Original Article

Evaluation of Serum Levels of Zinc, Copper, Iron, and Zinc/Copper Ratio in Cutaneous Leishmaniasis

*F Pourfallah[1], S Javadian[1], Z Zamani[1], R Saghiri[1], S Sadeghi[1], B Zarea[2], Sh Faiaz[1], F Mirkhani[1], N Fatemi[1]

[1]Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
[2]Qom Central Hospital, Qom, Iran

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Abstract

Background: The purpose of this study was to evaluate the levels of zinc (Zn), copper (Cu), iron (Fe) and zinc/ copper ratio in the serum of patients with cutaneous leishmaniasis in Qom Province, center of Iran.

Methods: Serum levels of zinc and copper were determined by flame atomic absorption spectrophotometer and serum iron concentration was measured by using an Auto Analyzer. The study group consisted of 60 patients with cutaneous leishmaniasis and the control group of 100 healthy volunteers from the same area who were not exposed to cutaneous leishmaniasis.

Result: There were no statistically significant differences in age and body mass index between the two groups. Serum Zn ($P< 0.001$) and Fe ($P< 0.05$) levels were lower in patients with cutaneous leishmaniasis than the control group. We also found serum Cu concentration ($P< 0.05$) in the patient group was significantly higher than that of the control group. However, zinc/ copper ratio ($P< 0.001$) was lower in patients with cutaneous leishmaniasis than in the control group.

Conclusion: Our data indicated that Zn/Cu ratio was significantly lower in patients with CL as compared to the controls. Earlier reports suggest that, this ratio imbalance could be a useful marker for immune dysfunction in leishmaniasis. There was also strong association of Zn, Cu and Fe with CL. It suggests the use of blood zinc, copper, iron concentration and the copper/zinc ratio (Zn/Cu), as a means for estimating the prognosis of CL.

Keywords*: Cutaneous leishmaniasis, Zn, Cu, Fe. Zn/Cu ratio, Iran*

Introduction

Leishmaniasis is a group of diseases caused by several species of the genus *Leishmania*, a protozoa transmitted by the bite of a tiny insect vector, the sandfly. The four clinical patterns of the disease in the human host are: cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL).The annual incidence of CL is 1 to 1.5 million cases. Iran is known endemic foci of CL (Desjeux et al. 1996).

Trace elements are needed for many metabolic and physiological processes in the human body (Mertz et al. 1981). Alterations in iron (Fe), zinc (Zn) and copper (Cu) levels in the sera change during inflammation and infections. These are associated with elevated levels of acute phase proteins, such as ceruloplasmin (Cousins et al. 1985, Barber et al. 1988).

Zinc is a component of more than 200 enzymes, involved in various activities, such as metabolic functions, immunity and wound healing (Tudor et al. 2005).

Copper is an essential nutrient that is widely spread in food and water. It is a part of several metalloenzymes that is required for oxidative metabolism, including cytochrome oxidase, feroxidase, amino oxidase, superoxide dismutase, ascorbic acid oxidase and ty-

*Corresponding author: Dr Fatemeh Pourfallah, E-mail: fpourfallah@ yahoo.com

rosinase (Panemangalore and Bebe 1996).

Iron plays a role in the oxygenation of tissues as it is incorporated in the heme structure of hemoglobin (Peralta et al. 1999).

In this study, we investigated the association of essential trace elements such as zinc (Zn), copper (Cu) and iron (Fe) levels in serum of patients with cutaneous leishmaniasis.

Materials and Methods

The subjects of this case-control study were collected from the Central Hospital of Qom, center of Iran and a known endemic foci of cutaneous leishmaniasis. The study group consisted of 60 leishmaniasis patients (28 F, 32 M; mean of ages= 33.9±17.2 yr), and the control group consisted of 100 healthy volunteers (63F, 37M; mean of ages= 36.5± 16.3 yr) from the same area who were not exposed to cutaneous leishmaniasis.

All individuals gave a written consent, and this study was reviewed and approved by Human subjects Ethical Community of the Pasteur Institute of Iran. The patients were interviewed with structured questionnaire requesting information related to various criteria. The age, height and weight of all of the groups were recorded.

The patients were selected based on clinical symptoms confirmed by laboratory diagnosis. It was based on the presence of parasites in Geimsa-stained smears that were prepared with material aspirated from borders of skin lesions and tissue imprints from biopsy. The patients should not have taken any antimonial treatment before the blood testing and had not any other or secondary infections. Both patient and control groups had the same socio-economic status and no mineral supplement should have been taken prior to the test.

Standard methods were used to determine height (cm) and weight (kg). Body mass index (BMI) was calculated by kg/m^2.

Five ml of the venous blood was drawn after overnight fasting, from all individuals and transferred into acid-washed test tubes without any addition of anticoagulants. The blood samples were allowed to clot at room temperature for about one hour, then, the blood samples were centrifuged at 3000 rpm for 15 min at room temperature to separate the sera. Hemolytic sera were discarded. Sera were aliquoted into the eppendorf tubes and stored at -20 °C until they were tested in the Biochemistry Department of Pasteur Institute of Iran.

Analysis of copper and zinc were measured by using flame atomic absorption spectrometry (Thermo Jarrel Ash, Germany) according to the method of Kirgbright et al. Serum samples were diluted by deionized water. Different concentrations of trace elements were prepared for calibration of standard graphs. Absorbances were read at 324.7 nm and 213.9 nm, for copper and zinc, respectively. For accuracy, the standard solutions were run for every 10-test sample. Serum samples were run in triplicate, and individual values were averaged (Kirgbright et al. 1980).

Serum iron concentration was measured by using an Auto Analyzer (Technicon, RA1000, USA) with commercial kit (Shim Enzym, Iran)

The SPSS software package (Windows version 14, SPSS, Chicago, Ill, USA) was used for all statistical analyses. The mean and standard deviation (SD) were used for reporting and a *P*-value of <0.05 was considered significant.

Result

Table 1 shows the patient and control group were similar in age, height, weight body and Body Mass Index (Table 1). When patients with cutaneous leishmaniasis were compared to control group, levels of serum Zn ($P< 0.001$), Fe ($P< 0.05$) and Zn/Cu ratio ($P< 0.001$) were significantly lower than the control group (Table 2). However, serum Cu concentration

was higher in patients with cutaneous leishmaniasis than the control group ($P<0.05$) (Table 2).

The mean duration of the disease was 34±27 d and there was no significant differences when it was compared these trace elements before and after this time.

Table 1. Physical characteristics of patients with cutaneous leishmaniasis and control group

	Patient group No.=60 Mean ±SD	Control group No.=100 Mean ±SD	*P* value*
Age (yr)	33.4±17.2	36.5±16.4	$P> 0.05$
Height (cm)	165±5	167.6±7	$P> 0.05$
Weight (kg)	62.2±12	64.2±11	$P> 0.05$
Body Mass Index (Kg/m^2)	22.5±4.1	22.43±3.7	$P> 0.05$
Duration of disease(day)	34±27	NA	NA

*The mean difference is not significant at $P> 0.05$, NA negative

Table 2. Comparison of serum zinc, copper, iron concentrations and zinc/copper ratio in patients with cutaneous leishmaniasis and control group

	Patient group No.=60 Mean±SD	Control group No.=100 Mean±SD	*P* value*
Serum Zn (µg/dl)	98.58±19.7	126.38±40.2	$P<0.001$
Serum Cu (µg/dl)	133.65±9.1	127.3±13.4	$P<0.05$
Serum Fe (µg/dl)	119.78±41.6	143.56±54	$P<0.05$
Zn /Cu	0.7385±**0**.14	1.008±0.35	$P<0.001$

*The mean difference is significant at $P< 0.05$.

Discussion

Trace elements play a part in the synthesis and structural stabilization of both protein and nucleic acid. Hence, imbalances in the optimum levels of trace elements may adversely affect biologically processes, and are associated with many diseases (Muralidhar et al. 2004). Determinations of trace elements in various diseases have been carried out for many years, but there are a few studies of these elements in cutaneous leishmaniasis. In this study, we found that serum Zn concentration was significantly lower in patients with CL (Table 2) and malnutrition was not a problem, because BMI, weight, and height were not statistically changed in patients and normal individuals (Table 1).

Decreasing serum Zn levels is due to several reasons, mainly, synthesis of methallothionein (MT) in liver, and other tissues. Methallothionein binds 7 g atoms of Zn per mol and serves to draw Zn away from free-circulating pools and it is induced by IL-1 in vivo (Svenson et al. 1985, Rofe et al. 1996).

In our study, we also observed that Cu levels were significantly higher in patients sera than normal individuals (Table 2). Increased serum Cu is associated with an increase in the synthesis of the copper-binding protein ceruloplasmin, (Cillarie et al. 1989, Liew et al. 1990). It was demonstrated that IL-1, but not TNF-α, induced hypercupremia when injected into the preoptic anterior hy-

pothalamus (Klassing et al. 1987).

We also found that serum iron concentrations were significantly lower in patients with CL as compared to controls (Table 2). Iron has a major role in chronic inflammatory diseases (Barollo et al. 2005). Lin et al, demonstrated in vitro that iron chelation effectively blocked NF-kappa B activation and upregulates TNF-α and IL-6 genes in a model of cholestatic liver injury, suggesting a basic role for iron in the activation of the inflammatory process (Lin et al. 1997).

Kocygit observed similar results, in which the CL patients had significantly lower Zn and Fe level and higher serum Cu level as compared to the control subjects (Kocyigit et al. 1998). The authors claimed that the changes could be a part of defense strategies of organisms and were induced by IL-1, TNF-α, and IL-6 (Kocyigit et al. 2002).

Another study in agreement with our data, demonstrated that serum Cu concentration was found significantly higher in patients with acute and chronic cutaneous leishmaniasis than those of control group. However, Zn and Fe levels were lower in patients with acute ($P< 0.001$) and chronic cutaneous leishmaniasis than in the control group (Faryadi and Mohebali 2003).

Our data indicate that Zn/Cu ratio were significantly lower in patients with CL as compared to the controls. Earlier reports suggest that, this ratio imbalance could be a useful marker for immune dysfunction in leishmaniasis (Weyenbergh et al. 2004).

Different studies were carried out on the effect of Zn on immune function. Oral ZnSO4 was administered to mice with CL and reported to be effective. It had been reported that deficiencies of trace elements can change the immune function from cellular Th1 to humoral Th2 prematurely and oral ZnSO4 seemed effective (Sprietsma et al. 1997). In vitro sensitivities of promastigotes and axenic amastigotes of both *L. major* and *L. tropica* to zinc sulfate were studied by Najim et al. (1998). The efficacy of 2% ZnSO4 both as an oral and intralesional injection was studied in treatment of CL but they showed inadequate therapeutic value (Yazdan Panah et al. 2003, Khatami et al. 2005).

Our data indicate that Zn/Cu ratio was significantly lower in patients with CL as compared to the controls. Earlier reports suggest that, this ratio imbalance could be a useful marker for immune dysfunction in leishmaniasis (Weyenbergh et al. 2004).

The findings of such studies indicate a strong association of Zn, Cu and Fe and the copper/zinc ratio with CL. A strategy can be devised to use blood zinc, copper, iron concentration and the copper/zinc ratio (Zn/Cu) as a means for estimating the prognosis of CL.

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