

Serum trace element levels in dogs with canine visceral leishmaniasis

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Abstract: Canine visceral leishmaniasis has been denoted as neglected despite being a very well-known disease. Trace element alteration has been recognized in humans with visceral and cutaneous leishmaniasis, together with canine visceral leishmaniasis. The trace elements occupy a vital position in the immunological system, and host immune responses mitigate defense against leishmaniasis. We aimed to select trace elements in a total of 45 dogs of several breeds; those at the age of 11 months to 6 and from both sexes (26 male and 19 female) were enrolled in the study. The dogs included in the study were divided into leishmaniasis-infected and noninfected groups. All cases in this study were included according to written owner consent. The trace element analysis of serum samples was carried out by using an inductively coupled plasma-optical emission spectrometry. The results of our study revealed that between the canine visceral leishmaniasis infected group and the uninfected group, *Leishmania*-positive dogs had significantly lower levels of Se ($p < 0.001$) and Zn ($p < 0.001$) compared to the negative ones. These results should be carefully elucidated in an attempt to analyze immune responses in dogs with canine visceral leishmaniasis.

Key words: Immune response, selenium, vector borne disease, zinc

1. Introduction

Canine visceral leishmaniasis (CVL) is a protozoal, zoonotic, vector-borne disease initiated by *Leishmania* spp. and endemic in a great majority of the world, including Türkiye [1-5]. The main etiologic agent among them is seen as *Leishmania infantum* [6,7].

It is known that CVL is a chronic systemic disease as it exhibits histological alteration including chronic mononuclear inflammation [8-10] and fibrosis [10,11]. The parasite is transmitted to hosts by the phlebotomine sand fly and then it can primarily survive and multiply in most organs being able to have phagocytic activity via scavenged mononuclear cells including macrophages [8, 12, 13]. Infected macrophages have opposite balance via destruction or replication of parasite producing reactive oxygen species (ROS), cytokines, and chemokines or antiinflammatory response, and both conditions affect clinical manifestation and progression of leishmaniasis [10, 14, 15].

Infectious diseases cause deficiency of main body substances by changing their metabolism, utilization, or

consumption [16]. Within this context, trace elements have got a critical role in various processes including cellular differentiation and replication, cell membrane stability, apoptosis, immunological functions, and attending enzyme structure [17-20]. Furthermore, they provide integrity with several enzymes involved in antioxidant activity against reactive oxygen species produced by infected macrophages [10, 21, 22]. Most studies demonstrate a negative association between trace element and CVL [10, 22-24].

In this study, it was aimed to detect trace element levels in different stages of canine visceral leishmaniasis.

2. Materials and methods

2.1. Study design and subjects

For this study, blood specimens were collected via *vena cephalica antebrachia* for immunofluorescence antibody titer (IFAT) and other hematological and biochemical analyses. A total of 45 dogs in different breeds, at 11 months to 6 years of age and from both sexes (26 male and 19 female) were enrolled in the study. The dogs were

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divided into 2 groups as *Leishmania*-positive (n = 30) and -negative (n = 15). All cases in this study were included according to the written consent of the owner. With regard to Kirikkale University Animal Experiments Local Ethical Committee, solely blood was withdrawn in which no invasive process was available, denoting that ethical paper would not be required as was evidenced by the official and signed paper (report no: 28.11.2022-E.134371)

In dogs from which blood was drawn for leishmaniasis, there were several clinical signs such as CVL-related ocular lesions, cachexia, lymphadenopathy, and onychogryphosis. Positive and negative dogs were confirmed by tentative diagnosis, positive point-of-care ELISA test (SNAP *Leishmania*; IDEXX Lab., Spain). All SNAP-positive sera were examined by indirect IFAT above 1/64 as described before [25, 26]. Negative group animals were selected based on negative point of care ELISA and IFAT analyses and other diseases. All negative dogs were included in the study after clinical hematological and biochemical evaluations.

2.2. Trace element examination

The trace element analyses were performed using induction coupled plasma-optical emission spectrometry (ICP-OES; Thermo iCAP 6000 series) in an experienced unit (Trace Element Analysis Laboratory resided in the Biophysics Department, Cerrahpaşa Faculty of Medicine, Istanbul University-Cerrahpaşa). The parameters of the ICP-OES device for the determination of zinc (Zn), magnesium (Mg), iron (Fe), copper (Cu), selenium (Se), chromium (Cr), manganese (Mn), arsenic (As), and cobalt (Co) elements are presented in Table. In the present research, suitable wavelengths of Cr, Cu, Fe, Mg, Mn, Se, Zn, Co, and As elements were used as 267.716 nm, 324.754 nm, 259.940 nm, 285.213 nm, 257.610 nm, 196.090 nm, 206.200 nm, 228.616 nm, and 189.042 nm, respectively. The calibration graph was obtained by using blank and

standard solutions from the ICP-OES device. Stock solutions were processed from standard solutions (Chem - Lab NV; Belgium) containing Cr, Cu, Fe, Mg, Mn, Se, Zn, Co, and As, and distilled water was used as a blank solution. Sera analytes were searched for trace element measurements as dilute 1:10 with distilled water. Each analysis was performed three times and the averages were used for the analysis. Trace element status was referred to as mg/L.

2.3. Statistical analysis

Data achieved from *Leishmania*-positive and -negative dogs were tabulated with mean and standard deviation. Normality and homogeneity analyses were performed, and the Mann-Whitney U test was used to detect the alterations among groups. A statistical package program (SPSS 26.0) was used, and the p-value was considered significant if lower than 0.05.

3. Results

Dogs included in this study were grouped as: *Leishmania*-positive and -negative matched with age and sex. Selected trace element levels in blood sera were presented in Table. *Leishmania*-positive dogs were selected from animals with clinical findings and positive SNAP *Leishmania* tests (SNAP *Leishmania* test kit, IDEXX). In the *Leishmania*-positive group, Se and Zn levels had significant decreases ($p < 0.001$) compared to the *Leishmania*-negative group dogs.

4. Discussion

Iron, Co, Zn, Cu, etc. are some of the trace elements that have a broad scope in human homeostatic processes [27]. Reduction or increments in trace elements in sera/plasma could make alterations in T-cell proliferation, B-cell activity, antibody creation, and various activities in immune status [28, 29]. As a result, both low and high levels

Table. Serum trace element levels of dogs positive with leishmaniasis.

	<i>Leishmania</i> ($\bar{X} \pm SE$)	Control ($\bar{X} \pm SE$)	p-value
Cr (mg/L)	0.008 ± 0.003	0.011 ± 0.004	0.110
Cu (mg/L)	0.720 ± 0.148	0.847 ± 0.317	0.146
Fe (mg/L)	1.426 ± 0.612	1.484 ± 0.407	0.549
Mg (mg/L)	20.559 ± 5.020	21.209 ± 1.128	0.146
Mn (mg/L)	0.004 ± 0.003	0.003 ± 0.001	0.116
Se (mg/L)	0.236 ± 0.091	0.316 ± 0.046	0.003
Zn (mg/L)	1.156 ± 2.976	3.150 ± 1.453	0.000
Co (mg/L)	0.004 ± 0.002	0.004 ± 0.002	0.761

\bar{X} : mean; SE: Standard error of mean

of trace element levels are directly related to increased susceptibility to infectious diseases [28]. The host immune response participates significantly in the pathogenesis and protection against leishmaniasis. A strong T helper 1 (Th1) cell activation against leishmaniasis triggers existence of proinflammatory cytokines, e.g., interleukin (IL)-2 and IL-12, as well as gamma interferon (IFN- γ) and tumor necrosis factor (TNF)- α . Macrophage and neutrophil activations, which are formed together with the abovementioned cytokine increases, play a critical role in killing parasites and protecting the body against infection [30, 31, 32]. Besides, both IFN- γ and TNF- α play a versatile role in the immunoprotection and immunopathology of leishmaniasis [30, 33]. Although *Leishmania* prevents the production of ROS by multiplying within macrophages [34], Paltrinieri et al. [35] stated that the increases in ROS are related to inflammation rather than the presence of the parasite.

According to the authors' knowledge, there are limited papers that analyzed trace element alterations in Leishmaniasis [10, 22, 23]. In previous studies, changes in Cu, Fe, Zn, Se, and Mg levels were evaluated. In both of these studies Fe, Zn, and Se levels were significantly decreased compared to the healthy control ones. Although we detected similar decreases in Se and Zn levels in our study, it was determined that there were no significant alterations in Fe levels. The role of products emerging in inflammatory processes in the regulation of Zn balance is explained by metallothionein, which is regulated by interleukins secreted by phagocytes [36, 37]. The metallothionein molecule helps to remove zinc from circulation by binding 7 g of zinc molecules. Increased IL-1, IL-6, and TNF- α levels in dogs afflicted with leishmaniasis also increase metallothionein production [38,39]. In a study by Wirth et al. [40], it was reported that zinc deficiency causes atrophy in the thymus gland, involution in the cortex, and a decrease in thymulin production and in lymphocyte and phagocyte counts in mice. Considering the outcomes of zinc on the immune system, numerous reports have been conducted to investigate the effects of various parasitic diseases on zinc levels in mice [41-44]. The effects of zinc deficiency on immune responses in parasitic infections and zinc deficiency have been described to cause insufficiency in immune responses in parasitic infections. In a study conducted on people with

visceral leishmaniasis, it was shown that the serum zinc level was significantly lower than the healthy group [45]. Similarly, Zn, Cu, and Fe levels were measured in people with cutaneous leishmaniasis, and Zn and Cu levels were initiated to be significantly lower than in the control group [46]. The low serum zinc level measured in our study is consistent with the results previously obtained with human and experimental animals.

In our study, we found no significant alterations in Fe status between healthy and *Leishmania*-positive dogs. Zafra et al. [47] stated that symptomatic dogs had higher parasitic load and lower serum nitric oxide levels compared to asymptomatic dogs. Souza et al. [10] found hemosiderin deposits in symptomatic dogs. Researchers also state that iron level is positively correlated with the degree of inflammation in all organs in symptomatic dogs, while it was negatively correlated to tissue antioxidant enzymes [10]. The lack of differences between the Fe levels found in our study may also be due to the differences related to the measurement technique in light of the information stated.

The results of our study revealed that dogs with canine visceral leishmaniasis had significantly lower Se concentrations than the negative group. Related reports have been presented in medicine [48]. Selenium is essential for several enzymes [49]. Also, Souza et al. [10] found higher superoxide dismutase activity in diseased dogs than in controls, besides lower glutathione peroxidase and catalase activity. In a study comparing serum Se, Zn, and Cu levels in visceral and cutaneous leishmaniasis in humans, a significant decrease was observed in especially Se and Zn levels, and at the end of the study, it was suggested that serum Se and Zn levels could be an appropriate marker for the pathophysiology of leishmaniasis [48]. The results obtained from our study show similarities with this study. Considering the antioxidant activity of Se on the immune system, its therapeutic efficacy is also being evaluated in patients with *Leishmania*.

5. Conclusion

As a result, Se and Zn levels were affected in leishmaniasis and including these trace elements in the treatment regimens could be investigated by further studies.

Conflict of interest

The authors declare no conflicts of interest.

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