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Comparison of serum zinc level in acute improved and chronic cutaneous leishmaniasis

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Background: Zinc is an effective factor in the immune response against infectious agents; its effect on the course of cutaneous leishmaniasis (CL) is unknown. This study aimed to compare the serum zinc level in patients with acute and chronic CL.

Methods: A descriptive study was conducted on 120 CL cases and controls. This included 30 cases of acute CL (less than one year of lesion recovery), 30 cases of chronic CL (period of illness over one year), and 60 healthy subjects with age and gender proportional to the patients. Volunteers entered the study with knowledge and consent. The serum zinc level was measured by atomic absorption spectrophotometry.

Results: The percentages of people with reduced serum zinc levels in the healthy, acute, and chronic groups were 13.3, 50, and 43.3%, respectively, whereby there was a significant difference between the leishmaniasis groups (acute and chronic) and the control group ($P < 0.001$). However, the mean serum zinc level did not differ significantly between the acute (75.36 ± 15.72 µg/dl) and chronic (73.96 ± 17.98 µg/dl) leishmaniasis groups ($P = 0.94$).

Conclusions: A reduced serum zinc level is associated with symptomatic CL, but does not affect the clinical outcome and recovery.

Keywords: leishmaniasis, acute, chronic, serum zinc level

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INTRODUCTION

Leishmaniasis, a parasitic disease, is endemic to 88 countries of the world (tropical and subtropical as well as temperate regions), 72 of which are developing countries. Over 350 million people are at risk of this disease, and 500, 000 new cases of visceral leishmaniasis along with 1-1.5 million new cases of cutaneous leishmaniasis (CL) are reported annually [1]. The leishmania strain is a diploid protozoon, and the parasite promastigote is transmitted from the female sandfly to the host's skin [2].

The clinical manifestations of CL depend on virulence factors of the leishmania strain as well as the host's immune responses, which are determined by both genetic and environmental factors [3].

Once the parasite enters the body, it stimulates both innate and adaptive immunity. $CD4^+$ T cells (T helper cells) are the most important part of the adaptive cellular immune response against leishmaniasis. By increasing the secretion of gamma interferon and interleukin (IL)-2, the Th_1 subtype of $CD4^+$ T cells promotes cellular immune responses and macrophage activity against the *Leishmania* parasite [4,5].

Th_2 subtype activity leads to the production of IL-4, IL-5, and IL-10, causing enhanced humoral immunity and increased immunoglobulin E (IgE) levels. The activity of Th_1 in comparison with Th_2

improves the clinical lesions of CL. In contrast, Th_2 hyperactivity is associated with chronic non-healing forms of the disease [4,5].

Zinc is the second trace element in the human body following iron. This element plays a significant role in the synthesis and metabolism of proteins and nucleic acids and is also involved in the stabilization of the cell membrane. It is also an essential cofactor for the activity of over 300 types of enzymes [6,7].

Among the available methods for measuring trace elements, the reference method is atomic absorption spectrophotometry (AAS), which is a sensitive and specific technique. Determination of the serum zinc concentration by AAS is the most reliable common test for estimating the nutritional state of zinc [8].

Zinc is an essential component of the thymulin hormone, which is important in maintaining a balance between $CD4^+$ and $CD8^+$ T cells. The number and activity of peripheral blood T cells and the blast transformation process are enhanced by zinc prescription and inhibited by zinc reduction [9]. In other words, T cell-mediated immune responses such as delayed hypersensitivity reactions and cytotoxic activity are suppressed when zinc is deficient [10]. Furthermore, zinc deficiency in humans causes a diminished immune response dependent on Th_1 but not Th_2 [11]. Various studies have been performed in relation to investigating the serum zinc level in patients with CL in comparison to healthy individuals. In the last decade, several studies have compared serum zinc levels in patients with leishmaniasis with control groups, but none have evaluated the effect of serum zinc on the clinical course of the disease [12-14].

In this study, we intended to compare serum zinc levels in patients with chronic (non-healed) and acute, improved CL.

PARTICIPANTS AND METHODS

Participants and study design

This cross-sectional study was performed from October 2016 to September 2017 in the dermatology clinic of Imam Reza Hospital, Mashhad. Considering the absence of similar studies in this regard, the present study was performed as a pilot on 60 patients (30 improved acute CL and 30 chronic, non-healed CL patients) as the case group along with 60 healthy people similar to the patients in terms of age and gender as the control group.

Clinical assessment

Patients with CL (after examination and parasitological diagnosis) and the control group individuals were informed about the study protocol. After completing a written consent form and meeting the inclusion and exclusion criteria, the individuals entered the study. The clinical diagnosis of leishmaniasis was made by a dermatologist, and confirmation was achieved by observing the parasite in the direct smear.

The control group was chosen from companions of patients visiting the clinic, who were similar to the patients in terms of age and gender, and had no underlying diseases or current or previous history of leishmaniasis. Then, 2 ml of blood was taken from the superficial veins of the elbow region to measure the serum zinc level; the measurement was made using an AAS device (AAnalyst 300, Perkin Elmer, USA). Zinc deficiency was considered as a serum zinc level lower than 70 µg/dl [7,15].

The inclusion criteria of the study were completing a written consent form and having improved acute CL (patient with CL who recovered at most one year after the onset of the disease with or without treatment) or chronic CL (patient with CL who had at least one year of disease and had not responded to one course of anti-leishmaniasis treatment).

The exclusion criteria included malabsorption disorders, consumption of zinc-containing supplements, hormonal drugs, steroids and anti-convulsants, a vegetarian diet, and underlying hepatic or renal disease.

Ethical considerations

Patients with CL and the control group individuals were thoroughly informed about the study and its protocol. If they agreed, they filled a written consent form.

Statistical methods

The recorded data were analyzed by SPSS 16 (SPSS Inc., Chicago, IL, USA). The characteristics

of the studied individuals were presented by descriptive statistical methods including central and distribution indicators as well as frequency distribution in the form of appropriate tables and charts. To compare the quantitative variables between the two groups, if the data were normally distributed, the ANOVA test was used; otherwise, the Kruskal-Wallis test was employed. To compare the qualitative variables between the two groups, the chi-squared test was utilized. Across all calculations, the significance level was considered as 0.05.

RESULTS

In this research, 120 people including 60 in the control group and 60 patients in the case group were investigated. The case group consisted of patients with CL (30 patients with chronic CL and 30 patients with improved acute CL). The demographic data of the participants in all groups are summarized in Table 1.

The number of people with reduced zinc level (relative to normal zinc level) in the control, acute improved, and chronic groups was 8 (13.3%), 15 (50%), and 13 (43.3%), respectively, whereby a significant difference existed between the subjects based on Fisher's exact test ($P < 0.001$). However, there was no significant difference between the chronic and acute improved CL groups in terms of the frequency of patients with reduced zinc levels based on the chi-squared test ($P = 0.79$).

The mean serum zinc level in the control, acute improved, and chronic group was 90.08 ± 16.30, 75.36 ± 15.72, and 73.96 ± 17.98 µg/dl, respectively, representing a significant difference between the subjects according to the ANOVA test ($P < 0.001$).

The mean serum level of zinc did not differ significantly between the acute improved and chronic CL groups based on the Tukey test ($P = 0.94$).

Table 2. The mean serum level of zinc in the subjects

Subject	Serum zinc level (µg/dl) Mean ± SD	Test result
Control group	90.08 ± 16.30	
Improved group	75.36 ± 15.72	*P*<0.001*
Chronic group	73.96 ± 17.98	

*Tukey test

However, there was a significant difference between the control and acute improved as well as chronic groups in terms of serum level of zinc ($P < 0.001$ and $P < 0.001$, respectively) (Table 2).

The mean serum level of zinc in women in the acute improved and chronic groups was 71.56 ± 15.93 and 73.68 ± 18.74 µg/dl, respectively. According to the t-test, there was no significant difference between the two groups ($P = 0.71$). The mean serum level of zinc was 79.71 ± 14.86 and 74.75 ± 16.86 µg/dl among men in the acute improved and chronic groups, respectively. Based on the t-test, there was no significant difference between the two groups ($P = 0.55$).

The mean serum level of zinc in the age group of below 18 years in the acute improved and chronic groups was 76.33 ± 19.4 and 72.8 ± 9.31 µg/dl, respectively. Based on the t-test, there was no significant difference between the two groups ($P = 0.55$). The mean serum level of zinc in the age group of above 18 years in the acute improved and chronic groups was 74.95 ± 14.40 and 75.22 ± 22.16 µg/dl, respectively. Based on the t-test, there was no significant difference between the two groups ($P = 0.96$).

DISCUSSION

In the current study on 30 acute improved CL and 30 chronic CL patients, along with 60 healthy people as the control group, the serum zinc level

Table 1. Demographic data of the patient and control groups

Subjects	Leishmaniasis patients		Control	*P*. value
	Acute improved	Chronic		
Gender, n (%)				
Male	14 (46.7%)	8 (26.7%)	22 (36.7%)	0.27
Female	16 (53.3%)	22 (73.3%)	38 (73.3%)	
Age (years), Mean ± SD	30.33 ± 19.7	28.40 ± 21.08	29.30 ± 19.53	0.93*
Intralesional treatment, n (%)	28 (93.3%)	29 (96.6%)		1
Systemic treatment, n (%)	6 (20%)	17 (56.7%)		0.007**

*ANOVA test
**Fisher's exact test

was significantly lower in CL patients than in healthy subjects. Although the serum zinc levels in the chronic group were lower, there was no significant difference between the acute improved and chronic groups, which can be affected by the small sample size.

Different studies have been done on serum zinc levels in patients with CL compared with healthy subjects. However, no study has so far compared this essential element among people with acute improved and chronic CL.

In a study by Faryadi *et al.*, the serum zinc level was evaluated among 54 people (18 controls, 18 acute CL, and 18 chronic CL patients). The serum zinc level was lower in the acute and chronic groups compared against the controls. Although there was no significant difference between the acute and chronic groups in that study, they did not consider the final clinical course of acute cases [12].

In a study by Van Weyenbergh *et al.*, the serum zinc level was compared across 31 patients with cutaneous, visceral, and mucocutaneous leishmaniasis and a control group. Serum zinc levels were lower in all three groups than in the control group. However, only in the visceral and mucocutaneous groups, there was a significant decrease in serum zinc level [15].

In a study conducted in Iraq, 60 patients with CL had low serum zinc levels and this decrease was significant in comparison with the control group. The same study showed that the ratio of CD_4 to CD_8 cells and the total count of CD_4 and CD_8 cells were significantly lower in patients with zinc deficiency compared to the control group [9].

In the study by Farzin *et al.*, a significant reduction of serum zinc level was observed in the cutaneous and visceral leishmaniasis groups compared with the control group [13].

Only in the study by Faryadi, the serum level of zinc was compared between acute and chronic CL, where there was no significant difference between the two groups [12]. Nevertheless, the sample size of that study was limited. Further, they had a definition of acute leishmaniasis limited to lesions with a lifespan of fewer than six months, without considering future improvements. Possibly, these lesions may be categorized as chronic cases in the case of one-year follow-up. A positive aspect of our study was considering the recovery of lesions in less than a year in the acute improved group.

The common finding across all of the above studies is the significant decrease of serum zinc level in patients with CL relative to controls, which was also confirmed in our study.

Malnutrition is the most common cause of immunodeficiency around the world. Nutritional deficiencies cause an increased risk of incidence of infectious diseases secondary to impaired immune responses [16]. They also have a significant role in the chronicity of some parasitic infections [17]. Although the incidence of leishmaniasis in endemic regions can be independent of the nutritional status of people [18], some studies have shown the relationship of CL with an improper nutritional situation, impaired growth, and iron deficiency among children [19,20]. Two major causes of impaired levels of micronutrients in the serum are nutritional deficiencies and underlying diseases [21].

In recent years, the investigation of zinc status has attracted a great deal of attention due to its important role in reducing the incidence and intensity of different types of infection [11], particularly in CL, as wound healing is impaired in patients with zinc deficiency [17]. In addition, zinc administration in acute CL has had a positive effect on the improvement of the lesions [12].

In CL, decreased serum zinc levels are associated with an inability of the host to eliminate the parasite [22,23].

Serum redistribution of micronutrients such as Se, Fe, and Zn along with an increased synthesis of acute-phase proteins seen in most infectious processes are a part of the defense mechanism of the host [24]. On the other hand, the release of endogenous leukocyte mediators (TNF-α, IL-1 and IL-6) from the activated phagocytes, which occur in response to various stimuli such as infections (e.g., CL), can reduce the serum zinc levels as the parasitic defense mechanism is intended to escape the host's immunity [25-27].

The possible mechanism of the effect of zinc on leishmaniasis treatment is that zinc deficiency causes an impaired Th_1-Th_2 balance. Without affecting Th_2 products, zinc deficiency reduces the Th_1 products (IL-2, IFN-α). On the other hand, it reduces the lytic activity of natural killer cells and minimizes the number of cytotoxic T cells [28].

In this study, there was no significant difference in the reduction of serum zinc levels between the acute improved and chronic CL groups,

suggesting that factors other than zinc deficiency are involved in the chronicity of leishmaniasis lesions. Furthermore, considering the lower levels of zinc in the patients relative to the controls, zinc deficiency might affect the susceptibility of a person to develop CL or may be a consequence of the disease. On the other hand, the limited sample size may also affect our findings.

CONCLUSION

In our study, which was the first to compare serum zinc levels among improved acute (in less than one year of follow-up) and chronic groups of CL, there was a significant increase in zinc deficiency in both acute improved and chronic groups compared to the control group. Although the serum zinc levels in the chronic group were lower, there was no significant difference between the acute and chronic CL groups.

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