membranes. The changes in the serum SA levels demonstrate the cellular or tissue damages. The monitoring of these changes provides useful data in diagnosis, differential diagnosis and prognosis of many diseases (Sydow et al., 1988; Taşkın, 2017). No study that aimed at the levels of TSA and LSA in the dogs with dirofilariosis was found in the literature review. Increased levels of sialic acid were detected in the distemper disease of dogs (Altıntaş et al., 1989), hydrocortisone and dexamethasone injections in the dogs with distemper (Engen, 1971) and those with tumoral and non-tumoral different diseases (Thougaard et al., 1998). Another study that investigated the correlation between TSA and serum α 1-acid glycoprotein (AGP) levels identified a positive correlation between AGP and TSA concentrations (Thougaard et

al., 1999) in the dogs with tumors.

In this study, serum TSA level was found significantly higher in the dogs with dirofilariosis compared with healthy dogs. That increase may be resulting from the increased synthesis of acute phase proteins (APPs) that contain sialic acid residues at the terminal position of the lateral oligosaccharide chain. Because, it has been reported that the levels of APPs increase both to meet the increased need for oxygen and as a response to the potential tissue damage in the dogs with dirofilariosis (Carretón et al., 2014). In our study, LSA levels of the dogs with dirofilariosis were detected to be significantly higher than the healthy dogs. Approximately 85-90% of the sialic acid are bound to the proteins. The remaining 10-15% portion is bound to the lipids. The increase in the level of APPs leads to only increased level of sialic acid fraction bound to the proteins in dirofilariosis, however, shows no impact on the sialic acid fraction bound to the lipids. Thus, increased level of serum LSA encountered in dirofilariosis may a consequence of the increased synthesis of lipoprotein in the liver that contains sialic acid. Accordingly, increased levels of VLDL and LDL were found to increase in our study in the dogs with dirofilariosis. Besides, the increase in the level of LDL was determined to be higher than reference value. On the other hand, the sialic acid residues released from oligosaccharides in the damaged cell and cell membranes due to the increased oxidative stress (Aslan et al., 2010) may be responsible for the increased levels of TSA and LSA in dirofilariosis.

CONCLUSION

We concluded in the light of the present study data that statistically significant differences were identified between the dogs with dirofilariosis and healthy dogs in terms of sialic acid levels and certain biochemical, venous blood gas and hematological parameters. On the other hand, the present study is the first to investigate the serum sialic acid levels in the dogs with dirofilariosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This study was supported by the Van Yuzuncu Yil University Scientific Research Projects Fund and registered with project No: TYL-2019-8057. Moreover, it was taken from the master thesis entitled "Dirofilariosisli Köpeklerde Serum Total ve Lipide Bağlı Sialik Asit İle Hematolojik-Biyokimyasal Parametre Düzeyleri".

REFERENCES

- Altıntaş A, Kurtkede A, Fidancı UR, Bökü MK (1989) Köpek gençlik hastalığında (distemper) serum sialik asit ve protein düzeylerinin klinik önemi. Ankara Univ Vet Fak Derg 36 (1) : 154-164.
- Anonymous (2020) <http://vetlab.com/Canine%20and%20Feline%20Normal%20Values.pdf>.
- Aslan O, Yıldırım A, Kanbur M, Altınordu S (2010) Detection of some biochemical and lipid peroxidation parameters in *Dirofilaria immitis* infected dogs. J Anim Vet Advan 9 (5) : 954-957.
- Atkins C (2005) Canine heartworm disease. In "Textbook of Veterinary Internal Medicine", Ettinger SJ, Feldman EC, editors. 6 th ed., St. Louis. Elsevier 1118- 1136.
- Atyha A H, Alani DAA (2017) Study the effects of naturally acquired canine dirofilariosis on some hematological and biochemical parameters. Iraqi J Vet Sci 41 (1) : 104-108.
- Balıkçı E (2005). Dirofilariosisli köpeklerin bazı klinik, hematolojik, biyokimyasal ve elektrokardiyogram bulguları. FÜ Sağlık Bil Derg 19 (1) : 43-48.
- Borthakur SK, Ali MA, Patra G (2011). Clinical, haematological and biochemical studies on *Dirofilaria immitis* in dog. J Vet Parasitol 25: 63-66.
- Bökü MK, Kurtkede A, Azizoğlu D, Kilit M (1996) *Dirofilaria immitis* ile doğal enfekte köpeklerde thiacetarsamide sodium uygulamaları. Ankara Univ Vet Fak Derg 43 (2) : 247-256.
- Carretón E, Corbera JA, Juste MC, Morchón R, Simón F, Montoya-Alonso JA (2011) *Dirofilaria immitis* infection in dogs: cardiopulmonary biomarker levels. Vet Parasitol 176 (4) : 313-316.
- Carretón E, Grandi G, Morchón R, Simón F, Passeri B, Cantoni AM, Kramer L, Montoya-Alonso JA (2012) Myocardial damage in dogs affected by heartworm disease (*Dirofilaria immitis*) : immunohistochemical study of cardiac myoglobin and troponin I in naturally infected dogs. Vet Parasitol 189 (2-4) : 390-393.
- Carretón E, Morchón R, González-Miguel J, Juste MC, Simón F, Montoya-Alonso JA (2013). Utility of cardiac biomarkers during adulticide treatment of heartworm disease (*Dirofilaria immitis*) in dogs. Vet Parasitol 197 (1-2) : 244-250.
- Carretón E, Morchón R, Simón F, Juste MC, Méndez JC, Montoya-Alonso JA (2014). Cardiopulmonary and inflammatory biomarkers in the assessment of the severity of canine dirofilariosis. Vet Parasitol 206 (1-2) : 43-47.
- Engen RL (1971). Serum sialic acid values in dogs with canine distemper. Amer J Vet Res 32 (5) : 803-804.
- Ertekin A, Keles I, Ekin S, Karaca M, Akkan HA (2000) An investigation on sialic acid and lipid-bound sialic acid in animals with blood parasites. Van Vet J 11: 34-35.
- Erdogan H M, Karapehlivan M, Citil M, Atakisi O, Uzlu E, Unver A (2008) Serum sialic acid and oxidative stress parameters changes in cattle with leptospirosis. Vet Res Commun 32:333-339.
- Esmailnejad B, Tavassoli T, Asri-Rezaei S, Dalir-Naghadeh B, Abtahi Froushani SM, Arjmand J, Golabi M (2014) Serum concentration of sialic acids in naturally occurring ovine babesiosis. Trop Anim Health Prod 46: 1217-1221.
- Fırat İ, Gülçubuk A, Çetinkaya H (2005) İstanbul'da üç köpekte *Dirofilaria immitis* olgusu. İÜ Vet Fak Derg 31 (1) : 187-193.
- Gazaygı S, Doğru MT, Yağci BB (2011) Cardiac troponin I levels in dogs with dirofilariosis and without dirofilariosis. Asian J Anim Vet Adv 6: 738-743.
- Gioia G, Lecová L, Genchi M, Ferri E, Genchi C, Mortarino M (2010) Highly sensitive multiplex PCR for simultaneous detection and discrimination of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood. Vet Parasitol 172 (1-2) : 160-163.
- Goggin JM, Biller DS, Rost CM, DeBey BM, Ludlow CL (1997) Ultrasonographic identification of *Dirofilaria immitis* in the aorta and liver of a dog. JAVMA 210: 1635-1637.
- Jacobs RM, Lumsden JH, Vernau W (1992). Canine and feline reference values. In "Current Veterinary Therapy XI Small Animal Practice". Philadelphia.
- Karagül H, Altıntaş A, Fidancı UR, Sel T (2000) Klinik biyokimya. Ankara: Medisan Yayınevi.
- Karapehlivan M, Atakisi E, Citil M, Kankavi O, Atakisi O (2007) Serum sialic acid levels in calves with pneumonia. Vet Res Commun 31: 37-41.
- Karapehlivan M, Uzlu E, Kaya N, Kankavi O, Ural K, Citil M (2007) Investigation of some biochemical parameters and the antioxidant system in calves with dermatophytosis. Turk J Vet Anim Sci 31: 85-89.
- Katopodis N, Hirschaut Y, Geller NL, Stock CC (1982) Lipid-associated sialic acid test for the detection of human cancer. Cancer Res 42 (12) : 5270-5275.
- Kına O (2009) *Dirofilaria immitis* 'li köpeklerde Paraoksonaz Aktivitesi ve Lipid Profilinin Belirlenmesi. Mustafa Kemal Üniversitesi, Yüksek lisans tezi, Hatay.
- Kitagawa H, Yasuda K, Sasaki Y (1993) Blood gas analysis in dogs pulmonary heartworm disease. J Vet Med Sci 55 (2) : 275-280.
- Loria AD Dattilo V, Santoro D, Guccione J, De Luca A, Ciarabella P, Pirozzi M, Laccino E (2020) Expression of serum exosomal miRNA 122 and lipoprotein levels in dogs naturally infected by *Leishmania infantum*: A preliminary study. Animals 10 (1) : 100.
- Meral Y, Bakırel U, Çakıroğlu D (2007) Bir köpekte kalp kurdu hastalığının (*Dirofilaria immitis*) ekokardiyografik teşhisi. JIVS 3: 1-10.
- Niwetpathomwat A, Assarasakorn S, Techangamsuwan A, Suvarnavibhaja S, Kaewthamasorn M (2006) Canine dirofilariosis and concurrent tick-borne transmitted diseases in Bangkok, Thailand. Comp Clin Pathol 15 (4) : 249-253.
- Niwetpathomwat A, Kaewthamasorn M, Tiawisirisup S, Techangamsuwan S, Suvarnavibhaja S (2007) A retrospective study of the clinical hematology and the serum biochemistry tests made on canine dirofilariosis cases in an animal hospital population in Bangkok, Thailand. Res Vet Sci 82 (3) : 364-369.
- Ok M, Öztürk AS, Er C (2010) Üç köpekte konjestif kalp yetmezliği. Eurasian J Vet Sci 26 (1) : 57-62.
- Pasca SA, Acatrinei D, Oprean OZ, Lazar M (2012) Vascular, hepatic and renal lesions by *Dirofilaria immitis* invasion in dogs. Arq Bras Med Vet Zootec 64: 841-846.
- Ranjbar-Bahadori S, Mohri M, Helan JA, Jamshidi K, Kashefnejad M (2010) Clinico-Pathologic Evaluation of the Canine Heartworm Infestation. Res J Parasitol 5: 90-98.
- Sarıtaş ZK, Akin F, Şahal M, Öcal N (2005) Open heart surgery applications in dogs suffering from natural infection of *Dirofilaria immitis*. Turk J Vet Anim Sci 29: 713-721.
- Sevimli KF, Kozan E, Bülbül A, Birdane MF, Köse M, Sevimli A (2007) *Dirofilaria immitis* infection in dogs: unusually located and unusual findings. Parasitol Res 101:1487-1494.
- Sribhen C, Kasemsant N, Kaewmukul S, Sribhen K (1999) Blood chemistry profile and cardiac troponin T concentration in Thai stray dogs infected with heartworms. Kasetsart J Nat Sci 33: 251-257.
- Sydow G, Wittmann W, Bender E, Starick E (1988) The sialic acid content of the serum of cattle infected with bovine leukosis virus. Arch Exp Vetmed 42: 194-197.
- Şahal M, Özlem M, Tanyel B, Öcal N, Sel T (1997) Köpeklerdeki dirofilariosis olgularında kan, idrar ve abdominal sıvıda biyokimyasal değişiklikler. Ankara Üniv Vet Fak Derg 44: 267-276.
- Tabrizi BA (2012) Evaluation of calcium, phosphorus and alkaline phosphatase in *Dirofilaria immitis* infection in dogs. World J Zool 7 (1) : 79-82.
- Taşkın E (2017) Ellajik Asitin Miyokart İnfarktüsü Oluşturulmuş Ratlarda Sialik Asit Düzeyleri ve Lipit Profili Üzerine Etkisi. Van Yüzüncü Yıl Üniversitesi, Yüksek lisans tezi, Van.
- Taylor MA, Coop RL, Wall RL (2007) Veterinary parasitology. 3. West Sussex: Blackwell Publishing.
- Thougaard AV, Hellmen E, Jensen AL (1998) Total serum sialic acid is a general disease marker rather than a specific tumour marker in dogs. J Vet Med A 45 (8) : 471-479.
- Thougaard AV, Hellmen E, Pedersen HD, Jensen AL (1999) Correlation between alpha 1-acid glykoprotein and total sialic acid in serum from dogs with tumours. J Vet Med A 46: 231-237.
- Voyvoda H, Sekin S, Karaca M (1996) Therapeutic efficacy of ooramectin and levamisole in *Dirofilaria immitis* infected dogs and changes of some blood parameters. YYÜ Vet fak Derg 7 (1-2) : 26-34.
- Yılmaz B (2000). Fizyoloji. Ankara: Feryal Matbaacılık.
- Yoon WK, Kim YW, Suh SL, Choi R, Lee SG, Hyun C (2017) Evaluation of cardiopulmonary and inflammatory markers in dogs with heartworm infection during treatment with the 2014 American Heartworm Society recommended treatment protocol. Parasit Vectors 10 (2) : 535.