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Effects of antimonial therapy on serum zinc, copper and iron concentrations in patients with cutaneous leishmaniasis

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Objective In the present study, effects of cutaneous leishmaniasis (CL) infection on total content of essential trace elements and alterations of these element contents during antimonial therapy were investigated.

Method 40 patients diagnosed with CL, and 32 healthy subject were included in the study. Pentavalent antimonial compounds (Glucantime) were given intramuscularly (20 mg/kg/day) for three weeks in the patient group. Serum copper (Cu) and zinc (Zn) concentrations were measured by Flame Atomic Absorption Spectrometry (FAAS), and iron (Fe) concentrations were measured by colorimetric method.

Results Before antimonial therapy; Cu concentrations were found to be significantly higher than those of healthy subjects ($p<0.05$). However, Zn and Fe concentrations were lower in the patient group ($p<0.01$). Cu levels were found to have tendency to decrease, while Zn and Fe levels were increase during the period of antimonial therapy ($p<0.001$, $p<0.01$, $p<0.01$ respectively).

Conclusion Our findings showed that serum trace element concentrations change in CL infection, probably parallel to host defense mechanisms, and these changes might partially be turned back to normal levels by antimonial therapy.

Key words Cutaneous leishmaniasis, pentavalent antimonials, copper, zinc, iron.

Introduction

Leishmania is dimorphic protozoon that spend part of their life cycle in a vector and partly in a mammalian host. The extracellular promastigote form of the parasite develops in the sand fly and inoculates into human (or other mammalians) skin. The promastigote subsequently undergoes phagocytosis by a macrophage and converts to the obligate intracellular amastigote stage (1). *Leishmania major*, the causative parasite of cutaneous leishmaniasis (CL) or oriental sore, is an intracellular protozoa of monocytic cells (2). CL is widespread through the Southeastern Anatolia of Turkey. It mainly affects the low-income people or rural and suburban populations.

The effects of many pathological conditions as congestive heart failure, pneumonia, rheumatic heart diseases, bronchitis, and various infections, hemolytic anemia, psoriasis, and on the concentrations of serum iron (Fe), zinc (Zn), copper (Cu), and other trace elements have been interest to investigators for a number of years (3).The changes of serum concentrations of essential trace elements Fe, Cu and Zn together with the synthesis of acute-phase proteins (like ceruloplasmin and transferrin), which take place during the course of most infections, is well established (4). The changes are part of the defense strategies of the organism, and are induced by the hormone- like substances, such as Interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-$\propto$) and interleukin-6 (IL-6) (5).

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Pentavalents antimonials (Sb V) remain the drugs of choice in the treatment of all forms of the disease in spite of their reported toxicity, difficulty in administration and high cost (6). It is not known whether the antimonial compounds is acting directly on the parasite or by activating the macrophage other components of the immune system which subsequently mediates an effect on the leishmania parasite (7-10).

The aim of this study was to investigate the status of trace elements, Fe , Cu and Zn in CL patients, and the effect of antimonial therapy on trace element concentrations .

Materials and Methods

Totally, 72 subjects were enrolled in the study, 40 patients, and 32 healthy persons who were non-exposed to CL. The study was held between May1996 and July 1996 in Harrankapı Leishmaniasis Treatment Center, Şanlıurfa which is an hyperendemic area for leishmaniasis in the Southeastern Anatolia of Turkey. Admission criteria for the patients group were any age or sex, any number of lesions, no pregnancy and no prior antimonial treatment. If patients had 6 months or older lesions, they were excluded from the study, because of spontaneous healing and immunity. Control group was selected among healthy parents or siblings non-exposed to CL. Age, weight and height were recorded. Body mass index (BMI) was computed as weight (kg) divided by height squared (m^2). Additionally, size, localization, number and

duration of lesions were recorded in the patients 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 was 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.

In the patients group, the drug pentavalent antimonial compounds "glucantime" was administrated intramuscularly once a day for 21 days (20 mg/kg) in a single injection. Ten ml venous blood was withdrawn before and three times during the treatment at one week intervals following overnight fasting. Blood was centrifuged at 1500 rpm for 5 min and serum stored -20^{o}C in plastic tubes until analyses. All the materials (glass and plastic) employed were thoroughly washed, soaked in 20% nitric acid and rinsed six times with demineralised water. Serum Fe was measured by commercial kits (Boehringer Mannheim, Germany) by automatic analyser(Hitachi 911, Boehringer Mannheim, Germany). Cu and Zn concentrations were determined by a SpectraAA 250 plus Zeeman Atomic Absorption Spectrometer (Varian, Australia) with a deuterium background correction. Deionised water and analytical-grade reagents were employed. Working standards were prepared daily from 1000 ppm stock solutions. Serum Cu and Zn were measured according to Parker et al (11). One volume of serum was diluted with four volumes of deionised water, and standards were prepared in 3 per cent bovine albumin in order to obtain the same viscosity with that of the serum. Serum Cu and Zn contents were expressed in µg/dl.

Statistical analyses were carried out by using a computer program (SPSS for Windows). Data were expressed as mean±SD. The difference of parameters between patients and controls were tested by Student's *t* test . The differences among patients groups that took antimonial therapy were tested by using Friedman Two-way ANOVA Test. Where a significant effect of antimonial therapy on elements concentrations were detected, paired student's *t* test was used.

Results

As seen in Table I, the cases and unmatched controls were similar in age, height, body weight, and body mass index (BMI). Serum iron and zinc concentrations were significantly lower in patients group that was not under therapy than those of healthy subjects (p<0.01). Copper concentration in serum of patients were higher than those of controls (p<0. 05) (Table II).

Table I. Physical Characteristics of CL Patients and Healthy Subjects

	Patients	Healthy Subjects	
	n= 40	*n= 32*	*p*
Sex (M/F)	22/18	17/15	>0.05
Age (year)	25.2±4.3	26.3±4.5	>0.05
Height (cm)	167±7	168±6	>0.05
Weight (kg)	64.1±7.9	65±12.4	>0.05
BMI (kg/m^2)	23.2±3.8	24.2±5.4	>0.05

Table II. Comparison of Serum Iron, Copper and Zinc Concentrations of Patients and Healthy Subjects.

Parameters	Patients	Healthy Subjects	
	n=40	*n=32*	*p*
Fe (µg/dl)	58.39±7.2	72.28± 7.7	<0.01
Cu (µg/dl)	144.06±24.8	113.65±26.8	<0.05
Zn(µg/dl)	83.8±16	94.15±12.4	<0.01

Serum iron concentration increased gradually by weeks. There were significant differences between, before and during therapy. There was also significant difference between the first and the third weeks. Copper concentration gradually decreased during therapy. Significant differences were found between before therapy and during therapy in all patients groups. No significant difference appeared during the therapy. Serum zinc concentration also gradually increased but only the value of the last week was significantly higher than before therapy (Table III). As seen in Table 3, the changes of iron and copper concentrations are faster than zinc.

Table III. The Changes of Serum Iron, Copper and Zinc Concentrations of the Patients During Antimonial Therapy

Parameters	Before Theraph	First Week	Second Week	Third Week	*p*
	n=40	*n=40*	*n=40*	*n=40*	
Fe (µg/dl)	58.39±7.2	63.27±6^{b}	65.14±8.8^{b}	66.52±8^{bc}	<0.001
Cu (µg/dl)	144.06±24.8	138.34±22.3^{b}	128.84±26^{b}	123.75±24.3^{b}	<0.01
Zn (µg/dl)	83.88±13.2	87.69±16	89.59±13.8	91.28±15.3^{b}	<0.01

Data are mean ± one S.D. a: the significance value of Friedman Two Way ANOVA test performed among all patients group. b: p<0.05 vs before therapy, c: p<0.05 vs 1st week according to paired student's *t* test.

Discussion

There is no considerable evidence for bi-directional interactions between essential trace elements and tropical infections. However, it is clear that research to date has only touched the surface of problem and much remains to be learned about the basic science and public health levels. Much research has been directed towards Fe and an increasing interest Zn and Cu (12, 13).

There are two general classes of abnormalities associated with trace elements: specific deficiency-from dietary inadequacies, imbalances, or secondary to other diseases. Both kinds of abnormalities can be diagnosed by analyses of trace elements in serum or other tissues. Furthermore, secondary changes occur as a result of diseases, but they are not exactly understood. In the present study, we have demonstrated that serum Zn and Fe concentrations were decreased and Cu concentrations were increased in patients with cutaneous leishmaniasis than those of control subjects.

Research effort has shifted from experiments to describe the changes in mineral metabolism associated with immune response to investigations of the mediators responsible(13). 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, although there is some species specificity (14).

The role of certain inflammatory products in the regulation of Zn balance has been well documented. Thus, leukocyte endogenous mediators (Interleukins), released from activated phagocytic cells, induces hypozincemia in experimental animals by redistribution of Zn from plasma to the liver (15). Decreasing serum Zn levels apparently result from the synthesis of methallothionein 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 was induced by IL-1 in vivo (16). However, we were unable to detect methallothionein.

Both IL-1 and TNF-∝ induce hypoferriemia and hypoferriemia induced by IL-1 at least in part, due to the releasing of apolactoferrin by granulocytes. Fe is removed from the circulation because apolactoferrin can remove Fe from transferrin and thus, Fe is sequestered in compartments which are nutritionally unavailable to bacteria or parasites (17).

Increased serum Cu is also associated with ceruloplasmin (Cp) and induced by IL-1 (18). It was demonstrated that IL-1, but not TNF-∝, induces hypercupremia when injected into the preoptic anterior hypothalamus (13). In our study we observed that Cu levels were significantly higher in patient's sera than that of healthy subjects. Increased Cu may be attributable to inflammation associated with the disease.

As seen above, the alterations of serum Zn, Fe and Cu may probably depend on cytokines specially IL-1 and TNF-∝. Although, we couldn't determine these immunocytokins, some observations shown that the production of IL-1 and TNF-∝ were induced by CL (19-21). Additionally**,** TNF-∝ appears to exert its leishmanicidal activity by activating macrophages, rather than by activating directly on the parasite.

The mechanisms of pentavalent antimonial agents against leishmania species, for which the drugs are still the primary chemotherapeutic agent, have appearently not been the subject of detailed investigation. It is not known whether the antimonial compounds are acting directly on the parasite or by activating the macrophage or other components of the immune system which subsequently mediates an effect on leishmania parasite. The latter possibility is more plausible since substantial evidence is now pointing toward on immune system involvement as a crucial element in mounting a full response to the drug (22). Ibrahim et al (22), suggested that the inhibitory action of pentostam was mediated through the macrophage rather than through a direct toxic effect exerted on the parasite. Vouldoukis et al (23), also showed that during infection with CL, serum levels of TNF-α increased and correlated in situ expression of CD23, IL4 and TNFα-mRNA, and this expression disappeared following antimonial treatment. Although, we couldn't find any reference about the relationship among immunocytokins trace metals and antimonial therapy in CL, our findings demonstrated that by the treatment of CL, when the host defense mechanisms returned to normal the expression of cytokins which were released from activated macrophages would disappear at the same time. Consequently, serum Fe, Cu and Zn contents returned to normal levels. As seen in Table 3, the changes of iron and copper concentrations are faster than zinc. These difference may be dependent on the changes of acute phase reactants of host during treatment.

In summary, we suggested that the serum redistribution of essential trace elements took place during the course of CL infections as a part of the defense strategies of the organism, induced by the hormone-like substances or other mechanisms. During the antimonial treatment, these changes

began to return normal levels by unknown mechanisms.

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