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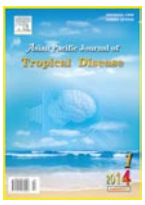

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Alterations of serum antioxidant trace elements (Se, Zn and Cu) status in patients with cutaneous leishmaniasis

Leila Farzin*, Mohammad Esmail Moassesi, Fattaneh Sajadi

Environmental Laboratory, Nuclear Science Research School, Nuclear Science & Technology Research Institute, Atomic Energy Organization of Iran (AEOI), P.O. Box: 11365-3486, Tehran, Iran

PEER REVIEW

Peer reviewer

Hassan Borji, Associate Professor, Department of pathobiology, University of Ferdowsi of Mashhad, Iran.
Tel: +98 5118805606
Fax: +98 5118763852
E-mail: hborji@um.ac.ir

Comments

This is a fair study in which the authors evaluated metal concentration in cutaneus leishmanniosis. Although the authors used a simple statistical method to analyze the data, such analyses should be encouraged. The findings are potentially very important. Details on Page S448

ABSTRACT

Objective: To assess the serum antioxidant trace elements selenium (Se), zinc (Zn) and copper (Cu) in cutaneous leishmaniasis patients.
Methods: In this study, serum Se, Zn and Cu was determined by using atomic absorption spectrometry in patients with cutaneous leishmaniasis (n=95). The values were statistically compared between patients and control group (n=100) using One-way analysis of variance (ANOVA).
Results: Our results showed that there was a significant difference in the values of Se and Zn between two groups (P<0.0001 and P<0.01, respectively). Meanwhile, no significant difference in level of Cu was observed between patients and healthy subjects (P>0.05). Se and Zn levels were found to be (4.33±1.06) and (70.23±19.12) μg/dL in cutaneous leishmaniasis cases, and these values were found statistically lower compared to the controls (11.10±2.37) and (119.61±26.18) μg/dL, respectively.
Conclusions: The observations that host products are released from stimulated leukocytes and could induce metabolic changes similar to an acute-phase response revealed an endocrine role for the immune system. Characteristic changes in trace-mineral metabolism are an integral part of the acute-phase response. The changes are usually reflected in decreased serum Se and Zn concentrations.

KEYWORDS

Cutaneous leishmaniasis; Antioxidant trace elements; Atomic absorption spectroscopy

1. Introduction

Many of diseases specifically associated with the tropics are caused by parasites. Parasites can be single-cellular celled (protozoa) or else multi-cellular and live within or on other organisms. Parasites exert an appalling toll on human health, causing diseases like leishmaniasis, malaria and sleeping sickness.

Leishmaniasis is caused by the parasitic *Leishmania* protozoa. These parasites are carried by the blood-sucking sandfly. Once the parasites are transmitted to humans or animals through a sandfly bite, the host's immune system attempts to consume the protozoa with immune cells called macrophages. Usually this defeats infection, but the *Leishmania* protozoa are capable of surviving and multiplying within the macrophages. Ultimately, these macrophages burst open, releasing the protozoa and allowing them to take over neighboring cells. This idea that cutaneous leishmaniasis was transmitted by man-biting insects of the genus *Phlebotomus* was suggested for the first time in 1905 by Sergent *et al*[1].

This disease became known as leishmaniasis after William Leishman, a Glasgwegian doctor serving with the British Army in India, developed one of the earliest stains of *Leishmania* in 1901[2]. Cutaneous leishmaniasis (CL) is the most common form of the leishmaniasis. It usually produces ulcers on the exposed parts of the body, such as the face, arms and legs. CL incidence ranges were estimated by country and epidemiological region based on reported incidence, underreporting rates if available and the judgment of national and international experts. Based on these estimates, approximately 0.7 to 1.2 million CL cases

*Corresponding author: Leila Farzin, Environmental Laboratory, Nuclear Science Research School, Nuclear Science & Technology Research Institute, Atomic Energy Organization of Iran (AEOI), P.O. Box: 11365-3486, Tehran, Iran.
Tel/Fax: +98 21 88221121
E-mail: LFarzin84@yahoo.com
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occur each year[3]. CL is more widely distributed, with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean Basin and Western Asia from the Middle East to Central Asia. The ten countries with the highest estimated case counts, Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru, together account for 70 to 75% of global estimated CL incidence. This disease 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[4,5]. Numerous studies over the past two decades indicate that trace elements play an important role in human health and disease. Trace elements that support antioxidant function, particularly selenium (Se), zinc (Zn) and copper (Cu) may be associated with a reduction in mortality in critically ill patients. Se, Zn and Cu are essential trace elements for all organisms and body growth and development is strictly dependent on these elements. They also play a role in supporting the immune system and affecting the integrity of the epithelial barrier and the function of white blood cells. The biologic role of Se, Zn and Cu is now recognized in structure and function of proteins, including enzymes, transcription factors, hormonal receptor sites and biologic membranes[6]. The trace elements essentially act as cofactors for antioxidant enzymes involved in the destruction of toxic free radicals produced in the body. The changes in the levels of trace elements are part of immunity of organism and are induced by different cytokines[7]. This study therefore, considers the assessment of Se, Zn and Cu in patients suffering from cutaneous leishmaniasis.

2. Materials and methods

2.1. Patients and controls

A total of 195 volunteers were enrolled in this study, 95 patients [mean age (37.2±10.2) years] suffering from CL and 100 healthy subjects [mean age (35.7±11.4) years] from the same areas who were not exposed to CL.

From April 2012 to December 2012, 95 patients infected with cutaneous leishmaniasis were collected from health service registry of three provinces of Ghom, Northern Khorasan and Esfehan. The patients were diagnosed paraclinically and the diagnosis was 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 study, because of spontaneous healing and immunity.

2.2. Laboratory measurements

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 was thoroughly cleaned with hot solution of nitric acid (20% v/v) for 48 h and rinsed three times with demineralized water. A total of 10 mL 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 mol/L) for Zn and Cu measurements. Determination of these elements were performed on a flame atomic absorption spectrometer (SpectrAA 220, Varian, Australia) equipped with deuterium background correction. Se was determined by the graphite furnace atomic absorption spectrometry (SpectrAA 220, GTA 110, Varian, Australia) equipped with pyrolytically coated graphite tubes and deuterium background correction. The corresponding hollow cathode lamps were used as light sources with each instrument operated under the optimized conditions indicated in Table 1.

Table 1
Instrument settings for determining of Se, Zn and Cu in human serum by atomic absorption spectrometry.

Element	Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Se	Concentration	Peak height	196.0	1.0	10
Zn	Concentration	Integration	213.9	1.0	5
Cu	Concentration	Integration	324.8	0.5	4

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 spectrometry in human serum samples were performed (pyrolysis temperature of 900 °C and an atomization temperature 2 600 °C) (Table 2). 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 modifier, thermal stabilization of Se in pyrolysis step increased.

Table 2
Furnace optimized parameters for analysis of Se in serum by graphite furnace atomic absorption spectrometry.

Step	Temperature (°C)	Time (s)	Argon flow-rate (L/min)
Drying	85	5.0	3
Pre last drying	95	40.0	3
Post last drying	120	10.0	3
pyrolysis	900	5.0	3
Pyrolysis	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

The accuracy of the measurement was evaluated based on recovery studies and analysis of quality control material (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.

2.3. Statistical analysis

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 taking into account 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[9].

3. Results

Biochemical data for the total subjects are presented in Table 3. A significant difference in Se and Zn levels was observed between patients and control group ($P<0.000\,1$ and $P<0.01$,

respectively). Se and Zn levels were found to be (4.33±1.06) and (70.23±19.12) μg/dL in patients with CL, and these values were found statistically lower compared to 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 cases with CL [(107.68±29.16) μg/dL] and healthy subjects [(91.42±27.54) μg/dL] (P>0.05).

Table 3

Biochemical data in patients with cutaneous leishmaniasis and control groups (mean±SD).

Element	Cutaneous leishmaniasis group (n=95)	Control group (n=100)	P value
Se (μg/dL)	4.33±1.06**	11.10±2.37	<0.000 1
Zn (μg/dl)	70.23±19.12*	119.61±26.18	<0.01
Cu (μg/dl)	107.68±29.16	91.42±27.54	>0.05

*: P<0.01, **: P<0.0001 compared with the control group.

4. Discussion

Old World cutaneous leishmaniasis, known as oriental sore, is an ancient disease and can be traced back many hundreds of years. This disease is characterized by single or multiple localized lesions on exposed areas of skin that typically ulcerate. CL is believed to be an autochthonous disease (natural to an area or country, *i.e.* not imported).

There are two general classes of abnormality associated with trace elements: abnormality as a result of a specific deficiency from dietary inadequacies and imbalances and abnormality secondary to other diseases. Both kinds of abnormality can be diagnosed by analysis of trace elements in serum or other tissues. Furthermore, secondary changes occur as a result of diseases; these changes are not exactly understood. In this study, we have shown that patients infected with CL had significantly lower status of Se and Zn than in controls (P<0.000 1 and P<0.01, respectively). The observed differences in the serum levels of these trace elements in patients support the results of previous articles published in the world[10,11]. Pourfallah *et al.*[11] reported that serum Cu concentration (P<0.05) in the patients was significantly higher than that of the healthy subjects. Meanwhile in this study, no significant difference in level of Cu is observed between two groups (P>0.05).

Trace elements like Se, Zn and Cu have a significant influence on the function of the immune system. In recent years, several studies have linked the concentrations of trace elements to various infectious diseases[12-16].

Our findings suggest that the decreased contents of Se and Zn may be a part of the defense strategies of the organism. Intensive research has focused on the mechanism(s) by which defense cells kill microrganisms. We know that nonspecific products are generated during the respiratory burst by macrophages to kill protozoa. Once parasites inoculated in the skin are phagocytosed by macrophages which in turn produces reactive oxygen species (ROSs) such as superoxide anion radicals ($O^{2-}\cdot$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·) as a host defense mechanism. It has recently come to light that ROSs are involved in intracellular killing and suppression of each radicals inhibits macrophage activity[17]. ROSs have been shown to kill protozoa such as *Toxoplasma gondii*, *Leishmania* and *Trypanosoma cruzi*[18].

Se is essential nutrient for humans, being necessary for activity of several enzymes, such as glutathione peroxidase (GSH-Px). GSH-Px is a glutathione recyling enzyme that catalyses the oxidation of reduced glutathione by H_2O_2 and other hydroperoxides to form oxidized glutathione and water[19,20]. The activity of GSH-Px was lower in CL patients[21,22]. The decreased activity of GSH-Px reflects an inefficient removal of H_2O_2 from the cellular milieu[23]. However, it is not known whether the causes of this depletion were dependent on Se content or on other factors. We were able to find any report on Se status and the relationship between this element and GSH-Px activity in patients with CL.

The role of certain inflammatory products in the regulation of the Zn balance has been well documented. Thus, leukocyte-endogenous mediators (interleukins) released from activated phagocytic cells, induce hypozincemia in experimental animals by the redistribution of Zn from plasma to the liver[24]. Decreasing serum Zn levels apparently results from the synthesis of metallothionein in liver and other tissues. Metallothionein binds Zn and serves to draw Zn away from free circulating pools; it was induced by hormone-like substances interleukin 1 and tumor necrosis factor-alpha (TNF-α)[25].

As seen above, the alteration of serum Zn probably depends on cytokines, especially interleukin 1 and TNF-α. Some observations have shown that the production of interleukin 1 and TNF-α were induced by CL[26]. Additionally, TNF-α appears to exert its leishmanicidal activity by activating macrophages, rather than by directly activating the parasite[27]. It was demonstrated that incubation of macrophages with TNF-α in the presence of bacterial lipopolysaccaride resulted in leishmanicidal activity[28].

In conclusion, the patients suffering from CL are in oxidative stress. Parasite invading the macrophages causes respiratory burst releasing different reactive oxidative species as a host defense. It is expected that the organism generates increased amounts of ROSs, prevents damage by killing protozoa as a host defense strategy. Lack of Se leads to a deficiency of GSH-Px enzyme activity and consequently, to a decreased ability to degrade H_2O_2. In addition, we concluded that serum Zn concentration was probably altered by the some immunocytokines as a host-defense strategy of organism during CL infection.

Our findings indicate a strong association of Se and Zn with cutaneous leishmaniasis. This study shows that Se and Zn may play an important role in the pathophysiologic processes of CL. More data are needed to properly define the role of these elements as a factor in the pathophysiology in patients with CL. A strategy can be devised to use serum Se and Zn concentration as a means for estimating the prognosis of CL. In addition, we postulate that oral Se and Zn supplementations may have an additive effect in the chemotherapy and prevention of CL.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Leishamaniasis disease is of great concern in public health. It is the third most common vector-borne disease and a very important protozoan infection. This study tries to determine Se, Zn and Cu in patients suffering from CL.

Research frontiers

Studies are being performed in order to determine occurrence of oxidative stress in CL.

Related reports

There is lots of published data in this field which you could follow some of them: Kocyigit A *et al*. (1998) analysed the alterations of serum selenium, zinc, copper, and iron concentrations and some related antioxidant enzyme activities in patients with CL. Ozbilge H *et al*. (2005) evaluated oxidative stress in CL. Serarslan G *et al*. (2005) determined serum antioxidant activities, malondialdehyde and nitric oxide levels in human CL.

Applications

Based on the results of this study, some information are available which can be useful for treatment strategies.

Peer review

This is a fair study in which the authors evaluated metal concentration in cutaneus leishmanniosis. Although the authors used a simple statistical method to analyze the data, such analyses should be encouraged. The findings are potentially very important.

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