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# Serum zinc and copper concentrations in patients with Beta-thalassemia major

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**Key words**
thalassemia – zinc – copper

**Abstract.** Objective: There is no consensus about zinc and copper deficiency in patients with thalassemia major. We have therefore investigated serum copper and zinc concentration in this condition as it may contribute to the morbidity seen in this group of patients. Material and methods: Serum copper and zinc were measured in 140 thalassemia major patients (79 male and 61 female) by Atomic Absorption spectrophotometer (AAS) and their status was compared with 140 gender and age-matched healthy subjects. Data that was normally distributed was analyzed using Student t-test. Results: In the control group, zinc concentration was higher in males than in females but no significant difference was found between copper levels. Thalassemic patients had significantly lower zinc (60.1 vs. 87.1 µg/dl) and copper (90.4 vs. 105.6 µg/dl) concentration compared with healthy subjects ($p < 0.001$). The prevalence of low serum levels of zinc and copper in thalassemic patients was almost two times higher than healthy subjects for zinc (72.3% vs. 29.3%) and copper (36.1% vs. 14.5%). Conclusion: A high prevalence of low levels of serum zinc and copper concentrations were observed in thalassemic patients.

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## Introduction

Thalassemia is the most common genetic disorder in the world causing a defect in hemoglobin synthesis [1]. Hemoglobin consists of two main protein chains called α and β. There are two subtypes of the disorder depending on what portion of the hemoglobin is abnormally synthesized. Each of these subtypes can be further classified depending on the number of thalassemia genes involved. The molecular defects producing β-thalassemia are heterogeneous, and each ethnic group possesses its own specific set of mutations [2, 3].

Almost 150 million people throughout the world carry the thalassemia gene and it is more common in Middle East and Iran [4]. More than 25,000 people have thalassemia major disease in Iran [5]. The disease is associated with severe anemia, jaundice, splenomegaly, growth failure, osteopenia, expanded bone marrow space, hepatic, siderosis and cardiomegaly [6, 7].

Short stature, low weight, anorexia and hypogonadism which were found in most of the patients with thalassemia could be related to their zinc deficiency [8, 9]. There is a direct relation between blood levels of zinc and testosterone and changes in steroidgenesis provoked by zinc-deficiency caused hypogonadism [10, 11]. The first clinical manifestations related to zinc deficiency and its essential role in human nutrition were hypogonadism and growth retardation [12].

Shamshirsaz et al. [20] conducted a study to determine the prevalence of prominent thalassemia complications in Iran. Their results showed that short stature was seen in 39.3% of thalassemic patients and hypogonadism in 22.9% of boys. Low serum zinc and copper was observed in 79.6% and 68% of the study population, respectively.

Trace element zinc is important in maintaining the healthy growth of the human body, especially for infants and young children's growth and development [8].

Zinc is intimately linked to bone metabolism, thus, zinc acts positively on growth and development. Zinc also enhances vitamin D effects on bone metabolism through the stimulation of DNA synthesis in bone cells (Salgueiro et al. 2002 **■■■ Not listed in References!**).

Zinc has been shown to be essential in the structure and function of a large number of macromolecules and for over 300 enzymatic reactions playing both catalytic and structural roles in enzymes [13]. It also contributes to protein synthesis, cell division, wound repairing, improving visual acuity and immunity system. Zinc is crucial for taste perception, and is involved in antioxidant defense systems [14]. Copper is an essential structural coparticipant of many enzymes acting as a cofactor. Copper is a participant in structural of cytochrome C oxidase, lysyl oxidase, superoxide dismutase (SOD) and thyrosinase. This trace element acts as the cofactor for at least 30 enzymes [15]. Deficiencies of zinc and copper in patients with thalassemia major have been under debate [16, 17, 18, 19 vs. 14, 20, 21, 22]. Thus in this study we aimed to evaluate zinc and copper status in thalassemia major disease.

## Materials and methods

### *Performing location/organization*

This research was conducted in Dr. Sheikh Hospital (the specialized center for thalassemic children in Mashhad), Bu-ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

### *Subjects*

The study was carried out on 140 patients (61 males and 79 females) with documented thalassemia major (age 7 – 21 years) in Dr. Sheikh Hospital. All patients were free from HBV, HCV and HIV. The diagnoses of â-thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles for the patients.

140 age- and sex-matched healthy participants (mean age 14.6 ± 4.4) without a history of anemia, abnormal complete blood counts and abnormal hemoglobin electrophoresis results, who lived in the same city, were recruited as the control group. Blood transfusion-dependence was present in 98% of thalassemics in which 58.6% received blood transfusion every month.

Each participant gave an informed written consent for participation in the study, and the study was approved by the Mashhad University of Medical Science Ethics Committee.

Inclusion criteria and exclusion criteria are given in below.

#### Inclusion criteria

- Transfusion dependent β-thalassemia major
- Volunteers to participate in this research with their parents consent

#### Exclusion criteria

- less than 8 years or more than 18 years old
- Thalassemic patients with malabsorption diseases, other GI problems and asthma
- Chronic use of systemic corticosteroids
- Patients currently use zinc supplement
- use of any drugs, except routine supplements of folic acid and ferrous sulfate
- hepatitis B and C
- smoking
- infections
- hypertension
- diabetes mellitus

### *Anthropometric measurement*

For all participants, anthropometric parameters including weight and height were measured using a standard protocol. Weight was measured with the participants dressed in light clothing after an overnight fasting using a standard scale (ISAK 2001, Lee & Nieman 2007).

#### Collection of serum samples

10 ml of blood samples were collected in the morning from each subject after an overnight fast. After being allowed to clot, blood was then centrifuged at 2,500 rpm for 15 min

Table 1. Characteristics of patient and control groups.

| Data | Mean ± SD | | |
|---|---|---|---|
| | Thalassemia | Control | p-value |
| Height | 143.0 ± 17.1 | 151.5 ± 15.5 | < 0.001 |
| Weight | 37.4 ± 12.5 | 52.1 ± 21.9 | < 0.001 |
| Age | 14.34 ± 4.5 | 14.93 ± 4.3 | 0.242 |
| Hb | 13.54 ± 1.7 | 8.63 ± 1.1 | < 0.001 |
| BMI | 18.31 ± 1.0 | 22.80 ± 1.2 | < 0.001 |

Values were expressed as mean ± SD. Comparisons were made using student's t test between groups.

Table 2. Reference ranges by age and gender (according to Mustafa Akcam, "Serum Ferritin, Vitamin $B_{12}$, Folate, and Zinc Levels in Children Infected with Helicobacter pylori" (2007).

| | Age (years) | Gender | Reference ranges |
|---|---|---|---|
| Zinc (µg/dl) | 6 – 9 | F, M | 78 – 105 |
| | 10 – 13 | F | 78 – 118 |
| | | M | 78 – 98 |
| | 14 – 19 | F | 59 – 98 |
| | | M | 65 – 118 |

F = female, M = male.

Table 3. Comparison of Serum and Zinc in thalassemia patients and control groups.

| Thalassemia group | Control group | p-value | |
|---|---|---|---|
| Zinc (µg/dl) | 60.1 ± 19.0 | 87.1 ± 23.7 | < 0.001 |
| Copper (µg/dl) | 90.4 ± 29.8 | 105.6 ± 32.5 | < 0.001 |

Values were expressed as mean ± SD. Comparisons were made using student's t-test between groups. There was a correlation between weight, height and age of first transfusion in patients group. No significant association between height, weight age and zinc serum levels was observed.

at room temperature to obtain serum. Hemolyzed samples were excluded from analysis. Serum was stored at –20 °C prior to analysis.

### *Serum zinc and copper assay*

The serum contents of both zinc and copper were measured by Flame Atomic Absorption Spectrophotometer (AAS) technique model CTA 3000 (CHEMTECH AI LIMITED Company). All tests were performed in duplicate.

### *Statistical analysis*

All statistical analyses were performed with SPSS19 (IBM company). Values were expressed as mean ± SD. Data that was normally distributed was analyzed using Student t-test (for two groups). Parametric and non-parametric correlations were assessed using Pearson correlation coefficients and Spearman correlation coefficients, respectively. The level of statistical significance was set to $p < 0.05$. Values are expressed as mean ± SD for normally distributed data. Comparisons were made using Independent T-test.

## Results

Characteristics of patient and control group are shown in Table 1. In the control group hemoglobin levels usually ranged between 10 and 12 g/dl, whilst in the thalassemia subjects hemoglobin levels ranged between 7 and 11 g/dl.

Thalassemic patients had significantly lower weight and height compared with healthy subjects ($p < 0.001$).

## Comparison of serum zinc and copper concentrations in groups

Reference values of serum zinc are shown in Table 2. 27.3% of control group had zinc deficiency. Thalassemic patients had significantly lower zinc (60.1 ± 19.0 vs. 87.1 ± 23.7) and copper (90.4 ± 29.8 vs. 105.6 ± 32.5) compared with healthy subjects ($p < 0.001$) (Table 2). More than 70% of thalassemic patients had zinc concentration below 70 mg/dl, while 35.9% case had severe zinc deficiency (≤ 50). Our findings demonstrated serum copper deficiency in ~ 14.5% healthy subjects and 38.3% of thalassemic patients.

## Discussion

Our results indicate that there are significantly lower plasma levels of zinc and copper in patients with thalassemia major compared with normal subjects. The prevalence of low serum levels of zinc and copper in thalassemic patients was almost two times higher than healthy subjects (72.3% vs. 29.3%) for zinc and 36.1% vs. 14.5% for copper.

Abnormal growth is a common feature among patients suffering from β-thalassemia

Table 4. Correlation of zinc and copper with age, height and weight in patients with thalassemia major.

| | Parameter | r | p-value |
|---|---|---|---|
| Thalassemia group | Height and zinc | 0.032 | 0.695 |
| | Height and copper | –0.016 | 0.841 |
| | Age and zinc | 0.007 | 0.942 |
| | Frequency transfusion and zinc | –0.038 | 0.721 |
| | Weight and zinc | 0.087 | 0.289 |
| | Weight and age of fist transfusion | 0.270 | 0.009 |
| | Height and age of first transfusion | 0.328 | 0.001 |
| | Age and copper | 0.015 | 0.857 |
| | Frequency transfusion and copper | 0.962 | 0.310 |
| | Zinc and copper | 0.201 | 0.018 |

Parametric and non-parametric correlations were assessed using Pearson correlation coefficients and Spearman correlation coefficients, respectively.

Table 5. Correlation of zinc and copper with age, height and weight in healthy subjects.

| | Parameter | r | p-value |
|---|---|---|---|
| Control group | Height and zinc | –0.083 | 0.004 |
| | Height and copper | –0.081 | 0.506 |
| | Age and zinc | 0.384 | < 0.001 |
| | Age and copper | –0.123 | 0.136 |
| | Weight and copper | –0.122 | 0.310 |
| | Zinc and copper | 0.042 | 0.615 |

Parametric and non-parametric correlations were assessed using Pearson correlation coefficients and Spearman correlation coefficients, respectively.

major. Many patients with thalassemia have deficiencies of micronutrients. Yet, there is no consensus about zinc deficiency in thalassemia major patients.

In some reports it has been described that the patients with β thalassemia major suffer from zinc deficiency and therefore they have delayed maturity and short stature [20, 21]. In one study on thalassemic children in Bangladesh, about 60% patients had low serum level of Zinc [14]. Bekheirnia et al. [22] reported that the frequency of hypozincemia in thalassemia major patients was high (85.5%). Tabatabaee et al. [23] showed that 84.8% of thalassemic major patients had zinc deficiency. Yazdiha et al. [21] reported that the serum concentration of zinc in thalassemic patients was significantly lower than in control group, and there was a significant difference statistically. Similar reports were provided by other researchers [14, 19, 20, 21, 22, 23, 24]. Hashemipoor et al. ■■■ **Not listed in References!**.found that zinc concentration of hair in thalassemic patients was lower than control group.

On the other hand, Mehdizadeh et al. [16] have reported that mean serum zinc level was significantly higher in thalassemic patients. Rea et al suggested hyper transfusion can prevent zinc deficiency in thalassemia major. Mehdizadeh et al. [16] found that the mean serum zinc level was high in the thalassemic group and therefore, suggested that routine zinc supplementation is not necessary for most of TM patients [17, 18]. Some studies reported that the serum zinc level in major thalassemic patients and control group were within normal limits [25, 26].

Some researchers have reported that there was an increase in serum level of copper in thalassemia major patients [17, 27, 28]. However, other authors have observed a reduction in the serum level of copper [22, 23, 29, 30].

Although the study by Kassab-Chekir showed no difference in serum copper concentrations in thalassemic patients [31], Kwan and colleagues reported that only 3 of their 68 thalassemic patients had zinc deficiency in their study population.

Some reports indicate that desferrioxamine increases urinary zinc excretion and may decrease zinc body content (Faranoush et al., 2008 ■■■ **Not listed in References!**). They found a significant negative correlation between zinc level and the duration and dosage of deferoxamine treatment.

De-Virgiliis et al. (1988 ■■■ **Not listed in References!**) concluded that chelation of zinc, may be related to the low iron burden. They suggested that other factors, such as anorexia, nutritional status; psychological problems (such as depression) and different metabolic and endocrine complications have led to zinc deficiency (Ugsal and Akar, 1993 ■■■ **Not listed in References!**).

Low levels of zinc and copper can be related to nutritional intake in thalassaemic patients. Malnutrition was primarily caused by inadequate nutrient intake, as indicated by the capacity to gain weight appropriately when provided with nutrition support and by the absence of intestinal malabsorption (Fuchs et al., 1996 ■■■ **Not listed in References!**). Another study by Klevay (2001 ■■■ **Not listed in References!**) was interested in copper deficiency and found that the iron can interfere with copper utilization without anemia and the dietary requirement for copper of

people with iron overload may exceed that of the general population and there is deficiency among patients in zinc and copper levels concerned in bone mineral density in thalassemia.

## Conclusions

In this study we observed that there was significantly lower plasma levels of zinc and copper in patients with thalassemia major compared with normal subjects. It seems that deficiency of serum zinc and copper in our patients could be attributed to a high prevalence of deficiency of these two trace elements in Iranian general population. Thus hypozincemia similar to iron deficiency anemia needs to be considered as a nutritional problem in this population.

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## Author: ■■■ Tables 3 – 5 weren't mentioned in the text!

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