Iranian J Publ Health, Vol. 32, No. 4, pp.53-58, 2003

Alterations of Serum Zinc, Copper and Iron Concentrations in Patients with Acute and Chronic Cutaneous Leishmaniasis

***M Faryadi[1], M Mohebali[1]**

[1]Dep .of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran

Abstract

The aim of this study was to measure the alterations in serum zinc (Zn) copper (Cu) and iron (Fe) concentrations in patients with acute and chronic cutaneous leishmaniasis. Serum zinc and copper were measured by flameless atomic absorption spectrophotometer and serum iron concentration was measured by the Ferrozine method with commercial kits and assay was carried out by using an automatic analyzer. A total of 54 individuals were enrolled in this study: 18 patients with acute cutaneous leishmaniasis, 18 with chronic one and 18 healthy people who were not infected by Leishmania parasites. Serum Cu concentration was found to be significantly higher in the patients with acute ($p<0.05$) and chronic ($p<0.05$) cutaneous leishmaniasis than those of control group. However, Zn and Fe levels were lower in patients with acute ($p<0.001$) and chronic ($p<0.001$) cutaneous leishmaniasis than in the control group. There were no statistically significant differences in serum zinc, copper and iron levels in patients with acute and chronic cutaneous leishmaniasis. Our results showed that serum essential trace elements Zn, Cu and Fe concentrations have been changed in these patients. The changes may be a part of defense strategies of organism and are induced by the hormonelike substances.

Keywords: *Cutaneous leishmaniasis, Trace elements, Iran*

Introduction

Cutaneous leishmaniasis, caused by *Leishmania major* and *L. tropica*, is characterized by a skin ulcer which heals spontaneously, leaving an unsightly scar (1). It is widespread through some of urban and rural regions in Iran. Present study has been conducted for the first time in Iran. The mechanism(s) by which defense cells kill microorganisms has been the subject of intense research in recent years. One of the mechanisms, the serum redistribution of essential trace elements Zn, Cu and Fe together with the increase in synthesis of acute-phase proteins (like ceruloplasmin), which takes place during the course of most infections, is well established (2). The changes are part of defense strategies of organism and are induced by the hormone like substances interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (3, 4). These substances are immunocytokins liberated in a dose-dependent mode, mostly by activated macrophages, in response to several stimuli, including exercise trauma, stress, or infection (5). Several enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) which contribute to immune system responses, require the mentioned trace metals for their activity. For example, Cu-Zn SOD is an important intra-cellular enzyme that requires both Cu and Zn for normal enzyme activity; Zn stabilizes the enzyme and Cu is necessary for catalysis. It acts as an antioxidant by oxidizing free-radical oxygen and it is the major Cu enzyme in erythrocyte (6). The purpose of the present study was to investigate the status of essential trace elements Zn, Cu and Fe concentrations in serum of patients with acute and chronic cutaneous leishmaniasis.

Materials and Methods

Altogether, 54 individuals were enrolled in this study: 18 patients with acute cutaneous

***Corresponding author:** Tel: +98 21 8951400, E-mail: mansoorfaryadi@yahoo.com

leishmaniasis, 18 with chronic one and 18 healthy people who were not exposed by *Leishmania* parasites. Inclusion criteria for the acute patients group were any age or sex, any number of lesions, and exclusion criteria included no pregnancy, no prior antimonial or any treatment and 6 months or older lesions because of spontaneous healing and immunity. Admission criteria for chronic patients group were patients with at least one year old lesions which not respond to antimonial therapy. The control group was selected among healthy parents or siblings who were not exposed to cutaneous leishmaniasis. Age, weight, and height were recorded. Additionally, number and duration of the lesions were recorded in the patient group. Diagnosis was confirmed clinically, as well as by laboratory demonstration of the parasite in the lesions by direct smears. Lesions were cleaned with ethanol, and punctured at the margins of the lesion with a sterile lancet. Smears were made from exudating materials, air-dried, and fixed in methanol. The smears were stained with Giemsa stain for examination by light microscopy under high objective magnification (×100). A total of 5 ml of venous blood was withdrawn. The samples were transferred into tubes without any addition of anticoagulants and centrifuged for 15 min at 3500 rpm. Sera were separated to be examined for Zn, Cu and Fe concentrations. Serum samples were diluted with deionized, distilled water for Zn and Cu measurements. Zn and Cu were determined by an (AA-680G) atomic absorption spectrophotometer (Shimadzu) with Graphite Furnace Atomizer (GFA-4B) in deuterium background correction (BGC) method. Serum Zn and Cu values were expressed in mg/l. 10 micro liter of diluted serum injected to graphite tube and absorption readings were measured at 324.8 nm for copper and 213.9 nm for zinc as peak height. All determinations were run in triplicate and individual values were averaged. The variation coefficient for replicate measurement was <10%. Graphite furnace temperature programs for zinc and copper have been shown in (Table 1). Serum Iron concentration was measured by the Ferrozine method with commercial kits (Boehringer, Mannheim) and assay was carried out using an automatic analyzer (Hitachi 911). Statistics were calculated with the SPSS for Windows Version 11.5 program. The mean values obtained in the different groups were compared by the one way ANOVA test. All results were expressed as mean value ± SD; significance was defined as $P < 0.05$.

Table 1: Graphite furnace temperature program for Copper and zinc determination by AAS[a]

Stage	Temp (ºC) copper/zinc	Time(s) copper/zinc	Ramp[b]/Step[c] copper/zinc	Gas(l/min) copper/zinc
Drying	150/150	30/30	Ramp	1.5
Ashing	500/300	20/20	Step	1.5
Atomizing	2300/1300	4/3	Step	0
Cleaning	2700/2700	3/3	Step	1.5
Cooling	0/0	40/40	Step	1.5

[a] Atomic Absorption Spectrophotometery.
[b] Increasing temperature in step mode.
[c] Increasing temperature in ramp mode.

Results

As seen in (Table 2), the cases and matched controls were similar in age, height, body weight. When compared to control group, patients with acute and chronic cutaneous leishmaniasis had significantly lower levels of serum Zn and Fe (P<0.001) (Table 3, 4). However, serum Cu concentration was higher in patients with acute (P<0.05) and chronic

(P<0.05) cutaneous leishmaniasis according to the control group (Table 3, 4). There were no statistically significant differences in serum zinc, copper and iron concentrations between patients with acute and chronic cutaneous leishmaniasis (Table 5).

Table 2: Physical characteristics of patients with acute and chronic cutaneous leishmaniasis and control group

	Patients with ACL[a] N=18 Mean±SD	**Patients with CCL[b] N=18 Mean±SD**	**Control group N=18 Mean±SD**
Age (year)	31.4±14.5	32.2±12.6	33.3±16.3
Height (cm)	169.6±7.6	167.9±7.1	165.8±5.9
Weight (kg)	63.2±12	65.3±12.7	61±15.8

[a]Acute Cutaneous Leishmaniasis.
[b]Chronic Cutaneous Leishmaniasis.

Table 3: Comparison of serum zinc, copper and iron concentrations in Patients with acute cutaneous leishmaniasis and control group

	Patients with ACL N=18 Mean±SD	**Control group N=18 Mean±SD**	**P value***
Serum Zn (mg/l)	845±104.8	1161.7±191.9	P<0.001
Serum Cu (mg/l)	1310±184.2	1116.7±220.4	P<0.05
Serum Fe (mg/l)	871.1±257.4	1226.1±163.5	P<0.001

*The mean difference is significant at P<0.05.

Table 4: Comparison of serum zinc, copper and iron concentrations in Patients with chronic cutaneous leishmaniasis and control group

	Patients with CCL N=18 Mean±SD	**Control group N=18 Mean±SD**	**P value**
Serum Zn (mg/l)	788.9±102.3	1161.7±191.9	P<0.001
Serum Cu (mg/l)	1288.9±211	1116.7±220.4	P<0.05
Serum Fe (mg/l)	814.4±256.4	1226.1±163.5	P<0.001

Table 5: Comparison of serum zinc, copper and iron concentrations in Patients with acute and chronic cutaneous leishmaniasis

	Patients with ACL N=18 Mean±SD	**Patients with CCL N=18 Mean±SD**	**P value**
Serum Zn (mg/l)	845±104.8	788.9±102.3	p>0.05
Serum Cu (mg/l)	1310±184.2	1288.9±211	P>0.05
Serum Fe (mg/l)	871.1±257.4	814.4±256.4	P>0.05

Discussion

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 the present study, we have demonstrated that serum Zn and Fe concentrations were lower in patients with acute (P<0.001) and chronic (P<0.001) Cutaneous Leishmaniasis (CL) than control group. However, Cu concentration was significantly higher in patients with acute (P< 0.05) and chronic (P<0.05) CL than control group. There were no statistically significant differences in serum zinc, copper and iron concentrations between patients with acute and chronic CL. There is a similar study which conducted by Kocyigit et al (7) on patients with acute CL in Turkey, which our study confirmed their results. Research effort has shifted from experiments to describe the changes in mineral metabolism associated with immune response to investigations of the mediators responsible (5). The observations that host products are released from stimulated leukocytes 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 Zn and Fe and increased serum Cu concentrations (8). 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 (9). Decreasing serum Zn levels apparently results from the synthesis of methallothionein (MT) in liver and other tissues. Methallothionein binds 7 g atoms of Zn/mol and serves to draw Zn away from free-circulating pools; it was induced by IL-1 in vivo (10). However, we were unable to detect MT. Increased serum Cu is associated with Cp and induced by IL-1 (11). It was demonstrated that IL-1, not TNF-α, induces hypercupremia when injected into the preoptic anterior hypothalamus (5). In our study, we observed that Cu levels were significantly higher in the patients' sera than in controls. The increased Cu may be attributable to inflammation associated with the disease. As seen above, the alterations of serum Zn, Fe and Cu probably depend on cytokines, especially IL-1 and TNF-α. Although, we could not determine these immunocytokins, some observations have shown that the production of IL-1 and TNF-α were induced by CL (12-14). Additionally, TNF-α appears to exert its leishmanicidal activity by activating macrophages, rather than by directly activating the parasite (15). It was demonstrated that incubation of macrophages with TNF-α in the presence of bacterial lipopolysaccaride (LPS) resulted in leishmanicidal activity (16). However, the high production of IL-1 in cutaneous leishmaniasis is in contrast to visceral leishmaniasis, where infection of mice with *L. donovani* resulted in the suppression of the IL-1 response (17). These findings may reflect the distinct pathology and immune responses caused by the two species of *Leishmania*. Unfortunately up to now, there were no updated references in this field. In conclusion, we concluded that serum essential trace elements Zn, Cu and Fe concentrations were probably altered by the some immunocytokines as a host-defense strategy of organism during CL infection. Further investigations will be needed to study

immunocytokines together with trace elements in acute and chronic CL infection.

Acknowledgements

We would like to thank Dr. S. Sadr and A. Ahadi from the Department of Physiology, Faculty of Medicine, and Tehran University of Medical Sciences for their supports in this study. We also thank all the individuals and staff of the Razi Hospital, in Tehran for their helps and supports.

References

1. Dowtlati Y (1975). Cutaneous leishmaniasis. *Int J Dermatol, 18: 362-68.*
2. Mastousek AJ, Burguera JL, Burguera M and Anez N (1993). Changes in total content of iron, copper and zinc in serum, heart, liver, spleen and skeletal muscle tissues of rats infected with *Trypanosome cruzi*. *Biol Trace Element Res, 37: 51-69.*
3. Barber EF and Cousins RJ (1988). Interleukin-1 stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats.*J Nutr, 118: 375-381.*
4. Cousins RJ (1985). Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev, 65:p. 238.*
5. Klasing KC (1988). Nutritional aspects of leukocytic cytokines. *J Nutr, 118(12):1435-43.*
6. Panemangalore M and 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.*
7. Kocyigit A, Erel O, Gurel MS, Avci S and Aktepe N (1998). Alteration of serum selenium, zinc, copper and Iron concentrations and some related antioxidant enzyme activities in patients with cutaneous leishmaniasis. *Biol Trace Elem Res, 65(3) 271-81.*
8. Powanda MC and Beisel WR (1982). Hypothesis: Leukocyte endogenous mediator/endogenous pyrogen/ lymphocyte-activating factor modulate the development of nonspecific and specific immunity and affects nutritional status. *Am J Clin Nutr, 35(4):762-68.*
9. Svenson KL, Hallgren R, Johansson E and Lindh O (1985). Reduced zinc in peripheral blood cells from patients with inflammatory connective tissue disease. *Inflammation, 9(2): 189-99.*
10. Rofe AM, Philcox JC and Coyle P (1996). Trace metal, acute phase and metabolic response to endotoxin in metaliothionein-null mice. *Biochem J, 314(pt3): 793-97.*
11. Klassing KC, Laurin DE, Peng PK and Fry DM (1987). Immunological mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. *J Nutr, 117(9): 1629-37.*
12. Pirmez C, Yamamura M, Uyemura K, Oliveira M and Conceicao-Silva F (1993). Cytokine patterns in the pathogenesis of human leishmaniasis. *J Clin Invest, 91(4):1390-95.*
13. Wagner HM, Beuscher HU, Rollinghoff M and Solbach W (1991). Interferon-gamma inhibits the efficacy of interleukin-1 to generate a Th2-cell based immune response induced by *Leishmania major*. *Immunobiology, 182(3-4): 292-306.*
14. Cillari E, Dieli M, Maltese E, Milano S, Salemo A and Liew FY (1989). Enhancement of macrophage IL-1 production by *Leishmania major* infection in vitro and its inhibition by IFN-γ. *J Immunol, 143(6): 2001-05.*
15. Liew FY, Parkinson C, Millott S, Severn A and Carrier M (1990). Tumor necrosis factor (TNF-α) in leishmaniasis. I. TNF-α mediates host-protection against

cutaneous leishmaniasis. *Immunology, 69(4): 570-73.*

16. Liew FY, Millott S, Parkinson C, Palmer RM and Moncada S (1990). Macrophage killing of *Leishmania* parasite in vivo is mediated by nitric oxide from L-arginine. *J Immunol, 144(12): 4794-97.*
17. Reiner NE (1987). Parasite accessory cell interaction in murine leishmaniasis I. Evasion and stimulus-dependent suppression of the macrophage interleukin-1 response by *Leishmania donovani*. *J Immunol, 138: 1919-25.*