ORIGINAL ARTICLE

A comparison of serum selenium, zinc and copper level in visceral and cutaneous leishmaniasis

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Background: Leishmaniasis is a widespread tropical infection, which has a high incidence rate in Iran. Visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are two forms of this disease. In this study, we investigated if selenium (Se), zinc (Zn) and copper (Cu) levels differ in different forms of leishmaniasis. **Materials and Methods:** To determine if leishmaniasis has effects on trace elements status, they were determined by atomic absorption spectrometry (AAS) in patients (n = 155, 95 CL and 60 VL) and control group (n = 100). **Results:** Our findings indicate that there is a significant difference in the values of Se and Zn between control and patient groups ($P < 0.0001$ and $P < 0.001$, respectively). Se and Zn levels were 3.65 ± 0.88 and 67.24 ± 18.76 μg/dL in the leishmaniasis patients, and these values were observed to be statistically lower compared to the control groups (11.10 ± 2.37 and 119.61 ± 26.18 μg/dL, respectively). Meanwhile, no significant difference in status of Cu was found between the cases (110.55 ± 29.25 μg/dL) and healthy subjects (91.42 ± 27.54 μg/dL) ($P > 0.05$). When the patients were divided into two groups, there appeared to be a significant decrease ($P < 0.001$) in Se concentration for VL patients (2.57 ± 0.64 μg/dL) compared with CL patients (4.33 ± 1.06 μg/dL). **Conclusion:** Based on these results, serum Se and Zn levels could be a useful marker for the pathophysiology of leishmaniasis.

Key words: Antioxidant trace elements, atomic absorption spectroscopy, cutaneous leishmaniasis, trace elements, visceral and cutaneous leishmaniasis

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INTRODUCTION

Leishmaniasis is a parasitic disease with a prevalence of 12 million cases and an approximate incidence of 0.5 million cases of VL and 1.5 million cases of CL.[1] This disease is the second largest parasitic killer in the world (after malaria). Leishmaniasis is transmitted through bite of an insect vector, phlebotomine sand fly.[2] Cutaneous leishmaniasis is the most common form of the disease. It usually produces ulcers on the exposed parts of the body, such as the face, arms and legs. The visceral form, also known as black sickness or kala-azar in Asia, and occurs when parasite migrates to the internal organs such as liver, spleen and bone marrow. It is the most severe form of leishmaniasis and if left untreated, will always result in the death. Leishmaniasis is still a great health problem in Iran. The prevalence of infection has been reported as 1.8% to 37.9% in different provinces of Iran.[3,4]

Selenium (Se), zinc (Zn) and Copper (Cu) are the essential elements that play a crucial role in the immune system. These trace elements act as cofactors for antioxidant enzymes involved in the destruction of toxic free radicals produced in the body. The serum levels of antioxidants vary in many diseases including leishmaniasis. These alterations are part of defense strategies of organism and are induced by different cytokines.[5-7] In this study, we investigated whether the serum levels of Se, Zn, and Cu change in leishmaniasis patients. In addition, the concentration of these elements is compared between cutaneous and visceral forms of leishmaniasis disease.

MATERIALS AND METHODS

A total of 255 volunteers were enrolled in this study, 155 patients (95 CL and 60 VL) and 100 healthy subjects from the same areas who were not exposed to leishmaniasis. All patients infected with leishmaniasis were collected from health service registry of four Provinces of Ghom (hyper endemic), Northern Khorasan (endemic), Esfehan (hyper endemic) and Kerman province (hyper endemic). The patients

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were diagnosed clinically, paraclinically or both and the diagnoses were confirmed by a physician. A questionnaire about the name, sex, habitation area, date of onset, date of diagnosis, number of lesions and location of the lesions was completed for each patient group. Patients who had lesions for 6 months or longer were excluded from the study, because of spontaneous healing and immunity.

All chemicals used in this study were of analytical grade for spectroscopy and purchased from Merck Co. (Germany). All of the materials (glass and plastic) employed were thoroughly cleaned with hot solution of nitric acid (20% v/v) for 48 hours and rinsed thrice with demineralized water. A total of 10 ml of venous blood was withdrawn and transferred into tubes without addition of anticoagulants and centrifuged for 15 min at a speed of 1760 g. Serum was separated for determination of Se, Zn and Cu.

The serum samples were diluted five times with chloric acid (0.1 N) for Zn and Cu measurements. Determination of these elements were performed on a flame atomic absorption spectrometer (FAAS) (SpectrAA 220, Varian, Australia) equipped with deuterium background correction. The corresponding hollow cathode lamps were used as light sources under the optimized conditions indicated in Table 1.[8]

For measurement of Se, samples were diluted 1+4 v/v with 0.1% v/v Triton X-100. Optimization of temperature program for Se determination by graphite furnace atomic absorption (GFAAS) in human serum samples were performed (pyrolysis temperature of 900°C and an atomization temperature 2600°C) [Table 2].[8] For the optimization of pyrolysis and atomization temperatures, a mixture of Pd + $Mg(NO_3)_2$ was used as matrix modifier.[8] In the presence of this modifier, thermal stabilization of Se in pyrolysis step increased.

The accuracy of the measurement was evaluated based on recovery studies and analysis of quality control material (QCM) (Seronorm™ Trace Elements Whole Blood, Level 1, Art. No. 201405, Norway). It was supplied freeze-dried and reconstituted by adding 3 ml of water. Accuracy was 97.5% for Se, 98.8% for Zn and 99.4% for Cu.

In this case control study, summary statistics (n, mean, standard deviation) were calculated. Values were statistically compared using one-way analysis of variance (ANOVA), also considering sex as grouping variable. All results were expressed as mean ± SD, statistical significance was defined as $P < 0.05$. Statistical evaluation was carried out by using the SPSS 11.5 version for windows.

RESULTS

In Table 3, the values of Se, Zn and Cu in the patients suffering with the leishmaniasis were compared to the control group. A significant difference in Se and Zn levels was observed between patients and healthy subjects ($P < 0.0001$ and $P < 0.001$, respectively). Se and Zn levels were found to be 3.65 ± 0.88 and 67.24 ± 18.76 μg/dL in patients, and these values were found to be statistically lower compared with the controls (11.10 ± 2.37 and 119.61 ± 26.18 μg/dL, respectively). Meanwhile, no significant difference in status of Cu was observed between the cases (110.55 ± 29.25 μg/dL) and control group (91.42 ± 27.54 μg/dL) ($P > 0.05$).

As shown in Table 4, when the subjects were divided into two groups, there were no significant variations ($P > 0.05$) in serum Zn and Cu concentrations between the CL (70.23 ± 19.12 and 107.68 ± 29.16 μg/dL, respectively) and the VL patients (62.50 ± 18.19 and 115.09 ± 29.39 μg/dL, respectively); however, there appeared to be a significant decrease ($P < 0.001$) in Se concentration for VL patients (2.57 ± 0.64 μg/dL) compared with CL patients (4.33 ± 1.06 μg/dL) [Table 4].

DISCUSSION

In this study, we have shown that patients with leishmaniasis had significantly lower status of Se and Zn compared with control groups ($P < 0.0001$ and $P < 0.001$, respectively). This result is according to the reported articles published elsewhere in the world.[5,9,10] A comparison of antioxidants status in CL and VL shows that the concentration of Se significantly reduces in patients suffering with VL. Our findings suggest that the decreased contents of Se and Zn may be a part of the defense strategies of the organism.

Reactive oxidative species (ROSs) that are generated during the respiratory burst by macrophages can protect against parasite attack.[11] ROSs such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals are generated as a host defense mechanism. Se acts as cofactor of glutathione

Table 1: Instrument settings for determining of Se, Zn and Cu in human serum by AAS

Element	Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Se	Concentration	Peak height	196.0	1	10
Zn	Concentration	Integration	213.9	1	5
Cu	Concentration	Integration	324.8	0.5	4

Table 2: Furnace optimized parameters for analysis of Se in serum by GFAAS

Element	Step	Temperature (°C)	Time (s)	Argon flow-rate (L/min)
Se	Drying	85	5.0	3
	Pre-last drying	95	40.0	3
	Post-last drying	120	10.0	3
	Ashing	900	5.0	3
	Ashing	900	1.0	3
	Gas stop	900	2.0	0
	Ramp stop	2600	0.8	0
	Atomization	2600	2.0	0
	Tube clean	2800	2.0	3

Table 3 Biochemical data (mean ± SD) in patients with leishmaniasis and control groups

Element	leishmaniasis (n = 155)	Control (n = 100)	P value
Selenium (µg/dL)	3.65±0.88	11.10±2.37	<0.0001
Zinc (µg/dL)	67.24±18.76	119.61±26.18	<0.001
Copper (µg/dL)	110.55±29.25	91.42±27.54	>0.05

Statistical significance was defined as $P < 0.05$; SD = Standard deviation

Table 4: Comparison of Se, Zn and Cu concentrations (mean ± SD) in patients with cutaneous and visceral leishmaniasis

Element	cutaneous leishmaniasis (n = 95)	visceral leishmaniasis (n = 60)	P value
Selenium (µg/dL)	4.33±1.06	2.57±0.64	<0.001
Zinc (µg/dL)	70.23±19.12	62.50±18.19	>0.05
Copper (µg/dL)	107.68±29.16	115.09±29.39	>0.05

Statistical significance was defined as $P < 0.05$; SD = Standard deviation

peroxidase enzyme (GSH-Px) to protect the body from ROSs that are produced during macrophage activity. In leishmaniasis patients the GSH-Px activity was lower than the controls.[12] The decreased activity of GSH-Px reflects an inefficient removal of hydrogen peroxide from the cellular milieu.[13]

Decreasing serum Zn levels apparently result from the synthesis of methallothionein (MT) in liver and other tissues. Methallothionein binds Zn and serves to draw Zn away from free circulating pools; it was produced by hormone-like substances interleukin 1 (IL-1) and tumor necrosis factor-alpha (TNF-α) that were induced by leishmaniasis.[14]

When comparing the patient groups, our results indicate significant decrease of Se in severe form of leishmaniasis (VL). So, we suggest severity of leishmaniasis can affect the serum Se status. In VL patients, there was severe oxidative stress accumulating ROSs.[15] Increased ROSs not only kill the parasites but also damage the cells.

The findings of such studies indicate a strong association of Se and Zn with leishmaniasis. A strategy can be devised to use serum Se and Zn concentrations as a means for estimating the prognosis of leishmaniasis.

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