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Short Communication

Transient Glomerular Dysfunction in Dogs Caused by *Dirofilaria immitis* Infection

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Abstract

The present study aimed to examine two glomerular markers (urinary albumin, uALB; urinary C-reactive protein, uCRP) in healthy dogs and in dogs infected with *Dirofilaria immitis,* and to identify some possible changes in these markers after therapy with ivermectin and doxycycline. Twenty dogs with *D. immitis* infection positive by both the Knott method and SNAP 4Dx (IDEXX, USA) test were included in the research, as well as twelve clinically healthy dogs of similar age which served as controls. Glomerular biomarkers (mean, SD) increased significantly in dogs with heartworm disease (uAlB/Creatinine, Cr mg/g: 527.57±312.54; uCRP/Cr mg/g: 0.520±0.624), compared to control dogs (uAlB/Cr mg/g: 94.44±56.50; uCRP/Cr mg/g: below detection limit). Six months after the initial examination and the simultaneous treatment, all glomerular markers were considerably decreased and did not differ from those in healthy animals. In conclusion, the observed changes in glomerular biomarkers clearly indicated the transient nature of glomerular dysfunction caused by the heartworm infection in dogs, which may be of clinical relevance.

Keywords: *Dirofilaria immitis infection, Dogs, Nephropathy, Glomerular markers*

Köpeklerde *Dirofilaria immitis* Enfeksiyonu Nedenli Transient Glomerüler Disfonksiyon

Özet

Bu çalışmanın amacı sağlıklı ve *Dirofilaria immitis* ile enfekte köpeklerde ve ivermectin ve doxcycline ile tedavi sonrasında iki glomerüler markırdaki (üriner albumin, uALB; üriner C-reaktif protein, uCRP) muhtemel değişimleri araştırmaktır. Çalışmada Knott metodu and SNAP 4Dx (IDEXX, USA) testleriyle *D. immitis* ile enfekte olduğu tespit edilen yirmi köpek ile birlikte kontrol olarak aynı yaş grubunda klinik olarak sağlıklı 12 adet köpek kullanıldı. Kontrol grubuyla (uAlB/Cr mg/g: 94.44±56.50; uCRP/Cr mg/g: tespit limitinin altı) karşılaştırıldığında kalp kurdu olan köpeklerin glomerüler biomarkırlarında (ortalama, Standart sapma) (uAlB/Kreatin, Cr mg/g: 527.57±312.54; uCRP/Cr mg/g: 0.520±0.624) artma olduğu belirendi. İlk bakıdan sekiz ay sonra ve takip eden tedavi sonrasında tüm glomerüler markırların dikkati çeken derecede azaldığı ve sağlıklı olan hayvanların değerlerinden farklılık göstermediği belirlendi. Sonuç olarak, biomarkır değerlerinde gözlenen değişikliklerin köpeklerde kalp kurdu enfeksiyonu nedenli glomerüler disfonksiyonun değişken doğasını açıkça ortaya koyduğu ve bu durumun klinik değeri olabileceği gösterilmiştir.

Anahtar sözcükler: *Dirofilaria immitis enfeksiyonu, Köpek, Nefropati, Glomerüler markır*

INTRODUCTION

Dirofilaria immitis is the causative agent of Heartworm infection in dogs, which is accompanied by abnormalities in the cardiovascular system and is often fatal. Adult worms are mainly localized on the right side of the heart and the pulmonary artery, but microfilariae are circulating with the blood and could be found in the vessels of various organs, including kidneys [1], where they cause glomerular dysfunction manifested as mild to moderate proteinuria [2]. Early diagnosis of glomerular lesions allows for the selection of an appropriate therapeutic approach to prevent further progression of renal disease. Traditional diagnostic tests (serum creatinine - SCR and urea - BUN)

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detect renal disease in an advanced stage of development, when more than 75% of functioning nephrons are irreversibly damaged [3,4].

Unlike conventional tests, in the recent years, more attention has been paid to the urinary proteins and enzymes as early markers of kidney injury in dogs. According to Clemo [5] and Price [6], urinary markers have the potential to determine the localization and severity of renal lesions in different parts of the nephron. They include proteins classified according to their molecular weight: high molecular weight (HMW), intermediate molecular weight (IMW) and low molecular weight (LMW) proteins [7,8]. According to Finco [4], the occurrence of urinary protein results from increased glomerular filtration rate or abnormal tubular function, leading to decreased reabsorption of naturally filtered protein. Glomerular dysfunction leads to the appearance of IMW (albumin) protein in the filtrate and, at a more advanced stage - to HMW (e.g. CRP) proteins [6,7,9]. Grauer et al.[10] determine microalbuminuria (albumin from 1 to 30 mg/dl), as the earliest indicator of glomerular impairment in dogs.

C-reactive protein (CRP) is a HMW acute phase protein whose serum concentration is increased during inflammatory processes [11,12]. Recently, two studies have assessed urinary CRP (uCRP) and CRP-to-creatinine ratio (uCRP/Cr) in dogs with pyometra and chronic renal failure, as indicators of glomerular damage [8,13]. To the best of our knowledge, investigations on urinary microalbuminuria and CRP in dogs with microfilariaemia have not been conducted until now, which motivated us to perform this particular study.

The aim of the present work was to examine two glomerular markers (uALB, uCRP) in healthy dogs and in dogs infected with *Dirofilaria immitis*, and to identify some possible changes in these markers after therapy with ivermectin and doxycycline.

MATERIAL and METHODS

Dogs

Twenty dogs with *Dirofilaria immitis* infection positive by both the Knott method and SNAP 4Dx (IDEXX, USA) test, patients of Small Animal Clinic of the Faculty of Veterinary Medicine, Trakia University, Stara Zagora were included in the study. Animals were of both sexes at a different age (1.5-6.7 years), of different breed and weight (14-31 kg). Twelve clinically healthy dogs were used as controls. All dogs were privately owned and admitted in the clinic between June 2010 and December 2012. The owners signed an informed consent form to allow participation of their dog in the study.

All patients were submitted to complete clinical examination and routine hematological and chemical analysis of blood and urine. During the clinical examination, the bioelectric heart activity was carried out by ECG registration.

Diagnosis was based on the history, clinical signs, ECG, blood test, chemical blood and urine analysis, as well positive results by both specific Knott assay and Snap 4Dx test.

Urine was collected by cystocentesis under ultrasound control to determine the specific gravity (USG), creatinine (uCr), total protein (UP), albumin (uALB), C-reactive protein (uCRP) and bacteriological culture. Urinary protein-to-creatinine ratio (UPC), albumin-to-creatinine (uALB/Cr) and C-reactive protein-to-creatinine (uCRP/Cr) ratios were calculated according to the conventional methods.

After the disease has been diagnosed, all dogs were assigned microfilaricide treatment with ivermectin - 0.06 mg/kg, sc (Cevamec 1%; Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Budapest, Hungary, No 0604V1), once monthly (for 6 months) and daily oral administration of doxycycline - 10 mg/kg (Stada Arzneimittel-AG,-Germany) (for 4 weeks).

The control dogs were considered healthy from the absence of specific positive serological reaction and negative findings in Knott, as well as from blood and urine parameters within the normal range, including negative urine cultures. In 15 of 20 dogs initially diagnosed with heartworm infection, a secondary clinical examination, blood and urine tests were carried out, six months after the therapy. The remaining 5 dogs were excluded from the study because they died (2 dogs developed "caval syndrome", and 3 - decompensated heart failure).

Laboratory Methods

Routine Urinalysis: The urine specific gravity (USG) was determined on a refractometer. Routine analysis of the sediment was performed using dipstick, urine culture and microscopy. Urine was centrifuged (450 x g for 3 min), aliquoted and frozen at - 80°C until uCRP assay. The analysis was performed within 30 min after sampling on an automated biochemical analyzer and commercial kits for determination of urinary creatinine (uCr) and urinary albumin (uALB). The analysis of CRP was performed at the University of Milan, Italy, on a microplate spectrophotometric reader with species-specific commercial ELISA kit (TECO Medical Group, Switzerland) .

Statistical Analysis

The statistical analysis was performed using one way analysis of variance (ANOVA, software Statistica v. 7.0, StatSoft Inc., USA, 2004) and presented as mean (X) and standard deviation (±SD). The statistical significance of changes in parameters was determined in the LSD test at $P<0.05$.

RESULTS

Study Group Characteristics

Signalment and clinical data *(Table 1)* showed that reduced appetite and weight loss were among the commonest clinical signs, detected in 17 dogs. Cough was a frequent accompanying sign, observed in 14 out of 18 dogs. In 3 out of 20 dogs, ultrasound evidence for fluid in the abdomen and extremities swelling (2/20) were present. Seven dogs (35%) showed altered electric heart activity (ST segment depression, high or low amplitude of the ventricular complex). Ultrasound findings of kidneys showed signs specific for glomerulonephritis (enlarged kidney with hyperechoic and thickened renal cortex).

Insignificant changes in CBC parameters (Hb, Er and Hct) were established. A mild leukocytosis up to 17.31±6.21 10^9/L was observed. Sixteen out of 20 dogs with heartworm infection had hypoalbuminemia (<25 g/L), but none was hypoproteinemic (reference range: 25-37 g/L).

Routine Renal Tests in Healthy Dogs and Dogs with Dirofilariosis

Descriptive statistics of routine renal markers is shown in *Table 2*. Serum creatinine and urea concentrations in dogs with dirofilariosis before treatment and dogs with dirofilariosis included in the follow-up study after 6 months remained unchanged compared to control animals and reference ranges for dogs.

Average urinary protein-to-creatinine ratio (UPC) *(Table 2)* in control dogs was 0.29±0.11. Higher UPC values were established in dogs before treatment – 3.18±4.62 (P<0.05 vs control values). In dogs after treatment, UPC was restored to control and reference values.

Statistically significant changes were observed in mean urine specific gravity (USG). In group before treatment, USG attained 1.024±0.004 (P<0.001) as compared to control USG (1.033±0.008). In group after treatment, USG was recovered reaching 1.033±0.007 (P>0.05 vs control dogs).

Urinary Markers in Healthy dogs and Dogs with dirofilariosis

Urinary albumin-to-creatinine (uALB/Cr) and urinary C-reactive protein-to-creatinine (uCRP/Cr) ratio are presented in *Table 3*. The table shows that uALB/Cr markedly increased in dogs before treatment attaining 527.57±312.54 mg/g (110.8-1100.5 mg/g) at P<0.001 vs control values of 94.44±56.50 mg/g (36.05-112.01 mg/g). In dogs after treatment uALB/Cr normalized up to values similar to control ones.

In dogs with dirofilariosis, uCRP/Cr underwent considerable changes. In control dogs, no CRP (uCRP/Cr) was detected in urine. Significant changes in uCRP/Cr occurred in dogs before treatment, attaining 0.520±0.624 mg/g. Thirteen out of 15 treated dogs were negative for uCRP. Insignificant amounts of uCRP/Cr varying between 0.01-0.03 mg/g were detected in 2 animals.

Table 1. *Signalment and clinical signs, haematological and biochemical test results (mean±SD, n = 20) in dogs with dirofilariosis before treatment with ivermectin and doxycycline*

Tablo 1. *Ivermectin ve doxycyclin ile tedavi öncesi dirofilaria ile enfekte köpeklerin Klinik bulgu ve klinik belirtileri ile hematolojik ve biokimyasal test sonuçları (ortalama ± SD, n = 20)*

Signalment and Clinicals Signs		Haematological and Biochemical Results (mean ± SD)		Reference Range
Weight loss	17/20	Hb, (g/l)	127±36.76	120-180
Cough	13/20	Er, (10^{12}/L)	5.52±1.40	5.5-8.5
Ascites	3/20	Hct, (%)	37.0±9.40	37-55
Limbs oedema	2/20	Leuc, (10^9/L)	17.31±6.21	6-17
Retrograde pulse	2/20	TP, (g/L)	61.39±5.38	54-78
ECG changes	7/20	ALB, (g/L)	21.85±4.38	25-37

Table 2. *Routine parameters for renal function. Laboratory results in control dogs (n=12), dogs with dirofilariosis before treatment (n=20) and 6 months after treatment (n=15), (sCr, BUN, UPC and UPC, mean and SD)*

Table 2. *Renal fonksiyonların rutin parametreleri. Kontrol köpekleri, tedavi öncesi diroflarialı köpekler (n=20) ve tedaviden 6 ay sonrasının (n=15) laboratuar sonuçları (sCr, BUN, UPC and UPC, ortalama ve SD)*

Parameter	Control (n=12)	Before Treatment (n=20)	After Treatment (n=15)	Reference Range
sCr (µmol/l)	79.67±16.41	85.45±22.05^{ns}	81.87±14.86^{ns}	<125
BUN (mmol/l)	4.35±0.92	5.41±2.21^{ns}	4.57±0.99^{ns}	3.3–8.3
UPC	0.29±0.11	3.18±4.62^{a}	0.27±0.11^{ns}	< 0.5
USG	1.033±0.008	1.024±0.004^{c}	1.033±0.007^{ns}	1.015-1.045

^{ns} P>0.05, ^{a} P<0.05, ^{b} P<0.01, ^{c} P<0.001, ^{d} P<0.0001 - compared to control dogs

Table 3. *Urinary concentrations of glomerular markers: results in control dogs (n=12), dogs with dirofilariosis before treatment (n=20) and 6 months after treatment (n=15), (uALB/Cr, uCRP/Cr, mean and SD, BDL-below detection limit)*

Table 3.*Glomerüler markırların üriner konsantrasyonları: Kontrol köpekleri, tedavi öncesi diroflarialı köpekler (n=20) ve tedaviden 6 ay sonrasının (n=15) laboratuar sonuçları (uALB/Cr, uCRP/Cr, ortalama ve SD, BDL-tespit limitinin altı)*

Biomarker	Control Group	Before Treatment	After Treatment
uALB/Cr(mg/g)	94.44±56.50 36.05–112.01	527.57±312.54 [c] 110.8–1100.5	115.13±65.06 [ns] 39.54–248.6
uCRP/Cr(mg/g)	BDL (n=12)	0.520±0.624 0.142–3.012	BDL (n=13), 2 dogs (0.01–0.03)

[ns] P>0.05, [a] P<0.05, [b] P<0.01, [c] P<0.001 - compared to control dogs

DISCUSSION

In this preliminary study on dogs with heartworm disease a significant increase in glomerular markers, compared to control animals of similar age was established. Elevated uAlb and uCRP concentrations clearly show that dirofilariosis affects negatively the nephrons at a glomerular level. The appearance of urinary CRP in dogs with heartworm infection is an indication about glomerular membrane damage at an extent such that to allow filtration of high molecular weight proteins as CRP. The advantage of glomerular markers compared with routine renal parameters consists in their ability to detect the damage at an earlier stage [9]. The presence of 20 nonazotemic dogs with dirofilariosis, but with increased values of glomerular markers, as compared to healthy animals, clearly confirms this statement. According to IRIS, proteinuria is present when UPC values are greater than 0.5 [14]. In this study, the levels of the analyzed indicators were mildly to moderately elevated, but glomerular markers testing revealed significant differences between control animals and those with dirofilariosis. In addition, glomerular biomarkers (uALB, uCRP) provided guidelines for the origin of proteinuria, which is not possible with UPC.

Maddens et al.[13] reported a significant loss of ALB and CRP in the urine of dogs with pyometra, and determined uCRP as a glomerular indicator of unique diagnostic value. Along with glomerular markers, authors investigated some tubular biomarkers, and concluded that pyometra damages nephrons both at glomerular and tubular level. In a previous study Smets et al.[8] described a comparable increase in uALB and uCRP levels in dogs with chronic renal failure grade III and IV, but at a higher extent (uALB).

The most probable hypothesis explaining the significantly raised uAlb and uCRP levels is the chronic stimulation of the immune system by *Dirofilaria immitis* microfilariae causing formation of circulating immune complexes precipitated in glomeruli [15]. Morchón et al.[15] and Kramer et al.[16] consider that rickettsia of the genus Wolbachia (Rickettsiaceae) play an important role in the pathogenesis and immune response to microfilariaemia in dogs. Similar are the studies of Paes-de-Almeida et al.[17], who investigated electron microscopic renal lesions in dogs with experimentally induced heartworm disease. The authors suggest that the detected dense deposits of immune complexes associated to immature heartworms and microfilariae, as well eventually adult worms are causes for glomerulonephropathy.

In our study, six months after the initial examination and ongoing therapy, all glomerular markers were significantly decreased and did not differ from those in control animals. In two of the dogs slight traces of CRP in the urine were detected which may be due to an inflammatory response leading to increased plasma concentrations, as well as a slight damage of glomerular membrane.

In conclusion, the observed changes in glomerular biomarkers in this study clearly showed the transient nature of glomerular dysfunction caused by the heartworm infection in dogs. Future studies are needed to test additional glomerular and tubular biomarkers to evaluate the disease as a model of nephropathy.

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