Original Article

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

F Pourfallah¹, S Javadian¹, Z Zamani¹, R Saghiri¹, S Sadeghi¹, B Zarea², Sh Faiaz¹, F Mirkhani¹, N Fatemi¹

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

(Received 19 Jun 2009; accepted 28 Oct 2009)

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 protozoan 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-
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/m².

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. Absorances 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 $34\pm 27$ 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 | Control group | $P$ value$^*$ |
|------------------|---------------|---------------|--------------|
|                  | No. = 60      | No. = 100     |              |
|                  | Mean ±SD      | Mean ±SD      |              |
| 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 | Control group | $P$ value$^*$ |
|------------------|---------------|---------------|--------------|
|                  | No. = 60      | No. = 100     |              |
|                  | Mean ±SD      | Mean ±SD      |              |
| 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 metallothionein (MT) in liver, and other tissues. Metallothionein 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, (Cillaric 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-
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).

Kocyigit 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).

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.

Acknowledgements

The authors wish to thank Pasteur Institute of Iran for financial support and Qom Central Hospital for their technical help. The authors declare that they have no conflicts of interest.

References

Barber EF, Cousins RJ (1988) Interleukin-1 stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. J Nutr. 118: 375–381.

Barollo R, D'Inc M, ScarpaV, et al. (2005) Effects of iron manipulation on trace elements level in a model of colitis in rats. World J Gastroenterol. 11(28): 4396-399.

Cillarie E, Dieli M, Maltese E, et al. (1989) Enhancement of macrophage IL-1 production by Leishmania major infection in vitro and its inhibition by IFN. J Immunol .143(6): 2001-2005.

Cousins RJ (1985) Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev. 65: 238-241.

Desjeux P (1996) Cutaneous leishmaniasis:
public health aspects and control. Clin Dermatol. 14: 417-423.

Faryadi M, Moheballi M (2003) Alterations of serum zinc, copper and iron concentrations in patients with acute and chronic cutaneous leishmaniasis. Iranian J Publ Health. 32 (4): 53-58.

Khatami A, Firooz A, Gorouhi F, et al. (2005) Treatment of acute Old World cutaneous leishmaniasis: A systematic review of the randomized controlled trials. Journal of the American Academy of Dermatology. 57(2): 335-340.

Klassing KC, Laurin DE, Penk RK, et al. (1987) Immunological mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. J Nutr. 117: 1629-1637.

Kirkbright GF (1980) Atomic absorption spectroscopy, Elemental analysis of biological materials. Vienna Technical Report Series. Int Atomic Agency. 197: 141-165.

Kocyigit O, Erel MS, Gueral S, et al. (1998) Alterations of serum selenium, zinc, copper and iron concentrations, and some related antioxidant enzyme activities in patients with cutaneous leishmaniasis. Biol Trace Element Res. 65: 271–281.

Kocyigit A, Gur S, Erel O, et al. (2002) Associations among plasma selenium, zinc, copper, and iron concentrations and immunoregulatory cytokine levels in patients with cutaneous leishmaniasis. Biol Trace Elem Res. 90: 47-55.

Liew FY, Parkinson C, Millott S, et al. (1990) Tumor necrosis factor (TNF-α) in leishmaniasis. TNF-α mediates host-protection against cutaneous leishmaniasis. Immunology. 69(4): 570-73.

Lin M, Rippe RA, Niemela O, et al. (1997) Role of iron in NF-kappa B activation and cytokine gene expression by rat hepatic macrophages. Am J Physiol. 272: 1355-1364.

Mertz W (1988) The essential trace elements. Science. 213: 1332-1338.

Najim RA (1998) Zinc sulfate in the treatment of cutaneous leishmaniasis: an in vitro and animal study. Mem Inst Oswaldo Cruz. 93: 831-833.

Panemangalore M, Bebe FN (1996) Effect of high dietary zinc of plasma ceruloplasmin and erythrocyte superoxide dismutase activities in copper-depleted and repleted rats. Biol Trace Elem Res. 55(1-2): 111-26.

Peralta V, Cuesta MJ, Mata Iet al. (1999) Serum iron in catatonic and noncatatonic psychotic patients. Biol Psychiat. 45(6): 788-90.

Rofe AM, Philcox JC, Coyle P (1996) Trace metal, acute phase and metabolic response to endotoxin in metallothionein-null mice. Biochem J. 314: 793-797.

Shanker AH, Parasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. AJ Nutr. 68 (suppl): 447S-63S.

Sharquie KB (2001) Oral zinc sulfate in the treatment of acute cutaneous leishmaniasis. Clin Exp Dermatol. 26: 21-26.

Soleimani M (2003) Oral zinc sulfate in the treatment of acute cutaneous leishmaniasis. Iranian Journal of Dermatology. 2: 20-24 (In Persian).

Sprietsma JE (1999) Zinc-controlled Th1/Th2 switch significantly determines development of diseases. Med Hypotheses. 49: 1-14.

Svenson KLG, Hallgren R, Johansson E, Lindh U (1985) Reduced zinc in peripheral blood cells from patients with inflammatory connective tissue disease. Inflammation. 9(2): 189-199.

Tudor R, Zalewski PD, Ratnaike RN (2005) Zinc in health and chronic disease. Nutr Health Aging. 9(1): 45-51.

Weyenbergh V, Santana G, D’Oliveira A, et al. (2004) Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study. BMC Infect Dis 4: 50-59.
کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین اصول تنظیم قراردادها

کارگاه آنلاین پروپوزال نویسی

کارگاه آنلاین کاربرد نرم افزار SPSS در پژوهش

کارگاه های آموزشی تخصصی

سرویس ترجمه

سرویس های وب‌های

بانک مراکز اطلاعات علمی

سیاست ویراستاری STES

فیلم‌های آموزشی

کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین اصول تنظیم قراردادها

کارگاه آنلاین پروپوزال نویسی

کارگاه آنلاین کاربرد نرم افزار SPSS در پژوهش

کارگاه های آموزشی تخصصی

سرویس ترجمه

سرویس های وب‌های

بانک مراکز اطلاعات علمی

سیاست ویراستاری STES

فیلم‌های آموزشی