RESEARCH ARTICLE

International Journal of Veterinary and Animal Research
E-ISSN: 2651-3609 4(2): 49-52, 2021

Trace Element Levels in Naturally Infected Dogs with Giardiasis

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Received: June 16, 2021
Accepted: September 10, 2021

Abstract

As a well-known protozoan zoonotic giardiasis has manifested itself with various clinical findings in mammal species including human and dogs. Absorption of some essential biochemical elements required to growth has interrupted in giardiasis being tight relationship with malnutrition. Thus, the aim of the present study was to determine the serum trace element levels in dogs infected with giardiasis. For this purpose, totally 16 dogs enrolled in the study and 8 of these had diarrhoea compatible with giardia (infected group) and others were grouped into healthy. Diagnosis of giardia was performed by microscopical evaluation of cysts and supported by rapid test from faecal samples. In the infected group, copper (Cu), magnesium (Mg) and zinc (Zn) levels had significant differences to healthy control. Giardia infected dogs had a significant increase in Cu and significantly reduces in Mg and Zn levels. It was thought that unclear mechanism of decreased Zn levels might be related to elevated Cu involving enzyme systems. And also, malabsorption and diarrhoea were proposed mechanism for altered Mg levels, which could counteract by blocking of intestinal absorption. Consequently, Cu, Mg and Zn levels were affected in giardiasis and the addable of these elements to the treatment should be revealed by further studies.

Keywords: Diarrhoea, malnutrition, microelement, protozoal disease.

INTRODUCTION

Giardiasis is a well-known zoonotic protozoan disease of mammal species and still a health risk for low level social peoples in rural areas (van Keulen et al. 2009; Abdel-Moein and Saeed 2016). Diarrhoea, bloating, abdominal pain and increased peristaltic are common clinical reflection of disease and might be affected from many conditions such as age, host resistance and etc. (Adam, 2001). Dogs might be asymptomatic and symptomatic according to the aforementioned conditions and also, giardiasis could be detected independently both asymptomatic and symptomatic dog feces (Claerebout et al. 2009).

Giardia duodenalis is also responsible for negative impact of nutritional status, ocular and muscular complications, arthritis, and hypersensitivity reactions in humans (Çeliksöz et al. 2005; Hanevik et al. 2014). In children a tight relationship were giardiasis and malnutrition were defined before (Nematian et al. 2008) also this condition was associated with the malabsorption of many essential biochemical elements for growth (Simsek et al. 2004). Many oxidant and antioxidant pathways in mammals are depended on enzymes and trace elements. In addition to these meta-analytic studies reveals the usage of antioxidant, vitamin, and trace element supplementations during many illnesses in humans was in positive correlation to reducing mortality (Manzanares et al. 2012). Critical ill patients were under a process of metabolic stress conditions and needs requirements for trace elements (Agarwal et al. 2011; Lee et al. 2019).

Trace elements are very important for man and animal health and immunity, although they are required in small amounts in the body (Terpilowska et al. 2011). Manganese (Mn), cobalt (Co), copper (Cu), iron (Fe), zinc (Zn) and selenium (Se) are the most important of them. They act as cofactors of enzymes, and antioxidants. Trace elements also play a fundamental role in homeostatic mechanisms, immune functions, and in many physio-biochemical processes (Terpilowska et al. 2011; Nemec et al. 2012). While Eisa and Elgebaly (2010) are presented the role of iron in immunity, Sordillo et al. (1997) are reported the role of selenium, Zn and Cu elements in immune cells such as lymphocytes, neutrophils, and leucocytes.

Considering of variations in levels of trace elements were described in giardiasis in infant due to malabsorption and growth retardation (Karakas et al. 2001), It has been hypothesized that serum trace elements may be involved in the pathophysiology of canine giardiasis. Thus, this study was conducted to determine the levels of the serum trace element levels in dogs infected with giardiasis.

MATERIALS AND METHODS

Study design and subjects

The present study enrolled 16 dogs from different breeds, at the age of 9 months to 4 years old, and of both sexes (9 female and 7 male). Eight out of those 16 dogs presented clinical signs compatible with a susceptible giardia infection such as diarrhoea and vomiting. All cases enrolled were deemed eligible to participated within this study by written owner consent.

To ruling of other similar infection with giardiasis, native faecal examination was performed for intestinal parasites and rapid test kits for canine parvo/coronavirus infection (Antigen rapid CPV/CCV Ag test kit, Bionote, ELK Diagnostic, Istanbul) and for canine distemper (Antigen rapid CDV Ag test kit, Bionote, ELK Diagnostic, Istanbul) during routine clinical evaluation. And also, abdominal radiography and ultrasonography were performed in suspicious dog to eliminating of related gastrointestinal disease without giardiasis.

Dogs with naturally occurring giardiasis (n=8) were aged matched controlled to those of another 8 healthy dogs. Both group of dogs consumed commercially available and marketed dog food, were housed, and owned individually. All participant dogs initially were analyzed for the presence of giardiasis, and the absence of other relevant parasites/pathogenic bacteria via $ZnSO4$ flotation and culture, respectively.

Ethical Statement

This study was approved by the Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (HADYEK) with 64583101/2021/059.

Faecal Examination

All participant dogs were screened to confirming the presence/absence of *Giardia sp*. cysts. At least two consecutive and native smears were prepared, and stool sample was mixed with a 10-15 ml 33% ZnSO4 solution, which were then put into centrifuge tubes. Afterwards immediately centrifuged at 880 x g for 300 seconds, similarly to what has been described elsewhere (Karahallı and Ural 2017). Thereafter to centrifugation, two ml supernatant was handled, then were located on a microscope slide (with Lugol iodine) and analyzed at 40 x magnification for detecting Giardia cysts. This issue was repeated two times for individual samples for every case collected. Furthermore, giardia antigen in canine feces were tested by use of a rapid enzyme immunoassay (SNAP® Giardia Test, IDEXX, USA).

Trace Element Examination

The trace element analysis of serum samples has been carried out by using an inductively coupled plasma-optical emission spectrometry (ICP-OES Thermo iCAP 6000 series) at Trace Element Analysis Laboratory at Biophysics Department of Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa. The parameters of the ICP-OES device for the determination of chromium (Cr), Cu, Fe, magnesium (Mg), Mn, Se, Zn, Co and arsenic (As) elements are presented in Table 1. In the study, as the appropriate wavelengths of Cr, Cu, Fe, Mg, Mn, Se, Zn, Co and as elements were used 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 prepared 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. The serum samples were prepared for trace element measurements as dilute 1:10 with distilled water. Each measurement was performed three times and averages were used for the analysis. Trace element levels were expressed as mg/L.

Statistical analysis

Data obtained from healthy, and giardia infected dogs were tabulated with mean and standard errors. Homogeneity and normality test were performed, and Mann-Whitney U test were used to determine the differences in groups. Statistical Package for Social Science (SPSS 21.0) was used, and P was considered significant if it was less than 0.05.

RESULTS

Native smears were used for detected giardia cyst and trophozoites, revealed trophozoites in 3 out of 8 dogs and cysts were detected in all of the dogs. Supportive diagnosis by use of rapid diagnostic test kits applied to all dogs involved, were entirely positive.

Dogs enrolled in the study were classified as: giardia infected group and healthy ones aged, and sex matched. Serum selected trace elements levels were presented in Table 1. In infected group all cases were suffered from, abdominal pain, weight loss and intermittent diarrhea. In the infected group Cu($P<0.01$), Mg ($P<0.05$) and Zn ($P<0.01$) levels had significant differences to healthy control. Giardia infected dogs had a significant increase in Cu and significant reduces in Mg and Zn levels (Table 1).

Table 1. Serum trace elements levels of dogs infected with giardiasis.

Trace Elements	Giardia ($\bar{X} \pm SE$)	Control ($\bar{X} \pm SE$)	P value
Cr (mg/L)	0.01 ± 0.002	0.009 ± 0.001	0.918
Cu (mg/L)	1.653 ± 0.151	0.100 ± 0.099	**0.001***
Fe (mg/L)	1.636 ± 0.204	1.345 ± 0.136	0.142
Mg (mg/L)	21.570 ± 0.448	23.206 ± 0.372	**0.031****
Mn (mg/L)	0.003 ± 0.001	0.003 ± 0.001	0.918
Se (mg/L)	0.384 ± 0.032	0.309 ± 0.019	0.071
Zn (mg/L)	0.522 ± 0.111	2.427 ± 0.481	**0.000***
Co (mg/L)	0.004 ± 0.001	0.002 ± 0.001	0.731
As (mg/L)	0.193 ± 0.020	0.145 ± 0.012	0.072

$\bar{X} \pm SE$= Mean ± Standard Error, * $P<0.01$, ** $P<0.05$.

DISCUSSION AND CONCLUSION

In the present study the most striking results were evident to those of trace elements analyzed, Cu (mg/L) and Zn (mg/L), presented as ($\bar{X} \pm$ SE), in giardiasis vs. healthy groups [1.653 ± 0.151 vs. 0.100 ± 0.099, P<0.001] and [0.522 ± 0.111 vs. 2.427 ± 0.481, P=0.000], respectively. Those results might be comparable to what has been elucidated in human studies.

In a prior study regarding children, aged 1 to 6 years old, enrolled into Group 1 positive with enteric parasite and Group 2 (n: 60 in each) age and sex matched, without parasitic infection, a significant decrease in serum Zn and increase in serum Cu levels, were determined to those of group 1. Interestingly aforementioned changes in serum level of Zn and Cu were more evident in relation with *G. lamblia* infection in contrast to other parasitic infections (Yones et al. 2015). This finding was also presented in prior research (Schmidt et al. 1996; Bourdon and Blache 2001; Stief 2003). Brief explanation for this issue might involve; the antagonistic effect of Zn and Zn deficiency in relationship with parasitic invasion. From another point of view elevated Cu absorption via the gastrointestinal route might also participate (Yones et al. 2015). Furthermore, Zn is capable of antagonizing Cu absorption; through mechanisms of which induction of thionine synthesis, as the latter possesses increased affinity to Cu than Zn (Fischer et al., 1981). Elevated Cu concentrations denoted free radical generation could be triggered by inflammation. Three previous research (Ertan et al. 2002; Kilic et al. 2010; Yones et al. 2015) all claimed that Cu level was significantly elevated in humans infected with *G. lamblia*. It is unclear that underlying mechanisms should be highlighted, a probable explanation might involve elevated Cu involving enzyme systems (Yones et al. 2015).

In the present study Mg levels (mg/L) 21.570 ± 0.448 were deemed decreased in contrast to healthy dogs presented as 23.206 ± 0.372. Mg has long been known and recognized macro mineral presenting several important issues. In a prior research Mg levels were determined to those 11 diarrheic calves with naturally occurring giardiasis. Mg levels were determined (between 0.15-0.41 mmol/L) in naturally infected calves suggesting decreased Mg levels in comparison to healthy and age-matched calves (0.8-1.6 mmol/L) and hypomagnesemic calves without giardiasis (0.4-0.5 mmol/L). In that study malabsorption and diarrhea were proposed mechanisms for altered Mg levels, which could counteract by blocking of intestinal Mg absorption (Toplu et al., 2016). Consequently, Cu, Mg and Zn levels were affected in giardiasis and the addable of these elements to the treatment should be revealed by further studies.

Financial support

This research received no grant from any funding agency/sector.

Conflict of Interest

The authors declared that there is no conflict of interest.

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