EURASIAN JOURNAL OF HEALTH SCIENCES
ISSN 2651-3501

Eurasian JHS 2021; 4(1): 47-51

Research Article/ Araştırma Makalesi

Correspondence: Osman KINA
E-mail: osi.kina@hotmail.com

Determination Of Paraoxonase Activity as a Biomarker in Dogs with Dirofilariosis

Osman KINA [1,a], Tünay KONTAŞ AŞKAR [2,b], Murat GÜZEL[3,c]

[1] Department of Biochemistry, Institute of Health, University of Mustafa Kemal, Hatay, TURKEY
[2] Department of Dietetics and Nutrition, Faculty of Health Sciences, Çankırı Karatekin University, Çankırı, TURKEY
3 Department of Internal Medicine, Faculty of Veterinary Medicine, University of 19 Mayıs, Samsun, TURKEY
ORCIDS:[a] 0000-0001-8328-1693; [b] 000-0002-1121-8799; [c] 0000-0002-8937-5428

ABSTRACT

Dirofilariosis is a common asymptomatic zoonotic disease in which parasite adults are primarily found in the right-side of the heart and pulmonary arteries. Interest about the role of paraoxonase (PON) activity in cardiovascular diseases is increasing day by day. The aim of the present study is to define the changes in lipid prophile and enzyme activities (AST, ALT, LDH, CK, CK-MB) with paraoxonase activity in dogs with dirofilariosis. 15 healthy dogs and 30 dogs with dirofilariosis (antigen positive for adult D. immitis and negative for microfilariae) between 4-6 years of old age were enrolled in this study. Significantly higher serum trigliseride ($p<0.01$), very low-density lipoprotein ($p<0.05$), and low-density lipoprotein ($p < 0.001$) levels and aspartate aminotransferase ($p<0.001$), alanin aminotransferase ($p<0.001$), creatine kinase ($p<0.001$), creatine kinase MB fraction ($p<0.001$), lactate dehydrogenase ($p<0.01$) activities were found in dogs with dirofilariosis compared to healthy dogs. On the other hand, significantly lower total cholesterol ($p <0.001$) and high-density lipoprotein ($p < 0.01$) levels were found in dogs with dirofilariosis. Also, serum basal paraoxonase ($p<0.01$) activities of dogs with dirofilariosis were significantly lower than in the control group. In conclusion, serum bazal paraoxonase activity can be used as a marker of cardiac damage, such as LDH and CK-MB, in dogs with dirofilariosis.

Key words: Dirofilariosis, Dog, Paraoxonase, Lipid profile

Dirofilariosisli Köpeklerde Bir Biyomarker Olarak Paraoksonaz Aktivitesinin Belirlenmesi

ÖZET

Dirofilariosis, sivrisineklerle bulaşan özellikle kardiopulmoner hastalıklara yol açan dirofilaria türlerinin neden olduğu zoonoz bir paraziter hastalıktır. Bu çalışmada D. immitis ile enfekte köpeklerde paraoksonaz (PON) aktivitesi yanında, diğer enzim aktivite (AST, ALT, LDH, CK, CK-MB) ile lipid profili (trigliserit, total kolesterol, çok düşük dansiteli lipoprtotein, Düşük dansiteli lipoprotein, Yüksek Dansiteli lipoprotein) değişimlerinin ortaya konulması amaçlanmıştır. Çalışma, 4-6 yaş arası 30 sağlıklı ve 30 dirofilariosisli köpekde gerçekleştirilmiştir. Dirofilariosisli köpeklerde serum aspartat aminotransferaz ($p < 0.001$), alanin aminotransferaz ($p < 0.001$), kreatin kinaz ($p < 0.001$) ve laktat dehidrogenaz ($p < 0.01$) aktivitesi ile kreatin kinaz-MB izoenzim ($p < 0.001$) aktivitesi, sağlıklı köpeklere göre yüksek bulunmuştur. Serum bazal paraoksonaz ($p < 0.01$) ise sağlıklı köpeklere göre düşük bulunmuştur. Yapılan lipid profili analizlerinde ise, dirofilariosisli köpeklerde serum trigliserid ($p < 0.01$), çok düşük dansiteli lipoprotein ($p < 0.05$) ve düşük dansiteli lipoprotein ($p < 0.001$) düzeyleri sağlıklı köpeklere göre yüksek bulunurken, serum total kolesterol ($p < 0.001$) ve yüksek dansiteli lipoprotein ($p < 0.01$) aktivitesi ise düşük bulunmuştur. Araştırmadan elde edilen verilerin ışığında, serum bazal paraoksonaz aktivite düzeyi ölçümlerinin, dirofilariosisli köpeklerde meydana gelen kardiak hasarın belirlenmesinde, LDH ve CK-MB gibi yararlı bir indikatör olabileceği kanısına varılmıştır.

Anahtar kelimeler: Dirofilariosis, Köpek, Paraoksonaz, Lipid profili

Received:08.01.2021 Accepted: 25.01.2020

.

INTRODUCTION

Dirofilariosis is a zoonotic disease of canids which is caused by infection with the nematode *Dirofilaria immitis*. The disease is transmitted by mosquitoes and particulary causes cardiopulmonary diseases in their hosts (Montaya et al. 2006; Sevimli et al.2007). The distribution of Dirofilariosis is worldwide, esp. in tropical, subtropical and temperate regions (Sarıtaş et al 2005).

Dirofilaria immitis is the causative agent of canine heartworm disease, and the parasite adults are primarily found in the right-side of the heart and the pulmonary arteries of dogs (Çakıroğlu ve Meral 2007). The main hosts of *D.immitis* are dogs. Mosquitoes are the intermediate hosts of the Dirofilaria species. These species can also be transmitted to humans causing human pulmonary disease (HPD) which is responsible for the development of benign pulmonary nodules when people are bitten by culicide mosquitoes (Kartashev et al. 2011).

Dirofilariosis is rarely observed in dogs under the age of one. Dogs between the ages of 4–7 compose the most vulnerable group (McCall et al. 2004; Öncel ve Vural 2007). Dirofilariosis generally proceeds as clinical and subclinical. Despite the presence of microfilers in the peripheral blood in most of subclinical events, symptoms in dogs may be absent (Vencoa et al. 2014).

There is growing interest in the role of paraoxonase (PON, aryldialylphosphatase, EC 3.1.8.1) in cardiovascular disease. PON is a calcium-dependent esterase and it hydrolyse the aromatic carboxylic esters and organophosphorus insecticides (Juretic et al. 2001). Also PON has multifunctional roles in various biochemical pathways like protection against oxidative stress, contribution to innate immunity, and regulation of cell proliferation/apoptosis. PON seems to be the "hunters" of peroxynitrite-generated oxidized phospholipids which caused atherosclerosis or thrombogenesis (Martinelli et al. 2013).

Heartworm disease has importance in veterinary medicine and public health. Therefore diagnosis and screening of the disease are essential for control of the disease (Voyvoda et al. 2004; Carretóna et al. 2014). Diagnosis of Dirofilaria infection is made by clinical symptoms, native examination into peripheral blood, microfilariae detection with the Knott test and serological tests (Carretóna et al. 2014; Meral ve Bakırel 2007). In order to prevent, economic losses in countries, a determination of the porter animals with specific biochemical parameters is very important. The aim of the present study is to determine whether the measurement of paraoxonase activity may have a diagnostic or prognostic value in canine dirofilariosis, and whether change in paraoxonase activity is related with the arterial damage.

MATERIALS AND METHODS

A total of 60 big breed dogs between 4-6 years old, comprised of 30 clinically healthy and 30 dogs with dirofilariosis (antigen positive for adult *D. immitis* and negative for microfilariae) from Hatay, were enrolled in the present study. The animals were added to the proper groups after clinical examination, modified Knott test and antigen-ELİSA assay.

Blood samples were collected from the cephalic vein to the lithium heparine and silicone gel-coated test tubes. Serum/plasma samples were separated by centrifugation at 2500 rpm for 10 min at room temperature and kept at -80°C until processing. Samples were analyzed for lipid profile (triglycerides, total cholesterol, VLDL, HDL and LDL), and enzyme activities (AST, ALT, LDH, CK, CK-MB) besides paraoxonase (PON1).

Parasitological Analysis

The presence of microfilariae in peripheral blood was detected by the Knott test. After centrifugation of blood samples with 2 % formalin mixture (5 min, 1500 rpm), the sediment stained with 0.5 % methylene blue. Stained sediment was examined under a microscope at 400x magnification for the presence of microfilariae (15). The presence of adult D. immitis circulating antigens in blood were determined with a commercial Elisa kit (DiroCHEK®, Synbiotics, San Diego, USA) spesific for D. immitis. According to results of the Knott test and antigen-ELİSA assay, the dogs which have adult D. immitis parasite circulating antigen and no microfilaria in the blood were selected for the patient group.

Biochemical Analysis

In the present study,PON enzyme activity was determined with a method by Eckerson et al.(16). The principal of the method based is the enzymatic hydrolyses of paraoxon by PON enzyme into diethylphosphate and p-nitrophenol. Determination of the other ezyme activities (LDH, ALT, AST, CK, and CK-MB) in blood samples were performed in the Roche Modular System autoanalyzer by using Roche Diagnostic's reagent. Triglyceride, total cholesterol, and HDL analyses were also performed in the Roche Modular System autoanalyzer. The calculation of LDL and VLDL cholesterol levels were performed by Friedewald formulae (Friedewald et al., 1972)(17).

LDL = Total cholesterol – [(HDL) + (Triglycerides /5)]

VLDL = Triglycerides /5)

Statistical Analysis

For statistical evaluation of the results, SPSS 13.0 for windows packet program was used. Statistical differences between groups were evaluated by using the student t-test. Data were presented as mean ± standard error (± SE), and $P< 0.05$ was considered as significant.

RESULTS

The activity levels of PON, LDH, AST, ALT, CK and CK-MB in both the dogs with dirofilariosis and the healthy controls are shown in Table 1.

Table 1. The activity levels of PON1, AST, ALT, LDH, CK and CK-MB in the dogs with dirofilariosis and the healthy group.

Parameter	Control Group	Dogs with
Dirofilariosis	(n=30)	(n=30)
ALT (IU/L)	35,4 ± 7,6	70,7 ± 12,5^{a}
AST (IU/L)	29,8 ± 6,1	63,4 ± 13,7^{a}
LDH (IU/L)	197,7 ± 26,3	272,3 ± 32,8^{b}
CK (IU/L)	92,4 ± 28,6	158,5 ± 41,5^{a}
CK-MB (IU/L)	18,5 ± 5,7	54,1 ± 22,5^{a}
Basal PON1 (IU/L)	53,9 ± 15,4	33,2 ± 12,1^{b}

Data are expressed as mean ± standart error of the mean values.

a,b Significantly different from the control group (p<0.001, p<0.01 and p<0.05, respectively).

Serum LDH, AST, ALT, CK and CK-MB activity levels were significantly higher (P<0.001, P<0.001, P<0.01, P<0.001 and P<0.001, respectively) in the dogs with dirofilariosis in comparison to the control group. Whereas, PON1 activity levels were lower (P<0.01) than those of the control group.

In the study we also determined the lipid profile of dogs with dirofilariosis. Table 2 shows the changes in lipid profile (triglyceride, total cholesterol, VLDL, LDL, HDL) between the control group and the dogs with dirofilariosis. The dogs with dirofilariosis exhibited a significant increase in plasma triglyceride ($p < 0.001$), VLDL levels ($p < 0.01$), and LDL levels ($p < 0.001$), compared to the healthy dogs. On the other hand, the patient group had significantly lower total cholesterol ($p < 0.001$), and HDL levels ($p < 0.01$), when compared to the healthy controls.

Table 2. The changes in lipid profile between the healthy group and dogs with dirofilariosis.

Parameter	Control Group	Dogs with
Dirofilariosis	(n=30)	(n=30)
Triglyceride (mg/dl)	55,4 ± 16,3	72,9 ± 19,6^{b}
Total cholesterol (mg/dl)	170,6 ± 25,1	137,0 ± 18,2^{a}
VLDL (mg/dl)	9,2 ± 0,8	11,6 ± 1,3
LDL (mg/dl)	78,7 ± 9,2	103,4 ± 11,7^{a}
HDL (mg/dl)	81,7 ± 15,4	56,3 ± 8,6^{b}

Data are expressed as mean ± standart error of the mean values.

a,b Significantly different from the control group (p<0.001, p<0.01 and p<0.05, respectively).

DISCUSSION

In dogs with dirofilariosis, organs such as the heart, lungs, liver and kidneys are affected both functionally and morphologically in various rates. But in the late stage of Heartworm infection can cause the compliance of the pulmonary arteries and right-sided heart failure (Vencoa et al. 2014; Sarkar et al. 1978). In veterinary medicine, cardiac biomarkers are important for the early detection of cardiopulmonary diseases, the prognosis of disease and the efficiency of treatment (Vencoa et al. 2014). The determination of LDH, CK, CK-MB, AST and ALT enzyme activities which have great importance on diagnosing the disease in both human and dogs were determined in this study in order to determine heart and liver damage in dogs with dirofilariosis.

It was mentioned in the study that serum LDH, CK, CK-MB, AST and ALT enzyme activity volumes significantly increase as a result of cardiopulmonary lesions in dogs with dirofilariosis (Song et al. 2003; Bakırel ve Güneş 2009). High serum LDH, CK, CK-MB, AST and ALT enzyme activity volumes in dogs with dirofilariosis indicate that adult *D. immitis* nematode causes cardiovascular damage by settling in the right ventricular and pulmonary artery in dogs (Çakıroğlu ve Meral 2007). Besides, it was notified that increases of liver enzyme activities in dogs with dirofilariosis is due to ascites and passive congestion which develop due to right-sided heart failure in the disease.

It was mentioned that adult *D.immitis* cause endothelial damage, myocardial infarction and arteriosclerosis by settling in the right ventricular and pulmonary artery in dogs with dirofilaria disease (Bakırel ve Güneş 2009; Jarvik et al. 2003 It was mentioned that PON1 activity determination is the best indicator for diagnosis of vascular diseases. And reduced PON1 activity appears to be associated with increased cardiovascular risk (Martinelli et al. 2013). Although it was determined that PON1 activity decreases in many diseases of humans and animals such as ulcerative colitis (Baskol et al. 2006), reumatoid arthritis (Altındağ et al. 2007) and acute purpura (Ece et al. 2007), Behcet disease, atherosclerosis, coronary heart disease, myocardial infarction, hypercholesterolemia and diabetes (Farid ve Horii 2008; Efrat et al. 2009), there is no information about the PON1 activity levels in dogs with dirofilariosis. The finding of this study (Farid ve Horii 2008) showed that serum PON1 activity decreased in rats with N. brasiliensis infection. Reduced PON activity appears to be associated with increased cardiovascular risk. In the present study, the serum PON1 activity in dogs with dirofilariosis were found to be lower than in healthy dogs ($p<0.001$). Decrease of PON1 enzyme activity levels in dogs with dirofilariosis in the present study may be related with the decrease of hepatic PON1 production and the use of this antioxidant enzyme for the prevention of oxidative damage that is caused by adult parasites in the heart. PON1 also has multiple enzymatic activities, including esterase and lactonase.

In this study serum total cholesterol, triglyceride, VLDL, LDL and HDL cholesterol levels of dogs with dirofilariosis were also measured in relation with PON1. HDL-associated PON1 has functions in the control of oxidative stress and inflammation. The findings of these studies (Kitagawa et al. 1981; Jacobs et al. 1992) showed that serum triglyceride, free cholesterol, LDL cholesterol and total bladdery acids volumes increase, while HDL cholesterol levels decrease in dogs with dirofilariosis. Similar to the studies, serum triglyceride, VLDL and LDL cholesterol levels were found higher in dogs with dirofilariosis than in the healthy dogs in the present study, but the total and HDL cholesterol levels were found to be low. The increase in serum triglyceride and VLDL levels in dogs with dirofilariosis may be related with the lipolysis of fats in the host which stimulates the production of triglycerides and VLDL cholesterol in the liver. And high LDL levels in dogs with dirofilariosis may be related with the inhibition of LDL enterance to the cells in organs as a result of the parasite blocking on LDL receptors (28). The reason why total cholesterol and HDL volumes are found to be low in the study may be because that cholesterol's normal synthesis cannot be provided by hepatosites due to liver damage that occurred during the disease.

CONCLUSIONS

Parasites which are in dogs that are in close contact with humans have great importance on the environment and human health. The canine heartworm disease is a common zoonotic disease which causes economic losses not only in Turkey but also all around the world (Çakıroğlu ve Meral 2007).

Dirofilariosis is generally asymptomatic. As a result, there is need for new specific biomarkers for the diagnosis of the subclinical disease. Before treatment with adulticide, determination of the cardiac status of the dog for choosing the appropriate treatment is important (Carretóna et al. 2014). In this study, we determined the PON1 enzyme activity in dogs with dirofilariosis. In conclusion, serum bazal paraoxonase activity can be used as a biomarker of cardiac damage, like LDH and CK-MB, in dogs with dirofilariosis.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

Acknowledgments. This manuscript is obtained from the MS thesis of the first author at the Institute of Health, Mustafa Kemal University.

REFERENCES

Altındağ Ö, Karakoç M, Soran N, et al. (2007). Paraoxanase and arylesterase activites in patients with rheumatoid arthritis. Rheumatism, 22: 132-136.

Bakırel U, Güneş S. (2009). Value Of Cardıac Markers In Dogs Wıth Chronıc Mıtral Valve Dısease. ActaVeterinaria, 59(2-3): 223-229.

Baskol G, Baskol M, Yurci A, et al. (2006). Serum papoxonase 1 activity and malondialdehyde levels in patients with ulcerative colitis. Cell Biochem Function, 24:283-286.

Carretóna E, Morchónb R, Simónb F, Justea MC, Méndez JC, Montoya-Alonso JA. (2014). Cardiopulmonary and inflammatory biomarkers in the assessment of the severity of canine dirofilariosis. Veterinary Parasitology, 206; 43–47.

Çakıroğlu D, Meral Y. (2007). Samsun Bölgesinde, Köpeklerde Dirofilaria İmmitis Enfestasyonu İnsidansıİncelenmesi. JIVS, 2:1-12.

Ece A, Kelekçi S, Kocamaz H, et al. (2007). Antioxidant enzyme activies, lipid peroxidation, and total antioxidant status in children with Henoch-Schönlein purpura. Clin Rheumatol, 27:163-167.

Eckerson H, Wyte C, La Du. (1983). The human serum paraoxonase/arylesterase polymorphism. Am. J. Hum. Genet, 35:1126–1138.

Efrat M, Rosenblat M, Mahmood S, et al. (2009). Di-oleoyl phosphatidylcholine (PC-18:1) stimulates paraoxonase 1 (PON1) enzymatic and biological activities, 202:461-469.

Farid SA, Horii Y. (2008). Gastrointestinal nematode infection increases organophosphate toxicity in rats.Toxicology Letters, 180: 33–37.

Friedewald WT, Levy RI, Frederickson DS. (1972). Estimation of the Concentration of Low- Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge, Clinical Chemistry, 18: 499-502.

Jacobs RM, Lumsden JH, Vernau W. (1992). Canine and feline reference values. In: Current Veterinary Therapy XI Small Animal Practice. Philadelphia, 1250-1278.

Jarvik GP, Jampsa R, Richter RJ, et al. (2003). Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. Pharmacogenetic,13: 291–295.

Juretic D, Tadijanovic M, Rekic B, et al. (2001). Serum Paraoxonase activitiesin hemodialyzed Uremic patients: cohort study. Clinical science, 42(2): 146-150.

Kartashev V, Batashova I, Kartashov S, et al. (2011). Canine and Human Dirofilariosis in the Rostov Region (South Russia). Veterinary Medicine İnternational, 1-5.

Kitagawa H, Ishiao K, Kawokomi M. (1981). Cardiopulmonary fonction values before and after heartworm removed in dogs with caval syndrome. Am J Vet Res, 52: 126-132.

Knott J. (1939). A method for making microfilarial surveys on day blood. Trans. R. Soc. Trop. Med. Hyg, 33: 191.

Martinelli N, Consoli L, Girelli D, Grison E, Corrocher R, Olivieri O. (2013). Paraoxonases: ancient substrate hunters and their evolving role in ischemic heart disease. Adv Clin Chem, 59:65-100.

McCall JW, Guerrero J, Genchi C, et al. (2004). Recent advances in heartworm disease. Veterinary Parasitology, 105-130.

Meral Y, Bakırel U. (2007). BDR köpekte kalp kurdu hastalığının(dirofilaria immitis) ekokardiyografik teşhisi. JIVS, 3:1-10.

Montaya A, Morales M, Juste C, et al. (2006). Seroprevalence of canine heartworm disease (Dirofilaria immitis) on Tenerife Island: an epidemiological update. Parasitol Res, 100:103-105.

Öncel T, Vural. (2007). Seroprevalence of Dirofilaria immitis in stray dogs in İstanbul and İzmir. Turk J Vet Anim Sci, 29:785-789.

Sarıtaş Z, Akın F, Şahal M, Öcal N. (2005). Open Heart Surgery Applications in Dogs Suffering from NaturalInfection of Dirofilaria immitis. Turk J Vet Anim Sci, 713-721.

Sarkar P, Basak D, Bhattacharyya H. (1978). Pathology of Dirofilaria immitis infection in dogs. Indian Vet.J, 53 (1): 55-57.

Sevimli KF, Kozan E, Bülbül A, et al. (2007). Dirofilaria immitis infection in dogs: unusually located and unusual findings. Parasitol Res, 101:1487–1494.

Song KH, Lee SE, Hayasaki M. (2003). Seroprevalance of canine dirofilariosis in South Korea. Vet Parasitol, 114: 231-236.

Vencoa L, Bertazzoloa W, Giordano G, Paltrinieri S. (2014). Evaluation of C-reactive protein as a clinical biomarker in naturally heartworm-infected dogs: A field study, Veterinary Parasitology, 206, 48–54

Voyvoda H, Paşa S, Töz SÖ, et al. (2004). Aydın'ın bazı ilçe ve köyleri ile İzmir'in Selçuk ilçesindeki köpeklerde Leishmaniosis ve Dirofilariasis'in prevalansı. Turk J Vet Anim Sci, 28:110