

## Alterations in Serum Levels of Trace Elements in Cutaneous Leishmaniasis Patients in Endemic Region of Hatay (Antioch)

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Immune cells, like all other types of cells, require an adequate supply of trace elements for the structure and function of metalloproteins that participate in housekeeping processes such as energy production and protection against reactive oxygen species. In addition, trace elements are required for the activity of a number of enzymes that directly participate in host defense processes. In the present study we evaluated the alterations of zinc, copper, selenium and iron concentrations in serum of patients treated with meglumine antimonate (glucantime) and untreated cutaneous leishmaniasis. Serum levels of zinc, copper, selenium and iron were measured by atomic emission spectrometer. The serum levels of these elements were found to be significantly higher in untreated patients compared to those of healthy controls. However, the levels of zinc, copper and iron in serum were significantly reduced and selenium levels were increased in treated patients. These suggest that the increasing levels of trace elements in serum may be host defense strategies of organism infection. In addition the high trace element levels were a sequel of the disease rather than a cause.

**Key Words:** *Cutaneous leishmaniasis, Trace elements, Hatay (Antioch).*

### INTRODUCTION

*Leishmania* is an intracellular protozoan parasite that infects humans and causes a wide spectrum of diseases known as leishmaniases. Leishmaniases are endemic in 88 countries, where 350 million people live the risk of infection. Leishmaniases can be fatal, grossly disfiguring or relatively

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mild, localized and in some cases self-healing. *Leishmania* parasites inhabit two highly specific hosts, the sand fly, where they grow as motile flagellated promastigotes in the gut and the mammalian macrophages, where they survive and grow intracellularly as non-flagellated amastigotes<sup>1-3</sup>.

In Turkey, leishmaniasis is caused by a different number of species including *Leishmania infantum* and *L. tropica*. *Leishmania infantum* causes *Visceral leishmaniasis*, *Leishmania tropica* responsible for *Cutaneous leishmaniasis* (CL). *Cutaneous leishmaniasis* has been an important health problem in the south-east of Anatolia, Turkey, for several years. The incidence of the disease has been increasing in this region (mainly in the city of Sanliurfa) since 1980 and Çukurova (mainly the city of Adana and its districts) has been a new endemic region since 1985<sup>4-6</sup>. In Hatay province, the Mediterranean coast in south-eastern Turkey, human cutaneous leishmaniasis has posed an important public health problem for many years. *Cutaneous leishmaniasis* is characterized by chronic and painless, but often disfiguring ulcers that regress after some months to years, leaving atrophic scars<sup>7,8</sup>.

Several micronutrients such as trace elements (zinc, iron and selenium) modulate the immune function and influence the susceptibility of the host to infection. Deficiency of trace elements and infectious diseases are often concomitantly observed and result in complex interactions<sup>9</sup>. Many trace elements act as antioxidants or as modulators of the host immune response<sup>10,11</sup>. However, the changes of serum concentrations of essential trace elements iron, copper and zinc together with the synthesis of acute phase proteins (like ceruloplasmin and transferrin), which take place during the course of most infections, is well established<sup>12</sup>. Only few data are available on the role of trace elements in human leishmaniasis and especially there is no study with *Cutaneous leishmaniasis* in Hatay region.

The purpose of the present study was to investigate the serum levels of zinc, copper, iron and selenium in before and after-treatment with meglumine antimonate (glucantime) in *Cutaneous leishmaniasis* patients in Hatay region.

## EXPERIMENTAL

Patients were obtained from the Leishmania Treatment Center in Iskenderun and Antakya. All subjects were informed about the content of the study prior tests and their written consents were obtained. The study protocol was approved by the ethical committee of the Mustafa Kemal University Hospital.

A total of 50 subjects aged between 7-80 years were enrolled in this study. These patients were divided into two groups. The pre-treatment group contains 21 active *Cutaneous leishmaniasis* patients (11 females, 10 males) and post-treatment group contains 29 *Cutaneous leishmaniasis* patients (10

females, 19 males). These patients were treated with pentavalent antimonial compounds. This drug was administered intramuscularly once a day for 11 days (20 mg/Sb/kg/d) by a single injection. 26 Healthy people (14 females, 12 males) from same area who were not exposed to *Leishmania* parasites were used as controls. The clinical diagnosis was confirmed by parasitology laboratory upon 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. Material was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain for examination by light microscopy. Microscopic diagnosis was made when amastigotes were identified in the smears. Patients with negative smear were diagnosed by culture of the lesion aspirates material which was inoculated on NNN (Novy-Mac-Neal-Nicolle) medium with 10 % rabbit blood and incubated at 24 °C culture were observed for 4 weeks before being considered as negative<sup>5,8</sup>.

After diagnostic procedure, 5 mL of blood sample were withdrawn into tubes without any addition of anticoagulants and centrifuged for 15 min at 3500 rpm. Serum was removed and stored at -20 °C to measure trace element levels.

**Measurement of serum Se, Cu, Fe and Zn levels:** Melted serum samples were diluted by 1 % high purity nitric acid (laboratory grade) and diluted with ultra pure water (1:10). Selenium, copper zinc, iron, levels were measured by Varian® Liberty Series II inductively coupled plasma atomic emission spectrometer (ICP-AES) through preparing suitable calibration graphics of wave legends for each parameter. The standard of elements were obtained from High Purity® standards for ICP-AES. Total of five reference points were chosen prior to the determination of metal concentrations and at least 99.5 % correlation was achieved.

**Statistical analysis:** Data were evaluated by SPSS 13 for Windows (SPSS, Chicago, USA). Differences between the quantitative variables were evaluated by ANOVA and differences of  $p < 0.05$  were accepted as statistically significant level. All data were reported as mean  $\pm$  standard deviation (SD).

## RESULTS AND DISCUSSION

Research during the last quarter of the 20th century clearly established the importance of adequate trace element nutrition for protection of animals and humans against infections. Iron, zinc, copper and selenium have received the majority of attention. The data overwhelmingly support the following general conclusions: (1) an inadequate supply of these essential micronutrients is associated with suppression of numerous activities of cells in both the innate and acquired branches of the immune system; (2) the extent of the impairment in the immune system due to trace element

deficiency can be sufficient to increase the risk of morbidity and mortality due to viral, microbial and parasitic infections; and (3) reversal of the trace element deficiency restores immunocompetence<sup>11,13</sup>.

In this study, we measured the alterations in serum levels of trace element in pre-treated and post-treated patients with *Cutaneous leishmaniasis* in endemic region in Hatay. The results of the trace element analysis of the serum samples are summarized in Table-1. The levels of Cu, Fe, Se and Zn in the patient serum were higher than in the controls. However, in the treatment group the levels of Cu, Fe and Zn were reduced while Se level was increased.

TABLE-1  
SERUM LEVELS OF Cu, Fe, Se AND Zn  
IN CONTROLS AND PATIENTS

Parameters	Control (n = 26)	Pre-treated (n = 21)	Post-treated (n = 29)
Cu (mg/L)	2.79 ± 1.20	2.88 ± 0.85	0.89 ± 0.09*
Fe (mg/L)	2.08 ± 0.76	3.48 ± 1.35*	0.96 ± 0.08*
Se (mg/L)	0.99 ± 0.12	2.74 ± 1.15*	3.73 ± 0.56*
Zn (mg/L)	2.59 ± 1.06	4.96 ± 1.94*	1.27 ± 0.47*

\*p < 0.05 compared with control group.

There are some studies dealing with *Cutaneous leishmaniasis* in Turkey mostly endemic place in Sanliurfa. Koçyigit and colleagues determined serum Se concentration and blood glutathione peroxidase (GSH-Px) activity in *Cutaneous leishmaniasis* patients in Sanliurfa, hyperendemic area for Leishmaniasis in Center Southeastern in Anatolia. Selenium concentrations and GSH-Px activities were found to be lower in the patient group as compared to controls. These authors concluded that, selenium may play an important role in the pathophysiologic process of *Cutaneous leishmaniasis*<sup>14</sup>.

Wenbergh *et al.*<sup>15</sup> studied plasma levels of Cu and Zn in 31 patients with either localized cutaneous (LCL), mucosal (ML) or *Visceral leishmaniasis* (VL) and 25 controls from endemic and non-endemic area in Brazil. They observed that Zn levels were significantly decreased in all three patient groups as compared to controls. Plasma Cu levels were increased in LCL and VL patients but not in ML patients and it was strongly correlated to anti-leishmania IgG. Cu/Zn ratios were higher in patients with deficient cellular and exacerbated humoral immune response. They suggested that, Zn deficiency in VL and ML indicate possible therapeutic administration of Zn in these severe forms of leishmaniasis. Cu/Zn imbalance can be a useful marker for immune dysfunction in leishmaniasis and suggest that trace metals are implicated in both humoral and cellular anti-Leishmania immune response.

Faryadi *et al.*<sup>16</sup> measured the alterations in serum Zn, Cu and Fe concentrations in patients with acute and chronic cutaneous leishmaniasis. Serum Cu concentrations were found to be significantly higher in the patient group with acute and chronic cutaneous leishmania compared to those of control group. Zn and Fe levels were lower in patients with acute and chronic cutaneous leishmania in the control group.

Erel *et al.*<sup>17</sup> were measured the serum levels of Cu, Zn and Fe before and after pentavalent antimonial compounds (Glucantime) treatment. This drug was given intramuscularly (20 mg/kg/d) for three weeks in patients group with CL. Before antimonial therapy Cu concentrations were found to be significantly higher than those of healthy subjects, while Zn and Fe levels were lower in the patient group compared to controls. Cu levels were decreased, while Zn and Fe levels were increased during antimonial therapy. There was a negative correlation between serum Cu and Zn levels in *Cutaneous leishmaniasis* patients.

In conclusion, in the present study the increased trace element levels may be the cause, rather than a consequence of *Cutaneous leishmaniasis*. In addition, the increased levels of serum Cu, Fe, Se and Zn could be the consequence of the host defense strategies of organism infection. Trace metal levels have a predictive value for clinical evaluation of and susceptibility to leishmaniasis.

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(Received: 5 September 2007;

Accepted: 18 January 2008)

AJC-6221