Kafkas Univ Vet Fak Derg
18 (4): 585-588, 2012
DOI:10.9775/kvfd.2011.5899

RESEARCH ARTICLE

Evaluation of the Serum Lipid Profiles in Dogs with Symptomatic Visceral Leishmaniasis

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Makale Kodu (Article Code): KVFD-2011-5899

Summary

The aim of this study was to investigate the serum lipid profile in naturally infected symptomatic dogs with visceral leishmaniasis (VL). A total of 20 owned dogs, comprising 10 healthy controls and 10 infected dogs with L. infantum were enrolled in this study. The clinical history and physical examination were performed in all dogs. Infected dogs showed one or more signs of canine leishmaniasis such as skin lesions, loss of weight, conjunctivitis, alopecia, scaling, onychogryphosis, lymphadenopathy, weakness, anorexia, and epistaxis. The diagnosis of canine visceral leishmaniasis (CVL) was confirmed by the immunofluorescence antibody test (IFAT) with an antibody titer over or equal to 1:128. None of the healthy dogs had clinical signs consistent with any of the disease and were all seronegative for leishmaniasis. In this study, serum cholesterol, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels were significantly higher (P<0.01) in symptomatic dogs when compared to control animals. However, triglyceride and high density lipoprotein (HDL) levels were lower (P<0.01) than those of the controls. In conclusion, it was determined that CVL induce significant changes in serum lipid profile.

Keywords: *Visceral leishmaniasis, Lipid, Cholesterol, Dog*

Semptomatik Leishmaniasisli Köpeklerde Serum Lipit Profilinin Değerlendirilmesi

Özet

Bu çalışmanın amacı doğal enfekte visseral leishmaniasisli semptom gösteren köpeklerde lipit profilindeki değişiklikleri araştırmaktır. 10 sağlıklı ve 10 L. infantum ile enfekte toplam 20 sahipli köpek bu çalışmaya dahil edildi. Bütün köpeklerin anamnez bilgileri alındı ve fiziksel muayeneleri yapıldı. Enfekte köpeklerde visseral leishmaniasisin (VL) deri lezyonları, ağırlık kaybı, konjiktivitis, alopesi, kepeklenme, tırnaklarda uzama, lenfadenopati, güçsüzlük, iştahsızlık, epistaksis semptomlarından bir veya birkaçı tespit edildi. Canin visseral leishmaniasisin (CVL) teşhisi immunofluoresens antikor testi (IFAT) ve antikor titresinin 1:128'e eşit veya üzerinde belirlenmesi ile doğrulandı. Sağlıklı köpeklerin hiç birinde klinik semptom yoktu ve leishmania antikoru tespit edilmedi. Bu çalışmada semptomatik köpeklerde serum kolesterol, çok düşük dansiteli lipopretein (VLDL) ve düşük dansiteli lipopretein (LDL) seviyelerinin kontrol grubu verileriyle karşılaştırıldığında yüksek bulundu (P<0.01). Fakat trigliserit ve yüksek dansiteli lipopretein (HDL) seviyeleri kontrol hayvanlarından daha düşük belirlendi (P<0.01). Sonuçta CVL'in serum lipit profilinde önemli değişikliklere neden olduğu belirlendi.

Anahtar sözcükler: *Visseral leishmaniasis, Lipit, Kolesterol, Köpek*

INTRODUCTION

Canine visceral leishmaniasis (CVL) is a zoonotic, potentially fatal, systemic disease caused by a protozoan of the genus *Leishmania* in tropical and sub tropical climate zone of the world. *Leishmania spp*. is an intracellular protozoan that spends part of their life between sand fly vectors and the vertebrate host's macrophage [1,2]. VL can show different clinical manifestations from asymptomatic to symptomatic dogs, where a wide spectrum of clinical signs are evident such as skin lesions, weight loss, local and generalized lymphadenopathy, epistaxis, and lameness [3]. The appearance and severity of clinical signs depend upon dog's immunological response and the stage of the disease.

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Characteristic clinical signs of visceral leishmaniasis may detect infection, however; ancillary laboratory data in canine visceral leishmaniasis is helpful for contributing to diagnosis of the infection. The common clinical pathologic findings in CVL are the presence of hyperproteinemia, hyperglobulinemia, and thrombocytopenia. Proteinuria and azotemia are associated with glomerulonephritis. Liver failure occurs less commonly than renal failure and elevations in liver enzymes are not uncommon [4]. Different clinical pathologic findings may require establishing an early and accurate diagnosis/prognosis of CVL. There are few studies reporting an alteration in serum lipid profiles of leishmaniasis in humans [5-8]. Plasma lipid and cholesterol alterations have been demonstrated in naturally infected or experimental protozoan disease such as malaria, trypanosomiasis, giardiasis, toxoplasmosis, and babesiosis [9-11]. The aim of the present study was to evaluate lipid profile (triglyceride, total cholesterol, VLDL, LDL, HDL levels) in naturally infected symptomatic dog with VL.

MATERIAL and METHODS

A total of 20 owned dogs, comprising 10 clinically healthy control and 10 naturally infected owned dogs with VL from Hatay province in Turkey, were enrolled in this study. The dogs were between 1 and 8 years old and 12-40 kg body weight in different breed and sex. For all dogs, the clinical history and physical examinations were performed. The clinical status of each dog was carefully evaluated and defined according to weight loss, anorexia, enlarged lymph nodes, fever, epistaxis, conjunctivitis, dermatitis, skin ulcerations, alopecia, and onychogryphosis. None of the healthy dogs had clinical signs consistent with any of the disease.

A total of 8 ml blood sample was collected from the cephalic vein into the silicone gel coated tubes. All the dogs were fasted for eight hours before blood sample collecting. Serum samples were separated by centrifugation at 3000 rpm for 10 min at room temperature and kept at -20°C until processing. To detect *anti-leishmania* antibodies in dog sera, the indirect immunofluorescence assay (IFAT) was performed by using standard procedures. The IFAT was carried out using promastigotes from local *Leishmania infantum* stocks (*L. infantum* zymodeme MON-1). Briefly, following an incubation at 37°C for 30 min, slides were washed and stained with FITC-labeled anti-dog IgG conjugate (Sigma, A9042) diluted 1:200 in PBS. Slides were covered and examined under a fluorescence microscope. Antibody titer over or equal to 1:128 was scored as positive [1]. All healthy control animals were seronegative for CVL.

Serum samples were analyzed for triglycerides, total cholesterol, VLDL, HDL, and LDL- cholesterol. The analyses were performed on an automated analyzer (XL-600, Erba, India) using commercial test kits (Teco Diagnostics, California, USA) and VLDL levels were calculated by the following formula: triglycerides/5.

The Student t-test was used to compare serum triglyceride, cholesterol, VLDL, LDL, and HDL levels between the controls and CVL group. Data will be presented as mean ± standard error and a p value of less than 0.05 will be considered as significant.

RESULTS

Clinical signs of the infected dogs had typical clinical signs of CVL such as, skin lesions (9/10), loss of weight (8/10), conjunctivitis (7/10), alopecia (6/10), scaling (6/10), onychogryphosis (5/10), lymphadenopathy (4/10), weakness (3/10), anorexia (3/10), and epistaxis (2/10). A clinical diagnosis of the all infected dogs was confirmed by serologically by a positive (between 1:128 and 1:4096) indirect fluorescent antibody test (IFAT) for the CVL. Control dogs did not show any clinical signs and serologically positive titer.

Concentrations of serum triglyceride, cholesterol, VLDL, LDL and HDL levels and protein profiles in the dogs infected by leishmaniasis and the healthy controls were summarized in *Table 1*. Statistically significant increases in serum cholesterol, VLDL and LDL levels were observed

Table 1. *Concentrations of serum lipid and protein parameters in the dogs infected by leishmaniasis and the healthy controls*
Tablo 1. *Leishmaniasisle enfekte ve sağlıklı kontrol köpeklerinde serum lipit ve protein parametreleri konsantrasyonları*

Parameters	Healthy Control (n=10)	Visceral Leishmaniasis (n=10)	P
Triglyceride (mg/dl)	162.8±3.8	141.8±6.0	P<0.01
Cholesterol (mg/dl)	43.9±4.0	69.7±6.4	P<0.01
LDL (mg/dl)	72.9±5.6	102.9±6.2	P<0.01
HDL (mg/dl)	70.8±3.9	44.2±4.2	P<0.01
VLDL (mg/dl)	8.7±0.8	13.9±1.8	P<0.01
Total Protein (mg/dl)	5.5±0.4	7.8±0.8	P<0.05
Albumin (mg/dl)	3.6±0.3	2.0±0.2	P<0.01
Globulin (mg/dl)	1.9±0.4	5.8±0.9	P<0.01

in leishmania infected group when compared to controls. There was, however, a statistically significant decrease in serum triglyceride and HDL level (P<0.01) in CVL. In leishmania infected group, serum total protein (P<0.05) and globulin (P<0.01) levels were significantly raised. However, a significant decrease in albumin concentrations in dogs was observed when compared to healthy control (P<0.01).

DISCUSSION

In this study, clinical signs of infected dogs were similar to those reported in a previous study. Infected dogs showed mostly skin lesions, loss of weight, conjunctivitis, alopecia, scaling, onychogryphosis, lymphadenopathy, weakness, anorexia, and epistaxis, respectively. The clinical diagnosis of CVL is relatively easy in dogs with symptomatic dogs. Serodiagnosis and laboratory findings such as hyperproteinemia, hypoalbuminemia and hyperglobulinemia are helpful markers of the disease [4]. In this study, we observed that serum total protein and globulin levels were significantly higher in dogs with CVL than the healthy dogs. Most dogs affected with CVL show hyperproteinemia in advance stages due to increased production of β and γ globulins [12]. This can be observed by an increase of the total serum proteins with hyperglobulinemia and hypoalbuminemia, with decreased albumin/globulin ratio [13].

Plasma lipoproteins (VLDL, LDL, and HDL) function primarily in lipid transport among tissues and organs [14]. Besides their primary role in lipid transport, lipoproteins participate in innate immunity, since they have broad preventive effects against bacterial, viral and parasitic infections [15]. Changes in lipid profile occur in patients having active infections with most of the parasite. It has been reported that parasites are able to metabolize cholesterol, but the underlying mechanism has not fully been understood yet [10]. During the acute-phase response to infection and inflammation, it is well known that some alterations in lipoprotein metabolism occur and result in a variety of changes in the plasma concentrations and composition of lipids and lipoproteins [16]. It was reported that plasma lipid and lipoproteins could play important role in the development or maintenance of the immune response in human VL [8]. One of the responsible mechanisms for the altered lipid profile in patients with visceral leishmaniasis might be sequestration and/or degradation of lipoproteins in the enlarged spleen and liver [17]. In addition to several types of acute conditions are associated with a marked decrease in HDL cholesterol [18]. In this study, statistically significant increases in cholesterol, VLDL and LDL levels (P<0.01) were observed in the *leishmania* infected group when compared to control group. There were, however; a significant decrease in triglyceride and HDL levels (P<0.01). The decrease in serum cholesterol, HDL, and LDL levels and high levels of triglycerides and VLDL were observed in human patients with VL by Soares et al.[8]. These results are in disagreement with our findings. However, significant increases in cholesterol, LDL levels and decrease in HDL levels were observed in *leishmania* infected dogs by Nieto et al.[19] as observed in our study. *Trypanosoma brucei* infection in rabbits results in an increase in cholesterol and VLDL, and decrease in HDL due to lipoprotein lipase hypoactivity [14]. The significant alteration of lipid profile may be attributed to the consumptive evolution of the disease, hepatic disorders, interactions between the parasite and the normal cholesterol metabolism of the host [19], and lipoprotein lipase hypoactivity [14].

In conclusion, these results suggest that VL induced significant changes in serum lipid profile in the dog. The changes in lipid profile may be help in diagnosis in dog symptomatic visceral leishmaniasis.

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