Tr. J. of Medical Sciences
29 (1999) 291-295

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Decreasing Selenium Levels and Glutathione Peroxidase Activity in Patients With Cutaneous Leishmaniasis

Received: December 13, 1997

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Abstract: For most infectious diseases, increased formation of reactive oxygen species is secondary to the primary disease process. Some microorganisms are highly susceptible to exogenous reactive oxygen species such as hydrogen peroxide. Glutathione peroxidase (GSH-Px) remove H_2O_2, and selenium is requird for the activity of GSH-Px. Therefore, to assess the status of GSH-Px and selenium in cutaneous leishmaniasis (CL), we determined serum selenium concentration and blood GSH-Px activity in 52 patients with CL and in 38 healthy controls. Selenium concentrations and GSH-Px activities were found to be lower in the patient group than in the controls ($p<0.0001$). Serum albumin and hematocrit % (Htc%) levels were not different in patients than in controls. There were positive relationships between selenium concentrations and GSH-Px activities ($r=0.703$, $p<0.0001$), selenium and albumin ($r= 0.344$, $p<0.05$), GSH-Px and Htc%($r=0.48$, $p<0.01$) in CL patients. We suggest that selenium has an important role in the pathophysiologic processes of CL, and that the decreasing levels of selenium may be a host defense strategy of the organism against CL infection.

Key Words: *cutaneous leishmaniasis,* selenium, glutathione peroxidase

Introduction

Leishmania parasite exists in two forms, depending upon its host: the promastigote or leptomonad form and the amastigote or leishmanial form. The former is seen while the parasite is in the gut of the sandfly, where it is flagellated and extracellular. The latter form occurs in human hosts, where the organism exists intracellularly and is nonflagellated. The transmission occurs when an exposed area of the skin is bitten by an infected sandfly, at which time the organisms are engulfed by dermal macrophages (1, 2). *Cutaneous leishmaniasis* (CL) is widespread throughout Southeastern Anatolia of Turkey. It mainly affects low-income people or rural and suburban populations.

Intensive research has focused on the mechanism (s) by which defense cells kill microrganisms. It has recently come to light that nitric oxide (NO) has an important role in cytotoxicity, and that reactive oxygen intermediates (ROIs) are also involved in intracellular killing (3). ROIs have been shown to kill protozoa such as Toxoplasma gondii, Leishmania and Trypanosoma cruzi (4). Hydrogen peroxide (H_2O_2) play an especially important role in host defense (5).

Selenium is an essential nutrient for humans, being necessary for activity of Glutathione peroxidase (GSH-Px), a glutathione recyling enzyme that catalyses the oxidation of reduced glutathione by H_2O_2 and other hydroperoxides to form oxidized glutathione and water (6, 7). The decreased activity of GSH-Px reflects an inefficient removal of H_2O_2 from the cellular milieu (8).

The purpose of the present study was investigate the selenium status and define the relationship between GSH-Px activity in patients with CL and controls.

Materials and Methods

A total of 90 subjects were enrolled in the study, 52 patients and 38 healthy subjects not exposed to CL. The study was conducted between November 1996 and March 1997 at Harrankapi Leishmaniasis Treatment Center in Şanlıurfa hyperendemic area for *leishmaniasis* in Southeastern Anatolia. Admission criteria for the patient

group were any age or sex, any number of lesions, no pregnancy and no prior treatment, antimonial or other wise. Patients who had lesions for 6 months or longer were excluded from study, because of spontaneous healing and immunity. The control group was selected from healthy parents or siblings who had not been exposed to CL. Age, weight, height 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 material, air dried and fixed in methanol. They were then stained with Giemsa's stain for examination by light microscopy. Microscopic diagnoses were made when amastigots were identified in the smears. Materials were cultured on Novy-Mac Neal-Nicolle (NNN) with rabbit blood agar medium for up to 3 weeks for detection of leishmanial promastigotes.

After the diagnostic procedure, all of the material (glass and plastic) employed was thoroughly cleaned with hot solution of nitric acid (20% v/v) for 48 hours and rinsed six times with demineralized water. A total of 10 ml venous blood was withdrawn, 5 ml was transferred into tubes without addition of anticoagulants and centrifuged for 15 min at a speed of 1760g. Sera were separated for determination of selenium and albumin levels, and 5 ml were transferred to heparinised tubes and GSH-Px activities were measured immediately.

Serum selenium determination was performed with a Spectra AA 250 plus Zeeman atomic absorption spectrophotometer with graphite furnace GTA-96 (Varian, Australia), with deuterium background correction. Varian hollow cathode lamps were employed at 196-nm wavelength and 1.0-nm bandpass. Pyrolytically coated graphite tubes with pyrolytic graphite platforms (Varian) were used. Selenium concentration was determined by an internal standard addition method, as previously described (9). Serum was diluted (1:4) with 0.05% Triton-X100 in 0.125% nitric acid All determinations were run in duplicate, and individual values were averaged. By means of an autosampler, 10 L of the solution was dispensed on the atomizer platform together with 10 L of 1 mg/mL palladium-chloride and 2 mL 2% (w/v) ascorbic acide solution. The temperature program of the furnace is shown Table 1. Absorption readings were measured as peak height. The variation coefficient for replicate measurement was < 3%. The lowest threshold of selenium detection was 10 g/l. The accuracy and precision of the procedure were regularly checked with commercial samples with the recommended selenium contents (Seronorm serum, Nycomed AS, Oslo, Norway).

GSH-Px activities were measured in whole blood by the method of Paglia and Valentina (10) with the Ransel kit (Randox, Belfast) Measurement was carried out at 37°C. Internal quality control was maintained by use of a standardized control supplied by Randox. Measurements were made by automatic analyzer (Hithachi 911, Germany) according to the Randox application procedure. Blood Hb and hematocrit % (Htc%) concentrations were measured by automatic blood analyzer (Coulter, STKS) All values are expressed as units GSH-Px activity/g Hb. Serum albumin levels were measured with commercial kits (Boehringer Mannheim, Germany) by automatic analyzer (Hithachi 911, Germany).

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

Results

As seen from the Table 2, cases and unmatched controls were similar in age, height, body weight and body mass index. When compared to controls, patients with CL had significantly lower levels of serum selenium ($p<0.0001$) (Table 3). There was also a significant decrease in GSH-Px activity in patients with CL ($p<0.0001$). For selenium, the mean levels were decrease by 30%, and GSH-Px activities were lower by 22% in CL patients. Serum albumin and blood Htc levels were lower in the patient group than in the controls, but it was not statistically significant (Table 3).

There was a significant positive correlation between selenium level and GSH-Px activity in the CL patient group ($r=0.550$; $p<0.0001$) and controls ($r=0.705$; $p<0.0001$). There was also significant correlation between selenium and GSH-Px in the total values of the two groups, as seen in Table 4 and Figure 1 ($r=0.703$, $p<0.0001$), as well as positive correlation between selenium and albumin ($r= 0.344$; $p<0.05$), and GSH-Px and Htc ($r=0. 482$; $p<0.01$), Htc and selenium ($r=0.289$;$p<0.05$) (Table 4).

Table 1. Furnace temperature program

Step	1	2	3	4	5
Temperature (°C)	200	1100	2500	2700	50
Hold (s)	60	22	2	3	-
Ramp(s)	10	10	0.7	0.1	13

Table 2. Physical characteristics of CL patients and controls

	Patients n=2 X±SD	Controls n=38 X±SD	p
Age (year)	29.2±4.3	28.3±4.5	>0.05
Height (cm)	168±6	169±5	>0.05
Weight (kg)	65.1±8.9	66±12.4	>0.05
Body mass index (Kg/m^2)	23.4±3.6	24.7±4.4	>0.05

Table 3. Comparison of laboratory findings of patients with CL and control

	Patients n=2 X±SD	Controls n=38 X±SD	p
Serum Selenium (µg/L)	75±20.37	106±26.28	<0.0001
Whole blood GSH-Px (U/gHb)	72.29±18.38	92.49±17.65	<0.0001
Serum albumin (g/dl)	4.3±0.3	4.5±0.2	>0.05
Hematocrit (%)	39.5±2.48	41.3±3.53	>0.05

Table 4. Relationships between blood GSH-Px activity, serum selenium, albumin and hemoglobin levels when two group were combined

Selenium	r=0.073		
	p<0.0001		
Albumin	r=0.185	r=0.344	
	p>0.05	p<0.05	
Hematocrit	r=0.482	r=0.289	r=0.189
	p>0.01	p<0.05	p>0.05
	GSH-Px	Selenium	Albumin

Discussion

The baseline selenium levels of the CL patients in our study were significantly lower than those of the healthy controls residing in the same area. We also observed that GSH-Px enzyme activity was significantly lower in CL patients.

In animals, many diseass 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 donovani (11). However, it is not known whether the causes of this depletion were dependent on selenium content or on other factors. We were also able to find any report on selenium status and the relationship between selenium and GSH-Px activity in patients with CL .

Drowkin et al. (12) demonstrated that selenium concentrations, GSH-Px activities and serum albumin levels were all significantly lower in patients and there was an important correlation between albumin and GSH-Px in acquired immunodeficiency syndrome (AIDS). They state that selenium deficiency seems to be a part of the protein-calorie malnutrition and impaired intestinal absorption common in AIDS. However, Drowkin's patients were predominantly malnourished drug users. Olmsted et al. (13) have also found that selenium concentrations were significantly lower in AIDS patients who were mainly homosexuals from higher socioeconomic classes where malnutrition is usually not a problem, and intestinal selenium absorption was not impaired in these patients. We also found that there was a good correlation between blood selenium values and GSH-Px activity (Fig 1), whereas there were weak corrrelation between serum albumin and selenium, and Htc and GSH-Px in these groups.

We determined the serum levels of albumin in order to assess nutritional because they depend on protein intake (14). We also measured the Htc levels, body mass index, weight and height of the patients and controls. These findings demonstrated that in our patients,

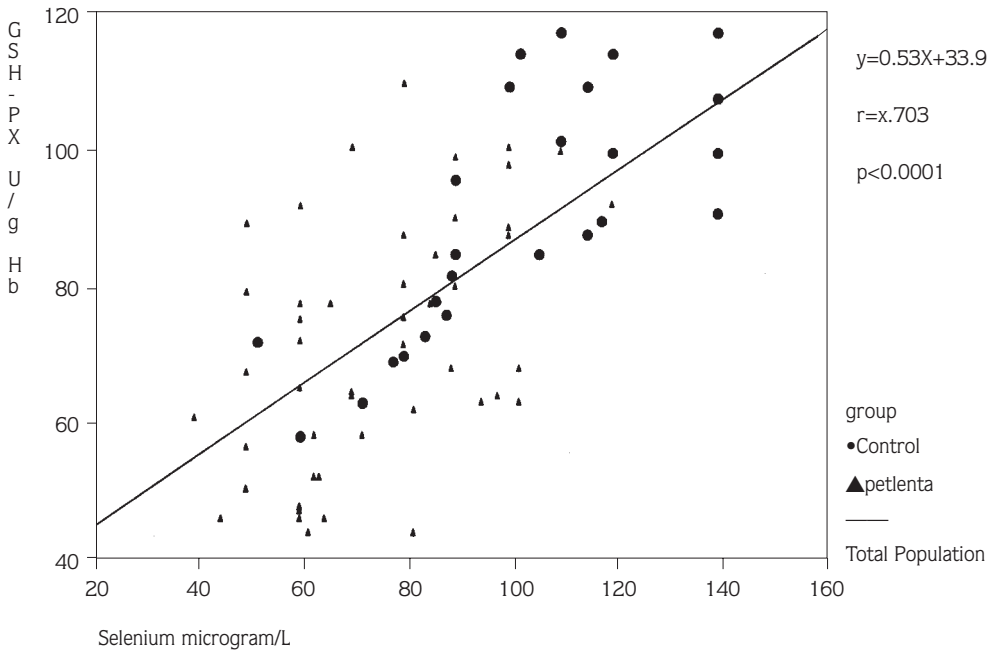

Figure 1. Relationship between serum selenium concentration and GSH-Px activiy in total group.

malnutrition was not a problem. Whereas, blood selenium concentrations were significantly lower in this group.

The serum redistribution of essential trace elements iron, zinc and copper together with the increasd synthesis of acute phase proteins which take place during the course of most infections is well established (15). The changes are part of defense strategies of the organism and induced by the hormone-like substances interleukin-1(IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) [16]. Our findings suggest that the decreasd contents of selenium may be a part of the defense strategies of the organism as is the case with copper, zinc and iron. Nevertheless, no report is yet available on the status of selenium concentrations and relationship between selenium and the GSH-Px in whole blood in CL patients.

We know that nonspecific products are generated during the respiratory burst by macrophages to kill protozoa, and H_2O_2 is an effector molecule against these parasites (16). It is expected that the organism generates increased amounts of hydroperoxides and prevents damage by killing protozoa as a host defense strategy. Lack of selenium leads to a deficiency of GSH-Px enzyme activity and, consequently, to a decreased ability to degrade H_2O_2 (17). Similarly, the unavailability of selenium for the growth of microorganisms could be important in the protective effect of selenium deficiency against *Salmonella typhimurium* infection in rats (18) and against *L. Monocytogenes*, *P. Bergeii and Pseudorabies* virus infections in mice (19), whereas in some infectious diseases, microbicidal activity is lower in the selenium-deficient animal than in the selenium-supplemented animal. For example, *C. albicans* multiplies in the selenium-deficient host. Selenium deficiency cannot protect against this infection and, it was claimed that if in vivo killing of any microorganism is particularly dependent on the neutrophil, and this microbicidal activity is impaired in the selenium deficient animal selenium deficiency may impair the response to infection (20). Macrophage is the exclusive host cell of the intracellular parasite *leishmania* (21). However, not much is known about the effects of selenium on macrophages (22).

We conclude that selenium may play an important role in the pathophysiologic processes of CL and the decreasing levels of serum selenium may be a host defense strategies of organism infection. More data are needed to properly define the role of selenium as a factor in the pathophysiology in patients with CL.

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