Research Article

Effect of Serum Ferritin on Serum Zinc Levels in Patients with Thalassemia Major: A Prospective Cross-sectional Study

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Abstract: Background: One of the most common cause of hereditary anemia is thalassemia, with carrier rate of 5.2% globally. Repeated blood transfusions lead to accumulation of iron in multiple organs which causes multiple complications. Among many complications, micronutrient deficiency of zinc does contribute to further hampering of overall general wellbeing of thalassemic patients. Keeping this in mind, a study is conducted to see the association of increased ferritin levels on serum zinc levels.

Objective: The objective of the study is to observe for a likely association between serum ferritin and zinc in patients with Thalassemia Major. Secondary objective is to assess growth and development of Thalassemic children as per growth centile charts.

Materials and Methods: A cross -sectional study was conducted at National Institute of Blood Disease & Bone Marrow Transplantation (NIBD), Karachi with the duration of 3 months from January 04, 2025 to April 14, 2025, after the approval of Ethical Review Committee bearing number NIBD/IRB/267/02-2024, dated: 31-05-2024. Data collected in this study included: age, sex, anthropometric assessment was done by CDC Growth centile charts. Serum ferritin and Zinc level were also estimated. Data was recorded and analyzed by SPSS 25.0 and the statistically significant level, considered as 0.05.

Result: Out of all patients, 40% exhibited zinc insufficiency, 35.7% had serum ferritin levels >3000 ng/ml, 28.6%had levels over 4000, and 21.4% had levels over 5000ng/ml. There was a substantial inverse relationship between high ferritin and low zinc levels. The growth of 62.9% of thalassemic patients were at fifth centile indicating deceleration in growth. Girls being dominant figure (57.1%) showing poor growth velocity.

Conclusion: Patients with Thalassemia major have showed substantial development retardation. Forty percent of the Thalassemic children had low serum zinc levels. Additionally, low zinc levels were significantly correlated with high ferritin levels.

Keywords: Thalassemia, Zinc deficiency, Serum ferritin levels, Beta globin, Transfusion.

INTRODUCTION

Thalassemia major is a most prevalent genetic disorder caused by quantitative deficits in hemoglobin synthesis. It is inherited through autosomal recessive manner. It is a disease caused by reduced or absent synthesis of beta globin chains that results in accumulation of unpaired alpha chains which eventually precipitate in erythroid precursors in bone marrow leading to their premature death and ineffective erythropoiesis [1].

One of the most common treatments of beta Thalassemia is blood transfusion. As a result of repeated transfusions, iron gets deposited in multiple organs causing oxidative damage leading to decline in their functions. Serum ferritin is used to measure iron levels. There are many complications of repeated blood transfusions which includes short stature, hypogonadism, heart failure, osteoporosis and zinc deficiency [2].

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One of the crucial micronutrient deficiencies patients with Thalassemia suffer from is zinc. The reduced zinc levels could be due to defective absorption, low intake, chelation therapy. Zinc is one of the essential micronutrients in humans and acts as a cofactor for more than 300 enzymes. It plays an important role in human growth and development. The importance of zinc deficiency has been shown by impaired wound healing, diminished immune response, growth retardation, decreased bone mineral density, impaired glucose tolerance and deformed nails [3, 4].

Growth retardation is one of the common complications of beta Thalassemia. There are many contributory factors which lead to growth failure. The notable causes are repeated transfusions due to low hemoglobin levels, micro and macro nutrient deficiencies, infrequent blood transfusion, iron accumulation and infrequent /excessive use of chelators eventually cause growth failure in children with Thalassemia Major [5].

Mild to moderate zinc deficiency is one of the most common findings in children with Thalassemia major. A large cross-sec-

tional study from Iran by Mashhadi *et al.* observed zinc deficiency in Thalassemia major patients as 98% [6].

The primary objective of our study is to observe for likely association between serum ferritin and zinc in patients with Thalassemia Major. The secondary objective is to assess growth and development of thalassemic children as per growth centile charts.

MATERIALS AND METHODS

A Cross-sectional study was conducted at National Institute of Blood Disease & Bone Marrow Transplantation, Karachi , Pakistan (NIBD). The duration of study was 3 months from January 04, 2025 to April 14, 2025, after the approval of Ethical Review Committee bearing number NIBD/IRB/267/02-2024, dated: 31-05-2024.

A total of 32 subjects were included in the study after calculating the sample size using online sample size calculator. With a correlation coefficient of 0.0232, 80 power of the study, and absolute precision of 0.05% was taken into account. The resulting sample size is taken as 35 thalassemia major patients. Thalassemia major patients with age more than 2 years and less than 16 years were included in the study. Patients having an active infection, (High grade fever, along with raised septic parameters), active liver disease, Hepatitis B, C and HIV, with auto immune disorders and malignancy, taking hydroxyurea and with acute and chronic malnutrition were excluded.

After informed consent, three to five milliliter of blood was collected in gel vacutainer from each subject. Blood samples were centrifuged at 3000 rpm for 15min, aliquoted with appropriate subject identification number and stored at -80ºC till further analysis. The sample of Serum Ferritin was centrifuged at 3000 rpm for 5 minutes. Serum was separated in a sample cup marked with patient MR and lab number. To maintain confidentiality patient's identification was deleted and codes were given to them. After sample collection, they were transported to the NIBD Imam Laboratory under manufacturer transportation recommendations for analysis.

The analysis was performed on Selectra Pro MR out in clinical chemistry analyzer by Colorimetric Method. The Zinc present in the sample is chelated by 5-Br-PAPS 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) phenol in the reagent. The formation of this complex is measured at a wavelength of 560 nm. Two level quality control materials will be run with each batch of samples. Serum Zn levels 65-150µg/dl will be taken as optimal, with <65µg/dl taken as zinc deficiency and >150µg/dl as elevated levels. Serum Ferritin levels are measured by chemiluminescence immunoassay.

STATISTICAL ANALYSIS

The collected data was analyzed on SPSS version 25. Descriptive data was analyzed with mean+/- SD and median (IQR) for quantitative variables. However, inferential statistics was applied as Chi-square to assess the association between categorical variables while Bivariate Correlation was applied to assess the effect of one variable on other. The statistically significant level was considered as <0.05.

RESULT

The study involved the recruitment of 35 thalassemic children. Approximately, 14 patients (40%) had zinc values below 60mcg/dl. Additionally, serum ferritin levels were evaluated which denoted that 35.7% of patients had serum ferritin levels > 3000ng/ml. Furthermore, 28.6% (4 patients) had ferritin levels above 4000 and 21.4% (3 patients) had serum ferritin levels above 5000ng/ml. Among 35 thalassemic children, majority were girls (57.1%) The median IQR of 10, the mean +/-SD was 9.03+/- 3.27 years. As shown in Fig. (**1**), it has been demonstrated that 62.9% of thalassemic patients were observed at 5th centile. The Huge number signifies the impact of disease and its complications over the growth and development of thalassemic children. The growth centile was further associated with serum ferritin levels which were found to be insignificant (p value =0.45). Thus, study findings reveals that serum ferritin alone does not contribute to growth impairment in thalassemic children.

Patients with serum ferritin above 4000 were given dual chelating agents (IV deferoxamine and deferiprone) with twice monthly follow up while patients below 4000 were taking only single chelating agent (Deferasirox) in maximum dose with a monthly follow up.

However, association of serum ferritin levels with serum zinc levels was found to be significant, i.e. (p-value=0.032), as shown in Table **1**. Further analysis revealed that higher Ferritin levels with Mean ± SD (2971.7±1275.5) ng/ml were substantially correlated with lower zinc levels i.e. (62.41±9.21) mcg/dl. As a result, Fig. (**2**) illustrates the observed weak negative correlation (r = -0.474 with a p-value of 0.004) in Thalassemic children.

Fig. (1). Pattern of Growth in Thalassemic Children.

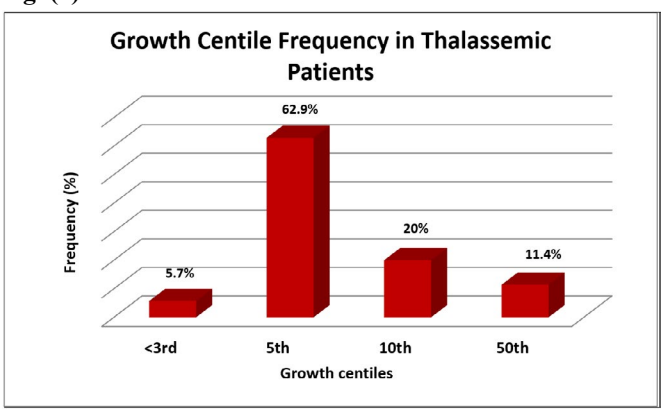
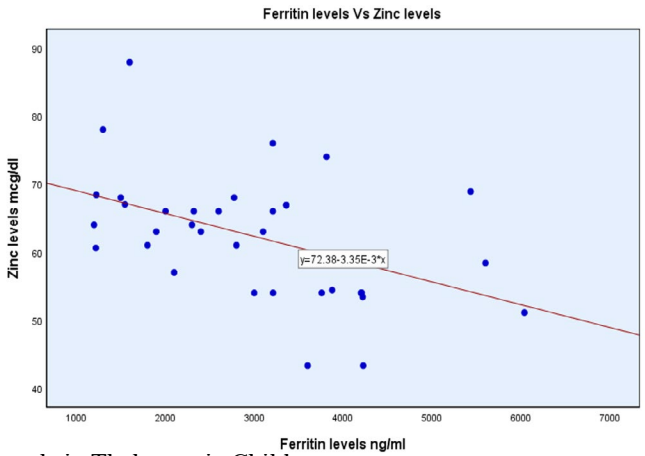


Fig. (2). Effect of Increasing Ferritin Levels over Serum Zinc



Levels in Thalassemic Children.

Table 1. Association of Rising Ferritin Levels with Serum Zinc Levels.

Serum Zinc Levels	<2000 ng/mL	2000–2999 ng/mL	3000–3999 ng/mL	4000–4999 ng/mL	≥5000 ng/mL	P-value
<65 µg/dL	2 (14.3%)	0 (0%)	5 (35.7%)	4 (28.6%)	3 (21.4%)	0.032
≥65 µg/dL	7 (33.3%)	7 (33.3%)	4 (19.0%)	2 (9.5%)	1 (4.8%)	

DISCUSSION

In our study serum ferritin have remarkable effects on serum zinc levels with a statistically significant correlation as increasing levels of serum ferritin contributes to declining levels of serum zinc levels (p-value 0.004). Study done by Hervita Yani in year 2019, found no association between serum ferritin and serum zinc levels in patients with Thalassemia major [2-7].

Previous research demonstrated that excessive iron accumulation from repeated blood transfusions and ineffective erythropoiesis effects the absorptive capacity of zinc in the gastrointestinal tract. It was documented in previous studies that ferritin levels are inversely related to serum zinc levels, so as ferritin levels increases, serum zinc levels decrease. In previous studies conducted by Arijanty *et al.* and Nima *et al.* serum Ferritin levels were found to correlate negatively correlated with plasma zinc levels. Similar, Mahyar *et al.* and Missiry *et al.* reported similar outcomes. El Missiry reported correlation between serum zinc levels and ferritin levels as not statistically significant [8-10]. Hervita in year 2019 described the similar findings as no major association in serum zinc levels observed between thalassemia children and healthy controls and no significant correlation noticed between serum ferritin and zinc in thalassemic children [11].

Zinc deficiency in thalassemia major patients could be due to diverse causes that includes hyperzincuria, increase ferritin levels, hepatic iron load and hepatic dysfunction [12]. Desferraxamine has zinc binding affinity and increases urinary zinc elimination resulting in zinc depletion [13]. Zinc deficiency was found to be 40% in our study. Hess *et al* reported 65% of thalassemic subjects had decreased serum zinc levels [14]. Nidumuru *et al.* reported 65% of thalassemic patients had low zinc levels [15]. Arcosoy *et al.* reported zinc deficiency in thalassemic children and found decrease zinc levels as important causative factor for delayed growth in thalassemic children. Mehzideh *et al.* from Tehran identified higher zinc levels in 64 thalassemia subjects [16]. Firdous *et al.* reported low serum zinc levels in 60% of Bangladeshi patients [17].

Growth failure is one of the common observations in thalassemic children. The etiological factors are various, the most common being the disease itself as chronic anemia along with excessive use of iron chelators, micronutrient deficiency and excessive iron accumulation contributes towards growth retardation. In our study, 62.9% of thalassemic children were observed to be plotted at 5th centile indicating growth disturbances as compared to healthy children of similar age and gender. The growth centile was found to have no correlation with serum ferritin levels (p value 0.45). This signifies that growth and development of thalassemic children are dependent on multiple factors instead of an impact of rising ferritin levels alone. Study indicated that growth failure was observed in 80 % of children born before 1978 and 75% of thalassemic subjects born between 1978 & 1995 [18].

Growth impairment in thalassemic children is well documented with a prevalence of 25 to 60% worldwide. In contrast, Moiz *et al.* revealed that 65% of patients were stunted and 40% were underweight [19]. Study done by El Missiry *et al.* in year 2014 found no correlation between serum zinc levels and growth parameters in regularly transfused thalassemic children [8]. Karunaratna observed that 50% of patients were stunted and that growth retardation were associated with chronic anemia, long term use of chelators, micronutrient deficiency and socioeconomic status [9]. A study in Italy showed that 18% of patients had growth parameters below fifth centile, this observation reflect the less effectiveness or inadequacy of therapeutic methods for thalassemic patients [10].

A study done by Ashraf in year 2009 found 56% of thalassemic children had growth velocity standard deviation score less than -1. The causes of decrease growth were multifactorial including, nutritional deficiency iron overload, extensive use of iron chelators, constant low hemoglobin levels below 9g/dl and growth hormone deficiency [20]. Another study done by Rathaur in year 2020 observed 65.7% of thalassemic children had short stature and among them 77% were underweight [21]. Another study conducted in year 2010 observed the effects of intensive chelation on growth in thalassemic children. It states that growth retardation was observed before 10 year of age which became evident in age between 10 & 15 years and thereafter growth failure was seen in relation to sexual maturity. The study observed the significant effect of chelation on growth & development of thalassemic children [22]. Harish K. Pemde in year 2011 observed around 33% of transfusion dependent thalassemia were of short

stature and all of them had significantly raised ferritin levels [23]. Fahim in year 2013 found 49% of thalassemia children were having short stature and 47% of them were underweight [24]. Origa in year 2016 revealed that thalassemic patients had reduced height & BMI associated with high ferritin & low hemoglobin [25]. A study done in Iran in year 2020 showed no association of Serum zinc levels with serum ferritin and chelating agents [26]. A study done by Akash in year 2025 accounted that growth failure is a prominent feature on transfusion dependent beta thalassemia major and oral zinc supplementation had no effect on linear growth, pointing that growth failure in thalassemia major is because of cumulative factors [27]. There is a significant correlation between serum ferritin level & growth disorders and combined chelating agents has a profound effect on serum ferritin levels which may improve growth in thalassemic children in long run [28-30].

LIMITATIONS

Only 35 thalassemic subjects were included in study, the results would be significant if large number of thalassemic patients were recruited in study. Most of patients were on regular chelation which could have shown profound decrease in levels of zinc if more patients were recruited in study. The dietary habits of thalassemic children were not taken into account which could have an impact on low serum zinc levels.

CONCLUSION

Thalassemia major, being a chronic disorder, as most prevalent inherited causes of morbidity in patients impairing not only quality of life but the overall general physical and mental health. Repeated transfusions, as one of the common treatment options, results into number of complications. The increased accumulation of iron leads to endocrinopathies resulting in growth failure and micronutrient deficiencies, with zinc deficiency, being one of them. The present study found significant negative weak correlation of serum ferritin on serum zinc levels and optimizing the rising ferritin levels could lessen the complications of this chronic disorder.

AUTHORS' CONTRIBUTION

Uzma Hayat: Conceptualization, Study design, writing draft.

Shabnam Dildar: Methodology, Data analysis and interpretation, Critical review and revision the manuscript.

Saima Siddique: Critical review and revision the manuscript, Final approval, final proof to be published.

Nizam-uddin: Study design, Methodology, Data analysis and interpretation.

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ETHICAL DECLARATIONS

Data Availability Statement

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board of National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan (NIBD), bearing number NIBD/IRB/267/02-2024, Dated: 31-05-2024.

Consent to Participate

Written informed consent was secured from all participants.

Consent for Publication

Consent for publication was obtained from all individual participants included in the study.

Conflict of Interest

Declared none.

Competing Interest/Funding

No funding was received for this study.

Use of AI-Assisted Technologies

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were utilized in the writing of this manuscript, in the creation of images/graphics/tables/captions, or in any other aspect of its preparation.

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