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Low Serum Levels of Selenium, Zinc, Iron, and Zinc/Copper Ratio in an Endemic Region of Cutaneous Leishmaniasis in Southwest Iran

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Abstract

Leishmaniasis is a widespread tropical infection; cutaneous leishmaniasis (CL) is the most common form of this disease known to cause significant morbidity. Trace metals, including selenium, zinc, iron, and copper, are required for the activity of several enzymes involved in immune system responses. The aim of this research was to measure the serum levels of selenium (Se), zinc (Zn), copper (Cu), iron (Fe), and Zn/Cu ratio in patients with CL. In this case-control study, 80 patients with CL and 80 healthy volunteers (not exposed to CL) from a CL endemic region in southwest Iran agreed to participate. Both clinical and parasitological verifications were made to include each subject as a CL-positive case. A questionnaire was completed for each participant which included the following criteria: age (year), height (cm), weight (kg), body mass index (kg/m^2), and duration of disease (day). The biochemical assays were performed according to the standard protocols, and the values of Zn, Cu, Se, and Fe were expressed in micrograms per deciliter (μg/dl). All results were expressed as mean ± standard deviation (SD), and the statistical significance level was defined to be less than 0.05 ($P < 0.05$). There were no statistically significant differences in terms of mean values of age, body weight, height, and body mass index between CL patients and the control group ($P > 0.05$). The mean ± SD concentrations of Zn, Fe, and Se in the control group were found to be 118.87 ± 6.35 μg/dl, 123.00 ± 8.40 μg/dl, and 11.26 ± 1.88 μg/dl, respectively. These trace elements (TEs) were statistically lower ($P < 0.001$) in patients with CL (case group) with values of 83.05 ± 7.32 μg/dl for Zn, 86.51 ± 10.09 μg/dl for Fe, and 3.83 ± 1.20 μg/dl for Se. We have also observed that serum levels of Cu in CL-positive group were significantly higher than in the controls ($P < 0.001$). Furthermore, CL patients had significantly lower Zn/Cu ratio than controls (0.63 ± 0.05 μg/dl *vs.* 1.11 ± 0.10; $P < 0.001$). The alternation in serum levels of TEs may be a part of the defense strategy of the organism. Based on these results, it can be suggested that serum levels of these TEs can be a useful marker to estimate the prognosis of CL infection.

Keywords Cutaneous leishmaniasis · Trace elements · Selenium · Zinc · Copper · Iron · Iran

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Abbreviations

BMI	Body mass index
CL	Cutaneous leishmaniasis
Cu	Copper
DALYs	Disability adjusted life years
EMR	Eastern Mediterranean Region
Fe	Iron
GSH-Px	Glutathione peroxidases
H_2O_2	Hydrogen peroxide
IFN-γ	Interferon-γ
IL-1	Interleukin 1
MCL	Mucocutaneous leishmaniasis
MT	Metallothionein
SD	Standard deviation
Se	Selenium
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences

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TEs	Trace elements
TNF-α	Tumor necrosis factor alpha
VL	Visceral leishmaniasis
WHO	World Health Organization
Zn	Zinc

Introduction

Leishmaniasis is a vector-borne infection caused by over 20 different species of obligate intracellular parasites of the genus *Leishmania* (family *Trypanosomatidae*, order *Kinetoplastida*). These infections are considered a serious public health problem affecting approximately 12 million people worldwide particularly in the tropical and subtropical regions [1–3]. According to the clinical signs and/or symptoms, the infection can be classified into three different categories: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) [1, 4, 5]. Unfortunately, there is still no licensed vaccine against leishmaniasis [6]. Pentavalent antimonials such as meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) have been administered since the 1940s for the treatment of all types of leishmaniasis and are still the first-line drugs used in most developing countries [7, 8].

During the past three decades, several publications have indicated that trace elements (TEs) are vital for human health [9–12]. Any imbalance in the amounts of TEs either by excess or deficiency may be seen in serum. Alterations of serum levels of iron (Fe), zinc (Zn), copper (Cu), selenium (Se), and other TEs have been assessed by investigators in several pathological conditions, including pneumonia, hemolytic anemia, rheumatic heart diseases, congestive heart failure, psoriasis, bronchitis, and some infectious diseases [9, 12–14].

Iron is an essential TE as it plays a critical role in oxygen transport to tissues as well as oxidative phosphorylation and metabolite oxidation in cells. This TE is pivotal not only for the metabolic functions of human and animals, but it is also an indispensable element in the life of protozoa and bacteria [15–17]. Copper is involved in the activity of several metalloenzymes, including cytochrome oxidase, dopamine-β-hydroxylase, ascorbic acid oxidase, tyrosinase, ferroxidase, amine oxidase, superoxide dismutase (SOD), and tyrosinase. The maintenance of proper Cu levels in the blood is very important as part of the defense mechanism and contributes to apoptosis, host metabolism, membrane stability, and enzyme activity. Copper also plays an indispensable role in the immune response to infection. Elevated serum Cu levels have been observed in some infectious and hepatic diseases. Thus, tracking serum Cu levels could be a useful strategy to monitor the progression of some diseases [9, 14, 18, 19]. Zinc is considered an efficient anti-inflammatory and antioxidant agent. This TE acts as a cofactor in over 200 metalloenzymes and other metalloproteins involved in biological systems, wound healing process, metabolic activities, and immunity [12]. The central role of zinc is clearly evident in the immune system. Its deficiency can interfere with the function of some cells of the immune system such as T lymphocytes [20, 21].

In this framework, the aim of the current research was to evaluate the serum levels of Se, Zn, Cu, Fe, and Zn/Cu ratio in CL patients and compare them with healthy individuals.

Materials and Methods

Sampling and Patients

In the current case-control study, a total of 160 volunteers agreed to participate, 80 patients with CL (cases group) and 80 healthy volunteers (control group) who were not exposed to CL. The 80 cases infected with CL were collected from health centers affiliated to the Abadan Faculty of Medical Sciences of two endemic cities (Abadan and Khorramshahr) in the southwest of Iran [22]. The 80 controls enrolled were defined as healthy individuals who have been living in the same area and at the same time with no previous history of CL.

The matching process was strictly performed by expert physicians for age criteria in both case and control groups to minimize bias in the results. Each CL-positive patient (case subject) was included in the study after they had undergone both clinical (by an expert physician) and parasitological (by trained personnel) confirmations. Laboratory confirmation was obtained for each case subject from a simple direct smear taken from the margins of the lesion to detect amastigote forms of the *Leishmania* species (*L. major* and *L. tropica*). The obtained samples were placed on a clean slide, stained with Giemsa, and examined with an optical microscope under × 1000 magnification. It should be noted that subjects with CL for 6 months or longer were excluded from the study due to normal healing and immunity ongoing processes.

Ethical Statement

All subjects in the case and control groups agreed to participate voluntarily in this study. A signed written informed consent was obtained from all individuals and parent or guardians of children under 15 years prior to their participation. We have followed all ethical considerations after receiving the approval from the Abadan Faculty of Medical Sciences Ethical Committee (IR.ABADANUMS.REC.1398.091).

Questionnaire

A questionnaire was completed for each participant which includes the following criteria, age (year), height (cm), weight (kg), body mass index (kg/m^2), and duration of disease (day). The last parameter was registered only for CL patients. All information gathered was imported to the Statistical Package for the Social Sciences (SPSS) software (version 21) (SPSS Inc., Chicago, IL, USA) for further analysis.

Biochemical Assays

All reagents and chemicals used in the current study were of analytical grade. All the materials either glass or plastic were thoroughly cleaned with a hot solution of nitric acid (20% v/v) for approximately 48 h and rinsed several times with demineralized water. A total of 10 ml of venous blood was taken from each subject and immediately transferred into acid-washed test tubes without any anticoagulant. The samples were centrifuged at 3000 rpm for 15 min at room temperature to separate the serum. The serum samples were aliquoted into microtubes and stored at − 20 °C until analyzed for determination of Se, Zn, Cu, and Fe in the central laboratory of the Abadan Faculty of Medical Sciences.

The biochemical assays were standardized according to the described protocols. The measurement of Cu and Zn were done as previously described by Farzin et al. [23]. Briefly, serum samples were diluted five times with chloric acid (0.1 N) and used in a flame atomic absorption spectrometer (FAAS) equipped with deuterium background correction to determine the concentrations of these TEs. For determination of Se, the samples were diluted 1 + 4 v/v with 0.1% v/v Triton X-100. The Se levels were quantified by graphite furnace atomic absorption spectrometry (GFAAS) equipped with pyrolytically coated graphite tubes and deuterium background correction [23]. The levels of Fe in serum were examined using an auto -analyzer (Model: Hitachi 911, Japan) with a commercially available kit based on the manufacturer's instruction. The values of Zn, Cu, Se, and Fe were expressed in micrograms per deciliter (μg/dl).

Statistical Analysis

Data analysis was performed using the SPSS software (SPSS Inc., Chicago, IL, USA) for Windows (version 21). For this purpose, the mean and standard deviation (SD) of all the measured values were computed to report the results. Furthermore, the values were statistically compared using Student's *t* test. All results were expressed as mean ± SD and the statistical significance was set at the 0.05 level ($P < 0.05$).

Role of the Funding Source

The funder of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Subjects

The population group in this study consisted of 80 patients with CL and 80 healthy individuals. As shown in Table 1, the participants in both patient and control groups were similar in terms of age with mean values of 31.98 ± 6.84 and 31.28 ± 7.40 years, respectively ($P > 0.05$). Furthermore, no statistically significant differences were recorded in the average values of body weight (kg), height (cm), and BMI (kg/m^2) between the two groups ($P > 0.05$), indicating a similar composition between the case and control groups without any confounding factor in terms of physical parameters. As an example, the observed mean values of BMI were 24.75 ± 4.43 kg/m^2 for CL patients and 24.68 ± 4.01 kg/m^2 for healthy subjects ($P > 0.05$). The physical characteristics of CL patients and control groups are summarized in Table 1.

Biochemical Assay

In this study, we have measured the serum levels of Zn, Cu, Fe, and Se and calculated the Zn/Cu ratio in CL patients and healthy volunteers. According to the results summarized in Table 2, the mean serum concentrations of Zn, Fe, and Se in CL patients (83.05 ± 7.32, 86.51 ± 10.09, and 3.83 ± 1.20 μg/dl, respectively) were significantly lower when compared with the values observed for the control group (118.87 ± 6.35, 123.00 ± 8.40, and 11.26 ± 1.88 μg/dl, respectively) ($P <$

Table 1 Physical characteristics of CL patients and control group

	Patient group (n = 80) Mean ± SD	Control group (n = 80) Mean ± SD	P value*
Age (year)	31.98 ± 6.84	31.28 ± 7.40	$P > 0.05$
Height (cm)	161.69 ± 7.52	163.33 ± 8.72	$P > 0.05$
Weight (kg)	64.13 ± 8.61	65.23 ± 7.35	$P > 0.05$
Body mass index (kg/m^2)	24.75 ± 4.43	24.68 ± 4.01	$P > 0.05$
Duration of disease (day)	31.51 ± 2.76	N/A	N/A

*No significant difference between the control and patient groups at $P > 0.05$

N/A not applicable

Table 2 Comparison of serum concentrations of zinc, copper, iron, and selenium and Zn/Cu ratio in CL patients and the control group

Serum concentration (mean ± SD)	Patient group (n = 80) Mean ± SD	Control group (n = 80) Mean ± SD	P value*
Zn (μg/dl)	83.05 ± 7.32	118.87 ± 6.35	$P < 0.001$
Cu (μg/dl)	131.82 ± 4.81	106.95 ± 7.61	$P < 0.001$
Fe (μg/dl)	86.51 ± 10.09	123.00 ± 8.40	$P < 0.001$
Se (μg/dl)	3.83 ± 1.20	11.26 ± 1.88	$P < 0.001$
Zn/Cu ratio	0.63 ± 0.05	1.11 ± 0.10	$P < 0.001$

*No significant difference between the control and patient groups at $P < 0.05$

0.001). On the other hand, we observed that Cu level was higher in CL patients (131.82 ± 4.81 μg/dl) than in the control group (106.95 ± 7.61 μg/dl) ($P < 0.001$). The Cu/Zn ratio was significantly lower in CL patients (0.63 ± 0.05) when compared with the healthy controls (1.11 ± 0.10) ($P < 0.001$). Comparison of serum Zn, Cu, Fe, and Se concentrations and the Zn/Cu ratio in both case and control groups are presented in Table 2.

Discussion

There are evidences in the literature supporting the association between essential TEs and tropical infections [11, 19]. In general, there are two main causes associated with abnormal levels of essential TEs, including (a) a specific deficiency arising from genetic defects, inadequate intake, excessive exposure, or impaired elimination and (b) imbalances and transient alterations as a result of several pathological conditions including infections. In both cases, the status of TEs can be measured in serum samples or other tissues. However, the imbalances and transient alterations in the levels of TEs due to some diseases are still not exactly understood [14, 19, 24]. TEs play key roles in different metabolic and physiological activities in the human body. They act as vital components in the synthesis and structural stabilization of proteins and nucleic acids. In addition, TEs are necessary for the activity of many enzymes involved in immune system response such as catalase, SOD, and glutathione peroxidase (GSH-Px) among others. Thus, any imbalance in the normal levels of the essential TEs adversely affects the biological processes [12, 25, 26].

In this research, we assessed the serum levels of Se, Zn, Cu, and Fe and determined the Zn/Cu ratio in CL patients and healthy subjects. As shown in Table 1, malnutrition, which can compromise the immune status of CL patients, was not found to be a potential issue because the physical characteristics such as height, weight, and BMI were not statistically different between the case and control groups. We have found that CL patients presented significantly lower serum Zn levels compared with the control group ($P < 0.001$). Our findings are in accordance with the previous studies where low serum zinc levels have been reported in CL [19, 25, 26] and VL [19, 24] patients. The relevance of zinc balance in the proper functioning of the immune system and the role of some inflammatory products in its regulation has been established [27]. Several immunocytokines (interleukins) are secreted by activated macrophages in response to several stimuli (including infection) and induce a redistribution of Zn in the organism as part of the defense strategy during the course of infection. Decreasing serum Zn levels result from increased synthesis of metallothionein (MT) in the liver induced by interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α). MT binds Zn and helps to remove Zn from serum to the liver and other tissues [24, 28–30]. Zinc deficiency enhances TH2 cytokines and leads to selective reduction in TH1 response and thereby results in the decrease of interferon-γ (IFN-γ) production [20, 21].

In this study, we observed that Cu concentration was significantly higher in the serum from CL patients than from healthy subjects ($P < 0.001$). Higher serum Cu level may be the result of the inflammation associated with the infection and may increase resistance to leishmaniasis [28]. It is believed that the enhanced Cu level in serum is correlated with an increase in the synthesis of the copper-binding protein, known as ceruloplasmin which is induced by IL-1 [27, 30]. It is documented that IL-1, but not TNF-α, induces hypercupremia following injection into the preoptic anterior hypothalamus [27, 31]. Under certain circumstances, the serum Cu levels tend to rise above the normal values to facilitate pivotal activities, including Cu-ATPases, which are required for liver function, connective tissues, central nervous system development, and several physiological processes. [24]. It has long been known that there are marked changes in the serum levels of TEs in the course of an infection [15, 16, 24]. These changes are a very important part of the acute-phase response within the frame of the defense strategies of the organism induced by IL-1, IL-6, and TNF-α [19, 27, 32–34]. Our results showed that Zn/Cu ratio was significantly lower ($P < 0.001$) in CL patients (0.63 ± 0.05) when compared with the healthy subjects (1.11 ± 0.10) which is in accordance with the recent published papers on CL [19, 25] and VL [19]. It has been

suggested that Zn/Cu imbalance changes the direction of T lymphocytes toward a TH2-type response and decreases TH1 cytokines, serving as a marker for immunodeficiency in leishmaniasis being more pronounced in the most severe and possibly fatal visceral form [35]. In general, with decreased serum Zn and increased serum Cu, humoral response (TH2) is elevated, resulting in a decrease in IFN-γ production. This imbalance could contribute to the clinical chronic state of leishmaniasis [24, 35].

The results of this study showed that CL patients had significantly lower serum Se levels than controls ($P < 0.001$); similar results were obtained in previous studies [19, 26]. Selenium is an indispensable micronutrient for humans and animals, being essential as a cofactor for some enzymes such as GSH-Px, a glutathione recycling enzyme which catalyzes the oxidation of reduced glutathione and other hydroperoxides by hydrogen peroxide (H_2O_2) to form oxidized glutathione and water. Any decrease in GSH-Px function leads to incomplete removal of H_2O_2 from cells [10, 36]. Biswas et al. [37] reported that GSH-Px activity was lower in patients with *L. donovani*. They advocate that "decreased activities of the protective enzymes suggest impairment of the defense mechanism against peroxidative threat" [37].

In the current study, we observed that the serum Fe levels were significantly lower in CL patients compared with the controls ($P < 0.001$); these results are consistent with those observed in earlier studies by others authors [25, 26]. Barollo et al. [38] stated that iron may play a major role in chronic inflammatory diseases. The decreased serum Fe level, known as hypoferremia, is part of the host acute-phase response to infection and inflammation aiming to reduce Fe availability to pathogens. During this process, IL-1 is released from activated phagocytes and induces the production of apolactoferrin by granulocytes. This enzyme mediates the removal of Fe transferrin from the serum sequestering Fe within the intracellular stores of the reticuloendothelial system in different tissues such as liver, spleen, and bone marrow. The serum Fe redistribution causes anemia during infection but maintains Fe nutritionally unavailable to parasites [17, 39, 40]

Conclusions

In conclusion, we have observed that CL patients had significantly lower serum levels of Zn, Fe, Se, and Zn/Cu ratio compared with the control group. Taking everything into account, our results suggest that the status of serum levels of the studied TEs in CL patients probably depend on IL-1 and TNF-α cytokines secreted by activated macrophages as part of the organism defense strategy in response to infection. In this study, we have not measured the presence or the levels of these cytokines in the serum of the participating subjects. However, other studies have reported that the production of IL-1 and TNF-α were enhanced by *Leishmania* infection [41, 42]. It seems that the Zn/Cu ratio is a beneficial marker for immune dysfunction in CL. Our results indicate that there is a significant association between TEs' (Se, Zn, Fe, Cu, and Zn/Cu ratio) status and CL. Thus, the assessment of TEs' profile in the serum of CL patients may be an essential tool for estimating the prognosis of the infection.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical statement All subjects voluntarily agreed to participate. A written informed consent was obtained from adult persons and the parent or guardian of children less than 15 years old. This study received the approval from the Abadan Faculty of Medical Sciences Ethical Committee (IR.ABADANUMS.REC.1398.091). Ethical issues (including plagiarism, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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