

SERUM SELENIUM, ZINC, COPPER AND IRON CONCENTRATIONS AND SOME RELATED ANTIOXIDANT ENZYMES IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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A. Koçyigit, M.D.* / O. Erel, M.D.* / MS. Gürel, M.D.
S. Avcı, M.D.*** / N. Aktepe, Ph.D.******

* Assistant Professor, Department of Biochemistry, Faculty of Medicine, Harran University, Sanlıurfa, Turkey.

** Assistant Professor, Department of Dermatology, Faculty of Medicine, Harran University, Sanlıurfa, Turkey.

*** Specialist, Department of Biochemistry, Faculty of Medicine, Harran University, Sanlıurfa, Turkey.

**** Biomedical Scientist, Department of Biochemistry, Faculty of Medicine, Harran University, Sanlıurfa Turkey.

ABSTRACT

Objective: This study has been carried out to measure the alterations of some trace element concentrations, their related antioxidant enzyme activities, and carrier proteins in patients with cutaneous leishmaniasis (CL).

Methods: Serum selenium, copper and zinc concentrations were determined by Atomic Absorption Spectrometer. Erythrocyte Cu-Zn superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities and ceruloplasmin (Cp) levels were measured with enzymatic colorimetric methods and transferrin (Tf) levels were measured with turbidimetric method.

Results: Erythrocyte Cu-Zn SOD activities, serum copper concentrations and Cp levels were found to be significantly higher in patients group, than those of controls. However, GSH-Px and CAT activities, and selenium, zinc, iron and Tf levels were lower in patients according to the control subjects. There were positive important correlations between Cu-Zn SOD and Cp, Cu-Zn SOD and copper, Cp and copper, GSH-Px and selenium, and iron and CAT in patients group.

Conclusion: Our results showed that serum essential trace elements selenium, zinc, copper and iron concentrations and their related enzymes Cu-Zn SOD, GSH-Px and CAT activities change in CL patients. The changes may be a part of defense strategies of organism and are induced by the hormone-like substances.

Key Words: Cutaneous leishmaniasis, glutathione peroxidase, Cu-Zn superoxide dismutase, catalase, selenium, zinc, copper, iron.

INTRODUCTION

Leishmaniasis is initiated when sand flies inject the extracellular promastigote form of the parasite into the skin. Promastigotes are rapidly phagocytized, after which they transform into the intracellular amastigote form. Promastigotes are not seen in clinical lesions; the multiplication of amastigotes within macrophages leads to clinical disease (1).

Cutaneous leishmaniasis (CL), caused by *L. Major* and *L. Tropica*, is characterized by a skin ulcer which heals spontaneously, leaving an unsightly scar (2). It is widespread through the Southeastern Anatolia of Turkey. It mainly affects the low-income people or rural and suburban populations.

The mechanism(s) by which defense cells to kill microorganisms has been the subject of intense research in recent years (3). Although, reactive oxygen intermediates (ROI) and nitric oxide (NO) that are generated during the respiratory burst by macrophages and polymorphonuclear leukocytes, are involved in intracellular killing it is now apparent that serum redistribution of essential trace elements also has an important role in cytotoxicity (4-6). 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) (7, 8). These substances are cytokines liberated in a dose-dependent mode, mostly by activated macrophages, in response to several stimuli, including exercise, trauma, stress, or infection (9). In animals, many diseases are also caused by simultaneous deficiency of selenium. However, the exact mechanism causing this deficiency remains unknown (10).

Several enzymes, including SOD, CAT and GSH-Px are capable of scavenging ROI *in vivo*. However,

these enzymes require mentioned trace elements for their activity. For example, Cu-Zn SOD is an important intracellular enzyme that requires both copper and zinc for normal enzymatic activity; zinc stabilizes the enzyme and copper is necessary for catalysis. Cu-Zn SOD eliminates O_2^- and hydroperoxides may oxidize cellular substrates, and they prevent propagation of free radical chain reaction (11). GSH-Px that is a glutathione recycling enzyme, catalyzes the oxidation of reduced glutathione by H_2O_2 and other hydroperoxides to form oxidized glutathione and require selenium for its activity (12). CAT is a tetrameric hemoprotein that catalyzes the dismutation of H_2O_2 to water and molecular oxygen (13).

The purpose of the present study was to measure the concentrations of the essential trace elements like selenium, zinc, copper and iron, their binding proteins, and some related antioxidant enzymes like Cu-Zn SOD, GSH-Px and CAT activities, together with the investigation of the relationship between these elements and their related enzyme activities in patients with CL.

MATERIALS AND METHODS

Totally 80 subjects were enrolled in the study, 42 patients, and 38 healthy persons who were not exposed to CL. The study was conducted between November 1996 and March 1997 in Harrankapı Leishmaniasis Treatment Center, Şanlıurfa which is an hyperendemic area for leishmaniasis in the Southeastern Anatolia of Turkey. Approval for the study was given by the Institution Ethical Committee. CL patients of any age and sex without regarding their lesion number were included in the study, while pregnant and those who were under antimonial or other treatments were excluded. If patients had 6 months or older lesions, they were excluded from study, because of spontaneous healing and immunity. Control group was selected among healthy parents or siblings which had not exposed to CL. Age, weight and height values were recorded. Additionally, size, localization, number and duration of 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 or cultures or both. 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, fixed methanol. The smears were stained with Giemsa's stain for examination by light microscopy. Microscopic diagnosis were made when amastigotes were identified in the smears. Also materials were cultured on Novy-Mac Neal-Nicolle (NNN) with rabbit blood agar medium for up to 3 weeks to detect the leishmanial promastigotes.

All of the materials (glass and plastic) employed were thoroughly cleaned with hot solution of nitric acid (20% v/v) for 48 hour and rinsed six times with demineralized water.

Total 10 mL venous blood was withdrawn. Of samples, 5 mL were transferred into tubes without any addition of anticoagulants and centrifuged for 15 min at a speed of 2000 x g. Sera were separated to determine selenium, copper, zinc and iron levels. Remaining 5mL of blood samples, 5 mL were transferred into heparinized tubes and erythrocyte Cu-Zn SOD, GSH-Px and CAT activities were measured immediately after preparation of erythrocyte hemolysate.

Serum selenium determination was performed by a SpectrAA 250 Plus Zeeman Atomic Absorption Spectrophotometer with a graphite furnace GTA-96 (Varian, Australia), with deuterium background correction. Varian hollow cathode lamps were employed at the 196-nm wavelength and 1.0-nm bandpass. Pyrolytically coated graphite tubes with pyrolytic graphite platforms (Varian, Australia) were used. Selenium concentration was determined by an internal standard addition method (14).

Serum copper and zinc were determined by a SpectrAA 250 Plus Zeeman Atomic Absorption Spectrometer (Varian, Australia) with a deuterium background correction. Serum copper and zinc values were expressed in mg/L.

Serum iron, Tf and albumin levels were determined with commercial kits (Boehringer Mannheim, Germany) by automatic analyser (Hitachi 911 Bohringer Mannheim, Germany) Serum ceruloplasmin (Cp) levels were determined by measurement of p-phenylenediamine oxidase activity according to the method of Sunderman and Nomoto (15).

In order to measure erythrocyte antioxidant enzyme activities, Erythrocytes were separated from heparinized blood by centrifugation at 2000 x g and washed with 0.9% isotonic NaCl solution repeatedly until a colorless supernatant was observed. Then, erythrocytes were lysed by addition of 4 volumes of double distilled water. The resulting suspension was centrifuged twice to eliminate all of the cell membranes: first by 10 min in the tube centrifuge at 2000 x g, followed by centrifugation in an eppendorf centrifuge at 4500 x g for 5 min. Hemoglobin was determined by Drabkin's method in an 0.1/mL aliquot of hemolysate. Blood hematocrit (Htc) concentrations (%) were measured by automatic blood cell analyzer (STKS, Coulter, USA).

GSH-Px and Cu-Zn SOD activities were measured in erythrocyte by method of Paglia and Valentine (16),

and McCord and Fridovich (17), employing the RANSEL and RANSOD kits (RANDOX, Belfast). Measurements were carried out at 37°C. Internal quality control was maintained by use of a standardized control supplied by RANDOX. Measurements were performed in an autoanalyzer (Hitachi 911 Boehringer Mannheim, Germany) according to the Radox application procedure. Erythrocyte CAT activity was determined by a spectrophotometric method (18)

Statistics were calculated with the SPSS for Windows program Version 6.0. The mean values obtained in the different groups were compared by Student's t-test. The Pearson's correlation test was used to evaluate the correlation between two variables. All results were expressed as mean values \pm SD; significance was defined as $p < 0.05$.

RESULTS

As seen from Table I, the cases and unmatched controls were similar in age, height, body weight, body mass index (BMI). When compared to controls,

patients with CL had significantly lower levels of serum selenium, zinc and iron ($p < 0.001$, $p < 0.01$, $p < 0.05$, respectively) (Table II). There were also significant decreases in serum Tf levels and GSH-Px and CAT activities in patients group ($p < 0.05$, $p < 0.001$, $p < 0.01$ respectively). However, serum copper concentrations, Cp levels, and Cu-Zn SOD activities were higher in CL patients according to the control subjects ($p < 0.01$, $p < 0.001$ and $p < 0.01$ respectively). Blood Htc and albumin levels were lower in patients group according to the controls, but it was not statistically significant (Table II).

There was a positive important correlation between selenium concentration and GSH-Px activity in total group ($r = 0.703$, $p < 0.0001$), as seen from Table III. In addition, positive correlations were noted between selenium and albumin ($r = 0.344$, $p < 0.05$), and GSH-Px and Htc ($r = 0.482$, $p < 0.01$), Htc and selenium ($r = 0.289$, $p < 0.05$) in patients. There was also positive relationships between Cu-Zn SOD and Cp ($r = 0.365$, $p < 0.01$), Cu-Zn SOD and Cu ($r = 0.255$, $p < 0.05$), Cp and Cu ($r = 0.67$, $p < 0.001$), and CAT and iron ($r = 0.43$, $p < 0.01$) in patients group (Table III).

Table I. Physical Characteristics of CL Patients and Healthy Subjects

	Patients (n= 42)	Healthy Subjects (n= 38)	p
Age (year)	29.2 \pm 4.3	28.3 \pm 4.5	>0.05
Height (cm)	168 \pm 6	169 \pm 5	>0.05
Weight (kg)	65.1 \pm 8.9	66 \pm 12.4	>0.05
BMI (kg/m ²)	23.4 \pm 3.6	24.7 \pm 4.4	>0.05

Table II. Comparison of Parameters in Patients and Healthy Subjects

Parameters	Patients (n= 42)	Healthy Subjects (n= 38)	p
Erythrocyte Cu-Zn SOD (U/g Hb)	570.96 \pm 77.04	425.4 \pm 64.26	<0.01
Erythrocyte GSH-Px (U/g Hb)	315.08 \pm 34.3	362.10 \pm 33.28	<0.001
Erythrocyte CAT (K act./g Hb)	99 \pm 0.57	153 \pm 0.54	<0.01
Serum Zn (mg/L)	0.74 \pm 0.112	0.847 \pm 0.111	<0.01
Serum Cu (mg/L)	1.44 \pm 0.298	1.227 \pm 0.415	<0.01
Serum Fe (mg/L)	0.722 \pm 0.183	0.924 \pm 0.1765	<0.05
Serum Se (μ g/L)	75 \pm 20.37	106 \pm 26.28	<0.001
Serum Cp(U/L)	1110 \pm 85	790 \pm 42	<0.001
Serum Tf (mg/dL)	263 \pm 28	296 \pm 35	<0.05
Serum albumin (g/dL)	4.3 \pm 0.3	4.5 \pm 0.2	>0.05
Hematocrit (%)	39.5 \pm 2.48	41.3 \pm 3.23	>0.05

Table III. Some Significant Correlations in Patients Group

Parameters	r	p
GSH-Px- Se	0.703	<0.0001
GSH-Px-Htc	0.482	<0.01
Albumin- Selenium	0.344	<0.05
Htc - Selenium	0.289	<0.05
Copper-Cp	0.67	<0.001
Cu-Zn SOD - Cp	0.356	<0.01
CAT-iron	0.43	<0.01

DISCUSSION

The serum redistribution of the essential trace elements, iron, copper and zinc together with the increase in synthesis of acute-phase proteins like ceruloplasmin and transferrin, which takes place during the course of most infections, is well established. The changes consist of a decrease in serum iron and zinc concentrations together with an increase in copper, and their magnitude depends on the intensity of the stimulus (6). In the present study we found that serum selenium, zinc and iron concentrations were significantly lower and copper concentrations were higher in patients with CL than those of control subjects. In addition, their related antioxidant enzymes GSH-Px and CAT activities were lower and Cu-Zn SOD activities were higher in CL patients.

It was demonstrated that the alterations of serum zinc, iron and copper concentrations particularly depend on cytokines specially IL-1 and TNF- α (9). Activated phagocytic cells release endogenous mediators that reduce plasma zinc concentrations in experimental animals by redistribution of zinc from plasma to the liver (19). Decreasing serum zinc levels apparently result from the synthesis of methallothionein (MT) in liver and other tissues. Both IL-1 and TNF- α decrease plasma iron concentrations and the decreasing concentrations of iron induced by IL-1 is at last in part, due to the release of apolactoferrin by granulocytes and apolactoferrin can remove iron from Tf, thus iron is sequestered in compartments which are nutritionally unavailable to bacteria or parasites (20). Increased serum copper is associated with Cp and also induced by IL-1 (21). It was demonstrated that IL-1, but not TNF- α increase plasma copper content (6). Increased Cp and copper content may be attributable to inflammation associated with the disease.

As seen above, the changes of these trace metal contents in CL patients may depend on cytokines

specially IL-1 and TNF- α , although we could not determine these cytokines. However, some observations have shown that the production of IL-1 and TNF- α were induced by CL (22-24).

In animals, many diseases are caused by simultaneous deficiency of selenium. However, the exact mechanism causing this deficiency remains unknown. It has been demonstrated that GSH-Px enzyme activity was lower in the patients with Leishmania Donovanii (25). However, it was not known whether the causes of this depletion depended on selenium content or the other factors. Also, we could not find any report about selenium status and relationship between selenium and GSH-Px activity in patients with CL. Dworkin (26), demonstrated that both selenium concentrations, GSH-Px activities and serum albumin levels were significantly lower in patients and there was an important correlation between albumin and GSH-Px in acquired immune deficiency syndrome (AIDS). He has claimed that selenium deficiency seems to be a part of the protein-calorie malnutrition and impaired intestinal absorption common in AIDS. However, Dworkin's patients were predominantly malnourished drug users. Olmsted et al. (27), have also found that selenium concentrations were significantly lower in AIDS patients. Whereas, they were mainly homosexuals from higher socioeconomic classes where malnutrition is usually not a problem and intestinal selenium absorption were not impaired in these patients. We also found that there was a good correlation between blood selenium content and GSH-Px activity. However, there were weak correlations between serum albumin and selenium, and Htc and GSH-Px in these groups. We assessed serum albumin levels because they depend on protein intake and it is frequently used to assess nutritional status (28). Also, we have measured Htc levels, BMI, weight and height of the patients and controls. These findings demonstrated that in our patients malnutrition is not a problem. Whereas, blood selenium concentrations were significantly

lower in this group. The decreased levels of selenium in our patients may be attributed to redistribution from the plasma pool into the tissues as a defense mechanism modulated by the effect of infection. The decrease in the activity of GSH-Px may also be related to decreased selenium levels in those patients.

Numerous studies have demonstrated for a long time that ROI such as O_2^- and H_2O_2 peroxide, which are products of the macrophage respiratory burst, were the major killing mechanisms (29). A number of groups reported evidences that ROI's were involved in macrophages leishmanicidal activity, and H_2O_2 is an effector molecule against these parasites (30). It would be expected that, increased amounts of hydroperoxides might be generated to kill protozoa as host defense strategies. It was known that there are strong relationships between antioxidant enzymes and their cofactors (31). We also found significant correlations between GSH-Px and selenium ($r=0.703$, $p<0.0001$), CAT and iron ($r=0.430$, $p<0.01$). Consequently, lack of selenium and iron leads to a deficiency of the GSH-Px and CAT enzyme activities and, consequently, to a decreased ability to degrade H_2O_2 (32).

We found that Cu-Zn SOD activity were higher together with serum copper and Cp levels in patients, and there was a positive significant correlation between copper and Cp, and Cp and Cu-Zn SOD, and Cu-Zn SOD and copper in patients group. We suggest that, cytokines induce not only trace element metabolism, but also antioxidant enzyme activity. It was demonstrated that both manganese (Mn) SOD and Cu-Zn SOD activities were significantly induced by IL-1 and TNF- α (33). Disilvestro et al. (34), and Assreuy et al. (3), also demonstrated that addition of SOD to macrophages, enhanced leishmanicidal activity. Although there is no available report about CL and antioxidant activity, our results are supported by mentioned reports. However, it was found that Cu-Zn SOD activity was lower together with GSH-Px and CAT activities in *Leishmania Donovanii* infection (25). This difference between visceral and CL may depend on a distinct pathology and immune response in two species of leishmania. For example, the high production of IL-1 in CL is in contrast to visceral leishmaniasis that resulted in suppression of the IL-1 response as seen above (35).

We conclude that serum essential trace elements selenium, zinc, copper and iron concentrations and their related enzymes Cu-Zn SOD, GSH-Px and CAT activities change probably by the effects of some cytokines as host defense strategies of organism during CL infection. However, there is no available report about selenium and cytokines. Further investigations will be needed to study the cytokines

together with trace elements specially selenium and antioxidant enzyme activities.

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