

# Zinc Status and its Correlation with Basic Parameters in Transfusion Dependent Thalassemic Patients: A Pakistani Perspective

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## Abstract

**Background:**  $\beta$ -thalassemia is an autosomal hemoglobinopathy with inconsistent universal distribution. Among patients with thalassemia diverse non-siderotic complications distinctly influence the attribute of life, including zinc deficiency due to varied etiologies.

The objective of the present study was to determine zinc levels in patients with  $\beta$ -thalassemia major and its correlation with maternal characteristics, hematological parameters, liver enzymes, serum ferritin, duration of chelation and number of transfusions among Pakistani patients.

**Patients and Methods:** Seventy-five  $\beta$ -thalassemia major patients on desferrioxamine were enrolled from August 2010 to July 2012. CBC, liver function tests, serum ferritin, HbsAg and Anti-HCV were evaluated. Zinc level was measured using atomic absorption spectrophotometer.

**Results:** The mean age of patients was  $10.57 \pm 3.5$  years. Forty one (54.7%) and 34(45.3%) patients were males and females respectively. The frequency of zinc deficiency was 24%. We established positive correlation between zinc deficiency and longer duration of chelation ( $P < 0.001$ ) and also with anemia ( $P < 0.001$ ). No correlation could be established with other parameters.

**Conclusion:** Our study revealed that hypozincemia is not unusual in  $\beta$ -thalassemic patients on desferrioxamine. We propose zinc levels should be regularly measured primarily in anemic patients with long duration of chelation.

**Keywords:**  $\beta$ -thalassemia major, zinc deficiency, desferrioxamine.

## Introduction

$\beta$ -thalassemia major symbolizes a group of recessively inherited hemoglobinopathies, first depicted by Cooley and Lee and exemplified by diminished synthesis of  $\beta$  globin chains <sup>1</sup>. The homozygous state typically needs medical care within the first year of life and is manifested by severe anemia that necessitates unremitting transfusion for continued survival <sup>1,2</sup>. Appropriate combination of blood transfusion support and iron chelation remedies has noticeably lengthened the life expectancy among these patients to the fourth decade of life <sup>1,3</sup>.

Nevertheless iron overload is still a major culprit of mortality and morbidity among thalassemics <sup>3</sup>. Cardiac impairment secondary to iron accumulation and oxidative injury represent the major cause of

mortality, which can be averted by chelation <sup>3</sup>. In the last four decades, the conventional drug used for iron chelation has been desferrioxamine (DFO). DFO has obviously improved the prospects of this disease as it is a powerful iron chelator, prevents iron accumulation and iron mediated organ damage <sup>4</sup>. But on the other hand it also chelates certain important minerals such as zinc, cobalt and copper <sup>4</sup>.

Zinc is a crucial trace mineral needed by all living tissues because of its decisive requirement in proteins synthesis and also acting as a cofactor in enzyme catalysis <sup>5,6</sup>. Zinc is found in nearly all cells and has fundamental role in immune system involving both innate and acquired immunity <sup>6</sup>. Zinc is also actively involved in many metabolic

activities in human body such as DNA synthesis, cellular growth, wound healing, fertility and conception<sup>5, 6</sup>. Beside it also has antioxidant properties thus protecting the cells from free radicals injuries, which are frequent finding in thalassemics, and is also involved in host defense mechanism such as chemotaxis of granulocytes<sup>6</sup>. The significance of zinc in human body is illustrated by the sound effects of zinc deficiency which includes impaired wound healing, osteoporosis, delayed skeletal maturation, growth retardation, brittle hairs, impaired glucose metabolism, impaired sense of smell and taste, reduced fertility, neurological disturbance, irritability and deformed nails<sup>3-5, 7</sup>.

Zinc deficiency in thalassemia is ascertained owing to various etiologies which integrate oral or parenteral iron chelation, inadequate diet, and augmented urinary zinc excretion and ongoing hemolysis<sup>1, 4, 8, 9</sup>.

In thalassemia major trace metal zinc deficiency has been debatable<sup>3</sup>. Many regional and international studies give conflicting results. Studies reported from Iran, Iraq and Egypt have revealed pronounced zinc deficiency in contrast to another study reported from Tehran, Iran which has revealed high levels of zinc in patients on DFO<sup>1, 2, 8, 10</sup>. Another study from Kuwait has reported zinc levels in thalassemia major patients to be within normal limits<sup>11</sup>.

Some studies recommend performing routine zinc assessment in thalassemic patients and to replenish it when required<sup>4</sup>. Currently no consensus for regular monitoring and no meticulous guidelines or recommendations for screening of these patients exists. However, it is well proven that zinc supplementation has a beneficial role and has been shown to enhance linear growth among thalassemic patients<sup>7</sup>.

The primary objective of our study was to evaluate the serum zinc levels in Pakistani transfusion dependent thalassemic patients on DFO. Whilst secondary objective was to ascertain any correlation of zinc levels with maternal characteristics, hematological parameters, biochemical markers, duration of chelation and number of transfusions.

## Patients and Methods:

### Patients

This was a prospective study extending from August 2010 to July 2012. Seventy five, non consecutive

patients with established  $\beta$ -thalassemia major were enrolled in this study. Thirty patients were in regular follow up prior to study; others were registered during the study period. Ethical endorsement was given by ethical and research committee of Liaquat National Hospital. An informed consent was attained from parents or Patients' guardians since all patients were under 18 years old.

All registered patients were on regular blood transfusions and parenteral iron chelation. Patients on oral iron chelation, having chronic liver disease (child score 2 and 3), diarrhea, malabsorption and those suffering from acute infections were excluded. Patients with  $\beta$  thalassemia intermedia and compound heterozygous form were also excluded from the study.

### Methods

Demographic data including age and gender (maternal characteristics), number of blood transfusions and duration of chelation were recorded by taking thorough history from parents or patients' guardians and from patients' medical records. Blood samples for accessing the zinc levels were collected prior to blood transfusion under aseptic conditions. To perform this 5cc of peripheral venous blood in sodium heparin bottle was taken from the patient and zinc levels were assessed using an atomic absorption spectrophotometry. The defined reference value for zinc was 50-150  $\mu\text{g}/\text{dl}$ , zinc deficiency was defined zinc serum level of  $<50\mu\text{g}/\text{dl}$ .

Hematological parameters including hemoglobin, hematocrit, WBC and platelets were determined by automated Sysmex analyzer XT 2000i (Sysmex, Japan). Serum ferritin and liver function tests were performed by HITACHI 912 using a photometric assay. Hepatitis B and hepatitis C infections were detected by chemiluminescence method (Abbott AxSYM, USA).

### Statistical Analysis

Data was entered and analyzed using SPSS software (Version 17, SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean  $\pm$  SD for quantitative variables and qualitative variables were presented as frequency and percentages. Student's t-test was applied for the comparison of means. Data were considered statistically significant at P value  $< 0.05$ . Correlation analysis was performed using

the spearman correlation method to identify relationship between the serum zinc levels and maternal characteristics, duration of chelation, number of transfusions, hematological markers and serum ferritin. Chi-square test was applied for evaluating the correlation between zinc levels and hepatitis B and C status.

## Results:

In this prospective study, 75 patients with  $\beta$ -thalassemia major were enrolled. Among these patients 41 (54.7%) were males and 34 (45.3%) were females. The mean age was  $10.57 \pm 3.5$  (range 5 to 15) years. The mean serum zinc level was  $71.45 \pm 21.98$  (range 30.4-117.0). The ratio of insufficiency was higher in male gender by a 2:1 ratio (male: female).

Overall 18 patients 24% were zinc deficient, out of which 12 were male and 6 were female. The patients were divided into two groups: zinc deficient and non zinc deficient. The mean zinc levels in zinc deficient and non deficient groups were  $44.03 \pm 5.48$  and  $80.11 \pm 17.64$  respectively ( $P < 0.001$ ). Baseline characteristics of both zinc deficient and non deficient patients are shown in table 1.

Zinc deficiency also revealed positive correlation with low hemoglobin (0.566, 0.014) as shown in figure 1. The mean hemoglobin was  $8.94 \pm 1.11$  in non deficient group, whereas was significantly lower,  $6.01 \pm 0.67$  in the deficient group (Figure 2) ( $P < 0.001$ ). Zinc deficiency also had positive

correlation with higher duration of chelation being  $7.72 \pm 3.06$  (years) and  $2.95 \pm 1.45$  (years) in zinc deficient and non deficient patients respectively ( $P < 0.001$ ).

Serum ferritin were significantly higher in all patients but no significant correlation was detected with zinc deficiency ( $P > 0.05$ ). HbsAg was found to be positive in 2.7% while Anti-HCV was reactive in 32% of patients. There were no statistically significant correlations ( $P > 0.05$ ) between low levels of zinc and maternal characteristic, TLC and platelets count, elevated liver enzymes, hepatitis B, hepatitis C and number of transfusions per month.

## Discussion:

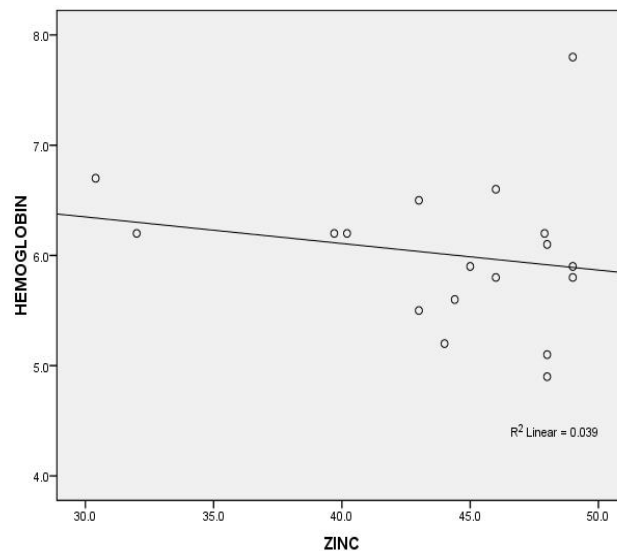
$\beta$ -thalassemia major causes decreased erythrocytes survival and iron burden owing to repeated blood transfusions and diverse pathological expressions of primary and secondary complications. The key treatment strategy is to decrease the iron overload which enhances the life expectancy of these patients.

The current study investigated the status of serum zinc in thalassemic patients on parenteral iron chelation. The explanation for zinc deficiency is the zinc binding affinity of desferrioxamine which augments urinary zinc elimination, hyperzincuria culminating in gradual zinc depletion in thalassemics<sup>4, 12-14</sup>.

Zinc is a key constituent which is essential for maintenance of cellular homeostasis. The principal

**Table 1:** Demographic findings among zinc deficient and non deficient thalassemic patients.

Evaluated parameters	Deficient group	Non deficient group	P value
	Serum zinc < 50 $\mu\text{g} / \text{dl}$ Mean $\pm$ SD	Serum zinc $\geq 50$ Mean $\pm$ SD	
Age	11.56 $\pm$ 3.51	10.26 $\pm$ 3.46	0.1
Serum zinc $\mu\text{g} / \text{dl}$	44.03 $\pm$ 5.48	80.11 $\pm$ 17.64	< 0.001
Duration of chelation(years)	7.72 $\pm$ 3.06	2.95 $\pm$ 1.45	< 0.001
Number of transfusion(per month)	2.22 $\pm$ 0.42	2.19 $\pm$ 0.97	0.9
Hemoglobin gm/dl	6.01 $\pm$ 0.67	8.94 $\pm$ 1.11	< 0.001
Hematocrit(%)	19.42 $\pm$ 3.76	26.57 $\pm$ 3.46	< 0.001
Ferritin $\mu\text{g} / \text{dl}$	4058.89 $\pm$ 2340.02	4040.5 $\pm$ 2090.07	0.9
Total bilirubin mg/dl	1.35 $\pm$ 1.12	1.43 $\pm$ 1.13	0.7
Direct bilirubin	0.55 $\pm$ 0.66	0.58 $\pm$ 0.67	0.8
Indirect bilirubin	0.78 $\pm$ 0.55	0.82 $\pm$ 0.59	0.7
AST u/l	55.83 $\pm$ 38.63	59.26 $\pm$ 34.69	0.7
ALT u/l	54.44 $\pm$ 45.72	67.91 $\pm$ 64.18	0.4



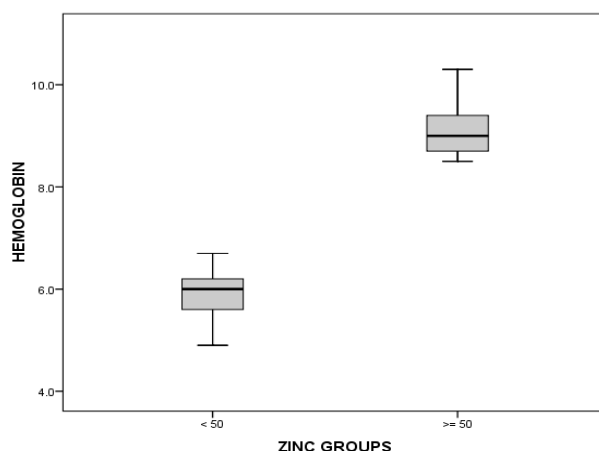
**Figure 1:** Positive correlation of zinc deficiency with low hemoglobin levels ( $P < 0.001$ ).

reason for its mandatory requirement is the fact that hundred of metallo enzymes require zinc as a cofactor to be functionally effective. Zinc insufficiency is deemed as one of the core causative factors for impaired linear growth, delayed sexual maturity, neuropsychological changes, immune dysfunction and endocrinopathies in thalassemic individuals<sup>3, 5, 7, 15</sup>.

Diverse studies have revealed considerably diminished zinc levels in thalassemia major patients. Al Samarrai et al. from Iraq demonstrated zinc deficiency among thalassemic patients and attributed it to hyperzincuria<sup>16</sup>. Shamshirsaz et al. have reported 79.6% of their thalassemic patients to have zinc deficiency and subsequent delayed sexual activity, retarded growth and significant association with lumbar BMD<sup>1</sup>. The authors emphasized that the cause of zinc deficiency was insufficient zinc intake which could be attributed to a high prevalence of deficiency of this trace mineral in general Iranian population<sup>1</sup>. Analogous results have been reported from Egypt, by Nasr et al. in their series of 64 patients<sup>2</sup>. They reported that the mean zinc level was significantly lower ( $12.4 \pm 5.4 \mu\text{g}/\text{dl}$ ) in patients versus the control group ( $95.1 \pm 10.3 \mu\text{g}/\text{dl}$ ). They also analyzed association of zinc insufficiency with duration of transfusion and chelation therapy

but no correlation were established, with sub optimal chelation<sup>2</sup>. In contrast our results shows statistically significant correlation ( $P < 0.001$ ) of zinc deficiency with duration of chelation and signifies the enhanced clearance associated with increased duration of chelation. Augmented urinary zinc excretion in patients receiving desferrioxamine supports our finding<sup>14</sup>.

The present study illustrated that hypozincemia is not uncommon in thalassemics regardless of age. Similarly a report by Dehshal et al. showed that 37% of thalassemic patients had zinc deficiency and authors proposed that serum zinc levels be routinely monitored in these patients<sup>4</sup>. Likewise Ferdaus et al. from Bangladesh also found that desferrioxime treatment was associated with zinc loss in 60% of their thalassemic patients<sup>3</sup>. Analogous to above mentioned studies, another regional study, by Ghone et al. reported from India showed diminished levels of serum zinc in their series of 72 patients<sup>17</sup>. A study on Iranian thalassemic patients have reported 10% of thalassemic patients to have zinc insufficiency while 52% of those had variable degree of depression<sup>18,19</sup>. Another study has identified a reverse correlation of zinc deficiency with blood transfusion rate ( $P < 0.05$ )<sup>18</sup>. Also Bekheirnia et al. from Iran found 84.8% of



**Figure 2:** Comparison of hemoglobin levels in zinc deficient and non deficient groups.

thalassemic patients to be zinc deficient<sup>19</sup>. Our findings are also in concurrence with studies reported from Turkey, Tunisia, Thailand and Egypt<sup>20-23</sup>.

In contrast to our results Bashir from Jordan found significantly increased zinc levels<sup>24</sup> in their thalassemic patients. In parallel Mehdizadeh et al. from Tehran also reported significantly higher serum zinc levels in 64 thalassemic patients<sup>10</sup>. The regularity of blood transfusion appears to somehow prevent such deficiency as a consequence of receiving healthy blood donation and it may clarify the normal or even elevated serum zinc levels<sup>10,12</sup>. As in our series, zinc deficiency was detected in under transfused patients with low hematocrit. Al Awadhi et al. found normal levels of serum zinc in 49 thalassemia major patients which correlates in their study with non compliance of patients with chelation therapy<sup>11</sup>. Similarly Kwan and colleagues reported only 3 out of 68 thalassemic patients to have zinc deficiency<sup>25</sup>. Kajanachumpol et al. reported plasma and hair zinc levels to be much lower but erythrocytes' zinc was higher in thalassemic patients than controls. The mechanism of increased erythrocytic zinc was not clear but may be the impact of impaired zinc metabolism<sup>22</sup>. Zinc deficiency in thalassemics has been debatable and controversial. The disparities in different reports can be accredited to regional factors, variable dietary habits, various chelation agents,

non compliance to chelation, difference in age and also transfusional practices.

### Conclusion:

Our study revealed that hypozincemia is not unusual in  $\beta$ -thalassemic patients on desferrioxamine. We propose zinc levels should be regularly measured primarily in anemic patients with long duration of chelation.

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