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Original article

Investigation of the bone metabolism in dogs with leishmaniasis

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Abstract

The present study was aimed at determining bone metabolism related changes in dogs with visseral leishmaniasis such as vitamin D, calcium, phosphate and ALP levels as compared to healthy ones. A total of 40 serums of male dogs aged between 4 and 6 years that were sent to Adnan Menderes University, Faculty of Medicine, Parasitology Laboratory from Kuşadasy and Bodrum districts between the years 2012 and 2013, suspected with leishmaniasis, by the veterinary physicians in accordance with the cold chain rules and 20 of which were evaluated as leishmania seropositive and 20 as leishmania seronegative by the IFA test, were included in this study. There was no any statistically significant difference between the serum 25-OH-D_3 levels of Leishmania positive and negative dogs ($p>0.05$). There was a statistically significant difference between the serum ALP ($p<0.05$), and P levels were observed to be higher in the seropositive dogs than in the seronegative dogs, while Ca level was low ($p<0.001$).

Key words: leishmaniasis, vitamin D, calcium, phosphate, alkaline phosphatase

Introduction

Leishmaniasis is an important zoonotic infectious disease which can be observed in many countries of the world. The disease is prevalently observed in the Mediterranean Region, Africa, Asia and Central Southern America (Papadopoulouveark 2005). While it is especially observed in the coastal regions in Turkey, cases from nearly all of the regions were reported (Özbel et al. 2002).

Dogs are the most important reservoir host for *Leishmania infantum,* which is the agent of the visceral disease (Oliva et al. 2010). Depending on the immune response of the animals which contracted the disease, the course of the disease can be asymptomatic or symptomatic (Desjeux et al. 2004). The primary symptoms observed on the skin can include ashyperkeratosis, skin dehydration, scleroderma, loss of elasticity, depigmentation, scurfy dermatitis (Gönul et al. 2002). Furthermore, anemia, neutropenia, thrombocytopenia, uremia, hyperproteinaemia, hypergammaglobulinemia, hyperalbuminemia, an increase in ALT, GGT, LDH and ALP levels, in urea and creatine level can be listed as mild or severe proteinuri a laboratory findings. The clinical findings may show variability depending on the course, phase and severity of the disease in Canine visceral leishmaniasis (CVL) (Freitas et al. 2012, Taher et al. 2015).

Calcium and phosphorus are important in terms of the development of bone structure and preservation of mineral density. These are also the most frequently found elements in the organism and they have

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important physiologic functions in many biological mechanisms, except for the skeletal system (Kalaycıoğlu et al. 2000, Pineda 2003). There are hormonal control mechanisms for the preservation of blood calcium and phosphorus levels. These mechanisms consist of 3 hormones, which are parathyroid hormone (PTH), calcitonin and vitamin D (1,25 dihydroxycholecalciferol). These hormones have also an effect on the bone and mineral metabolism (Oleszek and Marston 2000, Fidan and Dundar 2007).

Vitamin D is a group sterol with hormone-like functions. The most important effect of vitamin D is its effect on calcium homeostasis. It is responsible for the bone metabolism, passing of the calcium ions, which are necessary for neural functions in the bone structure, through the cell membrane, Ca^{+2} absorption from the intestines and mobilization from the bones (Kalaycıoğlu et al. 2006). The main activation stage in the vitamin D metabolism takes place in the kidneys and is catalyzed by 25-hydroxycalciferol--1α-hydroxylase. 1,25 dihydroxycholecalciferol (1,25 dihydroxyvitamin D) formed as a result of this hydroxylation is the most active form of vitamin D and it is responsible for all the effects of vitamin D (Gow et al. 2011, Dittmer and Thompson 2011).

Although the metabolic function of alkaline phosphatase is not completely understood, it is considered to play an important role in the intestinal lipid transport and calcification in the bones (Onat et al. 2002, Murray et al. 2004). It is found on the outer surfaces of absorptive cells and it helps the transport process. It is considered to be an enzyme that regulates the active transport *in* the cell membrane (Uzunoğlu 1998).

Alkaline phosphatase is found in the liver, bone tissue, kidneys, intestines, placenta, pancreas, thyroid gland, tooth enamel, leucocyte cell membrane, endoplasmic reticulum, mitochondria, nuclear membrane and cell membrane (Uzunoğlu 1998). Although its metabolic function is not completely understood, it is considered to have an important role in the intestinal lipid transport and calcification in the bones and regulates the active transport in the cell membrane (Onat et al. 2002, Murray et al. 2004). The alkaline phosphatase activity is one of the most frequently used biochemical indications in bone diseases and it is partially caused by osteoblasts. Thus, in osteoporotic patients, serum alkaline phosphatase activity reaches higher values when compared to the normal course (Çetiner et al. 2001, Ecer et al. 2005).

Changes in the alkaline phosphatase level observed in the laboratory findings in dogs with leishmaniasis suggest that leishmaniasis may cause bone formation (Çetiner et al. 2001, Ecer et al. 2005). In this study, in order to determine the effect of canine leishmaniasis on the bone metabolism, serum calcium, phosphate, alkaline phosphates and 25-OH-D_3 values, among the biochemical indicators of the bone, were investigated.

Materials and Methods

Samples

A total of 40 serums of male dogs aged between 4 and 6 years that were sent to Adnan Menderes University, Faculty of Medicine, Parasitology Laboratory from Kuşadasy and Bodrum districts in Turkey between the years 2012 and 2013, suspected with leishmaniasis, by the veterinary physicians in accordance with the cold chain rules, and 20 of which were evaluated as leishmania seropositive and 20 as leishmania seronegative by the Indirect Immunofluorescence Antibody Test (IFAT), were included in this study.

Determination of anti-leishmania antibodies by the indirect antibody fluorescence method

Leishmania antibodies were studied by the IFAT method in the serum samples. In the study, rabbit anti-dog IgG florescein isothiocyanate conjugated (Sigma, F-7884) was used at 1/100 dilution.

The samples were analyzed under the fluorescence microscope (Olympus CH-40) with an excitation filter at 490 nm wavelength, with 40 mm lenses by using a 20-mm ocular. In the study, 1/64 and above titers were evaluated as positive.

Biochemical Analyses

The indication of the calcium amount in the blood serum with the glyoxal-bis method was measured by the colorimetric method. This method is based on the measurement made by using the calcium chelator feature of glyoxal-bis in the presence of other +2 valent cations (Bellinger and Campbell 1965).

The serum inorganic phosphate level was determined as reductive in the presence of tin chloride, by the colorimetric method based on the formation of ammonium molybdiphosphate the reaction of phosphate ions with ammonium molybdate in an acidic medium (Davies et al. 1973). The analyses of serum alkaline phosphatase (Spinreact®, Catalogue no: BEIS44-I) and 25-OH-D_3 (Immunodiagnostic Systems Ltd, Catalogue no: UK51081) were made by using a kit.

Table 1. Visceral leishmaniasis seropositivity in the dogs according to the IFAT results.

Titers	Seropositive dog	
	number	%
1/64	1	5
1/128	3	15
1/256	4	20
1/512	12	60
Total	20	100

Table 2. Vitamin D, ALP, Ca and P results investigated in the dog serums.

	Leismaniasis negative (n:20)	Leishmaniasis pozitive (n:20)	p
25-OH-D_3nmol/L	70.01 ± 14.12	51.89 ± 6.59	0.252
ALP U/L	104.5 ± 8.7	137.1 ± 11	0.026*
Ca mg/100ml	8 ± 0.5	3.38 ± 0.48	0.000*
P mg/100ml	2.19 ± 0.17	3.01 ± 0.11	0.000*

Statistical Analyses

For the statistical analysis of the data obtained, SPSS (for Windows Release15.0 Standart Version-Copyright© SpssInc. 1989-2001) package program was used. The significance analyses were performed using the Mann-Whitney U Test.

Results

The titer rates of 20 Leishmania-positive male dogs that were included in the study are shown in Table 1.

Serum 25-OH-D_3, ALP, Ca and P results of Leishmania positive and Leishmania negative male dogs are given in Table 2.

There was not any statistically significant difference between the serum 25-OH-D_3 levels of Leishmania positive and negative dogs ($p>0.05$). There was statistically significant difference between the serum ALP ($p<0.05$) and P levels were observed to be higher in the seropositive dogs than in the seronegative dogs while Ca level was low ($p<0.001$).

Discussion

Leishmanias is is among the most neglected diseases in the world and it is reported by the World Health Organization (WHO) that the disease is endemic in a total of 98 countries, 350 million people are at risk, and there are estimated 2 million new cases in a year. The mortality and morbidity of leishmaniasis show a tendency to increase every year at an alarming rate (WHO 2010). Two epidemiologic forms, zoonotic and anthroponotic, are observed in leishmaniasis. The frequently observed form in the Mediterranean countries is the zoonotic form. The form observed in East Africa, Bangladesh and India is the anthroponotic form (Molano et al. 2003).

Many different methods are used in the diagnosis of canine leishmaniasis. These methods can be listed as cytohistological methods, serological tests, parasitological methods, molecular methods and determination of the cellular immune response. The sensitivity and selectivity of the IFAT method are reported to be close to 100%, and it is acknowledged as the reference serological method by the World Organization for Animal Health (WOAH) (Mettler et al. 2005).

The researchers reported that the IFA technique was a quite sensitive and specific test for the determination of leishmaniasis, and upon evaluating the clinical findings and laboratory results together, the titrations between 1/40 and 1/1024 were diagnostic in seropositive patients and the titers above 1/40 were sufficient for the diagnosis of canine leishmaniasis (Freitas et al. 2012). In our study, titers of 1/64 and above are accepted as positive.

In the investigation of the bone metabolism, vitamin D, alkaline phosphatase, Ca and P are generally evaluated together. It has been shown in the investigations that vitamin D is closely related to calcium and phosphor homeostasis, and plays a significant role in the bone model formation (Dittmer and Thompson 2011). In the study of Driel et al. (2006) conducted in order to investigate the autocrine/paracrine function of 1α, 25-OH-D_3, it was determined that 1α-hy-

droxylase is expressed in the human osteoblasts, and also there are megalin and cubilin protein receptors bound to vitamin D. It was observed that osteoblasts produced 1α, 25-(OH)2D_3, and ALP and osteocalcin expressions increased mildly with the addition of 25-OH-D_3 as a substrate to the osteoblast cell cultures while it did not have any effect on Ca and parathyroid hormone regulation. In the studies conducted on humans, 25-OH-D_3 showed a relation with osteoporosis, 25-OH-D_3 levels were observed to be low in patients with osteoporosis, while the parathormone levels were high (Sahota et al. 1999, Sahota et al. 2004, Garneo et al. 2007). The vitamin D metabolism in dogs was mostly investigated in the pathological cases of kidney failure (Galler et al. 2011). In the literature no study investigating the vitamin D metabolites in leishmania-infected dogs was encountered.

In this study conducted with the purpose of investigating the effect of leishmaniasis on the bone metabolism in dogs, serum 25-OH-D_3 levels in infected dogs were found to be lower than those in healthy dogs. However, this difference is statistically insignificant.

In the study conducted by Rallis et al. (2005), from 26 dogs infected with *Leishmania infantum,* anemia in 21 dogs, an increase in the total amount of protein in 23 dogs, low albuminemia in 6 dogs, an increase in the ALP activity in 15 dogs and hyperbilirubinemia in 2 dogs were determined. Similarly, in the studies conducted on dogs with leishmaniasis, the levels of ALP, ALT, SGOT, LDH, total protein, blood urea nitrogen, creatinine, creatine kinase, and cholesterol were observed to be higher than the normal values (Tyrphonas et al. 1977, Koutinas et al. 2001). In the present study, the serum ALP levels in the dogs naturally infected with leishmania were determined to be high similarly to the findings of other researchers.

Paşa et al. (2003) found that serum zinc and iron levels in the dogs with visceral leishmaniasis were significantly lower than those in healthy dogs, while the copper level was high. Any significant differences in the calcium, phosphor, and magnesium values were not observed. A positive correlation between hyperphosphatemia and the severity kidney failure was observed in 155 dogs with leishmaniasis at various stages of chronical kidney failure (Cortadellas et al. 2009). Upon evaluating the studies in general, it is observed that hypocalcemia and hyperphosphatemia occur in the cases in which kidney failure develops along with leishmaniasis. In the study conducted, the P and Ca results suggest that kidney failure may be found in the infected dogs.

As a result, the facts that clinical findings of leishmaniasis are not always clear and the symptoms vary depending on the immune system in patients make the clarification of the pathophysiology of the disease difficult. Upon evaluating the results in this study, which the bone metabolism in dogs with visceral leishmaniasis investigated, in general, an increase in the ALP level suggests that the findings of hypocalcemia and hyperphosphatemia may have adverse effects on the bone metabolism.

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