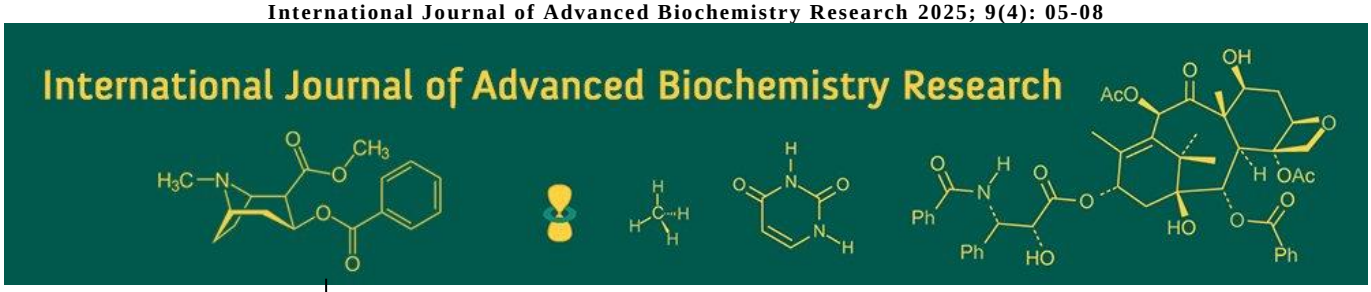

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Dr. Onesh Ch. Marak
Department of Biochemistry, M.G.M Medical College and M.Y Hospital, Jabalpur University, Indore, Madhya Pradesh, India

Dr. Rajeev Lohokare
Associate Professor, Department of Biochemistry, M.G.M Medical College and M.Y Hospital, Indore, Madhya Pradesh, India

Dr. Tshetij Rai
Department of Biochemistry, M.A.M.C, Delhi, India

Corresponding Author:
Dr. Onesh Ch. Marak
Department of Biochemistry, M.G.M Medical College and M.Y Hospital, Jabalpur University, Indore, Madhya Pradesh, India

Study of vitamin D in patients of beta thalassemia major at tertiary care hospital

Onesh Ch. Marak, Rajeev Lohokare and Tshetij Rai

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Abstract
Background: Beta-thalassemia major is a genetic disorder with marked deficiency of beta-globin production. Affected children require a critical need for regular blood transfusion which usually results in iron overload and secondary bone-related complications. Vitamin D is an important factor in bone turnover. Their levels are typically below normal in thalassemia patients. In this study, the intention was to estimate the serum levels of vitamin D, in patients with beta-thalassemia major and their correlation with the duration and frequency of transfusion.
Methods: A cross-sectional survey was carried out on 65 beta-thalassemia major patients aged 5-15 years. Serum levels of vitamin D was determined and analysed.
Findings: A high prevalence of vitamin D deficiency (55%) was found among the beta-thalassemia patients. The levels of vitamin D decreased with a significant rise in transfusion duration and frequency.
Conclusion: Since prolonged transfusion is one of the criteria in this study among the patients with beta-thalassemia, assessment of vitamin D levels should be checked routinely among these patients, and interventions to improve vitamin D status may be necessary to avoid bone complications.

Keywords: Beta-thalassemia major, blood transfusion and vitamin D

Introduction
Beta (β) Thalassemia syndrome refers to a group of genetic disorders mostly inherited in an autosomal recessive manner, which results from a quantitative decrease in β-globin chain of hemoglobin (Hb). This can result due to point mutations, or deletions in the β-globin gene (HBB). Synthesis of α-globin continues as normal, resulting in accumulation of excess α-globin chains in erythroid precursors. This results in ineffective erythropoiesis and RBCs which are prone to hemolysis. The β-thalassemia trait is prevalent at rates ranging from 1.4% to 3.4% in Central India, with β-thalassemia major (β-TM) reported at 0.94%. Patients with β-thalassemia major are in a state of chronic anemia for which they require frequent blood transfusion therapy to improve the quality of life and reduce complications [1]. One of the major complications of multiple blood transfusions is iron overload which consequently leads to the deposition of iron in various tissues (Hemosiderosis) like liver, pancreas, endocrine glands (e.g. thyroid, parathyroid) and heart [2]. A major significant challenge is Thalassemia bone disease, characterized by bone deformities, growth retardation, osteoporosis. The underlying cause for thalassemia bone disease is the dysregulation of bone remodelling and calcium homeostasis which is chiefly attributed to transfusion-induced hemosiderosis of liver and parathyroid gland [3].
Vitamin D plays a crucial role in bone health, and its deficiency can exacerbate the bone problems often seen in thalassemia patients, such as osteoporosis and bone pain [4]. Calcium is a mineral that serves as an essential component of bone structure, providing strength and rigidity to bones and teeth. Beyond its structural role, calcium acts as a signalling molecule involved in various cellular processes, including muscle contraction, neurotransmitter release, enzyme activation, and blood clotting [5]. In thalassemia, calcium metabolism is hindered due to Iron overload, Vitamin D deficiency, hormonal imbalance and chelation therapy.
A study by Sultana *et al*. concludes that transfusion dependent beta thalassemia patients have low calcium levels and high serum alkaline phosphatase level [7]. Another study by Ali *et al* demonstrated a high prevalence of vitamin D deficiency and low total serum calcium level in

thalassemia patients which was significantly lower than the healthy group [9]. Still, paucity of information regarding effects of multiple blood transfusion on serum levels of calcium, vitamin D and alkaline phosphate amongst patients with β-thalassemia exists. This study aims to address this knowledge gap by determining the serum levels of vitamin D and attempt to establish an association between them with the number and duration of blood transfusion in patients with β-thalassemia major.

Materials and Methods

This cross-sectional study was conducted at the Department of Biochemistry and the Department of Paediatrics at M.G.M Medical College and M.Y.H Hospital in Indore, Madhya Pradesh. The study involved a minimum of 65 patients diagnosed with Beta Thalassemia major, aged between 5 and 15 years, who were attending either the outpatient or inpatient departments. The duration of the study spanned one year, following approval from the ethical committee.

The primary focus was to measure and analyze serum levels of vitD in relation to the duration and number of blood transfusions received by the participants.

Inclusion criteria; Diagnosed cases of Thalassemia Major, verbal assent from children aged 7 to 15 years in the presence of their parents, along with written informed consent from the parents prior to study. For children in the age group of 5 to 6 years, only written informed consent from the parents was taken. Both male and female participants were included in the study.

Exclusion criteria; Individuals with Thalassemia minor were excluded, as well as those with other types of hemolytic anemia, including sickle cell disease, G6PD deficiency, and hereditary spherocytosis. Additionally, subjects with bone diseases such as rickets, osteomalacia, Paget's disease, bone fractures, or osteogenic bone cancer were excluded. Liver diseases, including intra and extrahepatic obstruction, hepatitis, and cirrhosis, were also excluded. Endocrine disorders like hyperthyroidism and hyperparathyroidism, as well as chronic conditions such as chronic kidney disease, were further grounds for exclusion from the study.

Venous blood samples (5 mL) were collected under strict aseptic conditions using disposable syringes and transferred into clot activator tubes, allowed to stand for 30 minutes at room temperature. The samples were then centrifuged at 3000 rpm for 10 minutes, and the serum was extracted using a clean pipette and stored at-20 degrees Celsius for further analysis.

Serum vitamin D levels were determined using an Enzyme Immunoassay (EIA) on an ELISA processor and reader in the biochemistry laboratory.

Statistical Analysis

The collected data were entered and stored in Microsoft Excel, and statistical analysis was performed using GraphPad Prism software version 10.2.3 (347). To determine the distribution of the data, the Kolmogorov-Smirnov test and Shapiro-Wilk test were utilized to classify the data as parametric or non-parametric. Parametric data were presented as means and standard deviations (SD), while non-parametric data were presented as medians and inter-quartile ranges (IQR). For parametric comparisons, the Unpaired t-test was used for two independent groups, and One-way ANOVA was employed for comparisons involving more than two independent groups. For non-parametric data, the Mann-Whitney U test was used for two independent groups, and the Kruskal-Wallis test was utilized for comparisons across more than two independent groups, with post-hoc analysis conducted using the uncorrected Dunn's test wherever required. Correlational analysis for parametric data was carried out using the Pearson correlation test, while the Spearman rank correlation test was applied for non-parametric data. A p-value of less than 0.05 was considered statistically significant for all tests.

Results

Table 1: Status of vitamin D level in serum of patients

Serum levels	Number of patients	Percentage	Mean concentration
Vitamin D-deficiency (<10 ng/ml)	36	55%	11.65 ng/ml
-insufficiency (10-30 ng/ml)	28	43%	
-sufficiency (30-100 ng/ml)	1	2%	

Comparison of concentration of serum vitamin D in male and female subjects

Table 2: Median, IQR, Median AND p-value for serum VitD across both the gender

Gender	Male (n=45)	Female (n=20)	p-value
Parameters	Median	7.4	0.32
Vit D	8.9		

Among the 65 β-thalassemia major patients included in our study, 45 (69%) were males and 20 (31%) were females. No significant difference was observed in vitamin D concentrations between males (Median = 8.9) and females (Median = 7.4); p = 0.32.

Comparison of concentration of serum vitamin D in 5-10 years age group vs. 11-15 years age group

31 (48%) were in the 5-10 years age group, and 34 (52%) were in the 11-15 years age group.

Table 3: Median, IQR AND p-value for serum vitamin D across both the age-groups

Age-groups	5-10 years (n=31)	11-15 years (n=34)	p-value
Parameters	Median (IQR)	Median (IQR)	
Vitamin D (ng/ml)	11.20 (7.2-18.6)	7.30 (4.92-13.93)	0.03

Non-parametric test-Mann-Whitney U test, was performed to compare the mean ranks of these parameters between the two age-groups.

34 children in the age-group of 11-15 years had significantly lower vitamin D concentrations with a median concentration of 7.30 ng/mL as compared to the 31 children in the age-group of 5-10 years (Median = 11.20); p = 0.03.

Comparison of concentration of serum vitamin D level with duration of blood transfusion in β-thalassemia patients

All the 65 patients in our study were grouped based on their duration of BT into 3 groups: i) 1-5 years, ii) 6-10 years and iii) >10 years.

Table 4: Median, IQR and p-value for serum calcium and vitamin D across the 3 groups based on duration of blood transfusions in years

Duration of BT	1-5 years (n=19)	6-10 years (n=25)	>10 years (n=21)	p-value
Parameters	Median (IQR)	Median (IQR)	Median (IQR)	
Vitamin D (ng/ml)	16.0 (7.8-24.2)	8.2 (5.25-14.05)	7.2 (4.7-14.6)	0.03

A Kruskal-Wallis H test was conducted to determine if there were statistically significant differences in serum vitamin D concentration between the 3 groups: patients with 1-5 years of BT (n = 19), 6-10 years of BT (n = 25) and >10 years of BT (n = 21). The test showed a statistically significant difference in vitamin D concentration between the groups, $\chi 2(2) = 6.813$, p = 0.03.

Dunn's post-hoc tests were performed without correction for multiple comparisons due to the exploratory nature of this study. The uncorrected Dunn's test indicated that there is a significant difference (p = 0.02) in vitamin D concentrations between patients with 1-5 years of BT (median = 16) and those with 6-10 years of BT (median = 8.2). The test also revealed significant difference (p = 0.02) in vitamin D concentrations between patients with 1-5 years of BT (median = 16) and those with >10 years of BT (median = 7.2)

Association between serum vitamin D concentration with duration of blood transfusion in β-thalassemia patients

Test for normality (normal distribution) was done for the data obtained from our study for serum concentrations of vitamin D using Shapiro-Wilk test and Kolmogorov-Smirnov test for each group. For parametric data, association was checked by using the Pearson correlation test, while for non-parametric data, association was checked by using the Spearman correlation test.

Strength of association denoted by the r-value was reported as following:

<0.3-Poorly correlated

0.3 to 0.59-Fairly correlated

0.6 to 0.79-Moderately correlated

0.8 and above-Strongly correlated

Table 5: Association between serum vitamin D concentration with duration of blood transfusion in β-Thalassemia patients

Duration of BT	1-5 years (n=19)		6-10 years (n=25)		>10 years (n=21)		Total (n=65)	
Parameters	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Vitamin D (ng/ml)	-0.39	0.09	-0.19	0.35	-0.30	0.18	-0.34	0.005

Comparison of concentration of serum vitamin D level with number of blood transfusions in β-thalassemia patients

Table 6: Median, IQR and p-value for serum vitamin D across the 3 groups based on total number of blood transfusions

Number of BT	<100 BT (n=15)	100-200 BT (n=27)	>200 BT (n=23)	p-value
Parameters	Median (IQR)	Median (IQR)	Median (IQR)	
Vitamin D (ng/ml)	16.0 (7.8-25.3)	11.2 (5.6-15.4)	6.9 (4.7-8.7)	0.005

Statistically significant difference in vitamin D concentration between the groups, $\chi 2(2) = 10.27$, p = 0.005 was observed on conducting Kruskal-Wallis H test. Uncorrected Dunn's test indicated that there is a significant difference (p = 0.001) in vitamin D concentrations between patients that had undergone <100 BT (median = 16) as compared to those with >200 BT (median = 6.9)

Table 7: Association between serum vitamin D concentration with number of blood transfusion in β-Thalassemia patients

Number of BT	<100 BT (n=15)		100-200 BT (n=27)		>200 BT (n=23)		Total (n=65)	
Parameters	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Vitamin D	-0.07	0.79	-0.33	0.10	0.78	-0.40	-0.40	<0.001

Discussion

β-Thalassemia major is an autosomal recessive, hereditary haematological disorder characterized by mutations in the β-globin gene (HBB), leading to deficiency or absence of functional beta-globin protein. It leads to ineffective erythropoiesis causing a chronic state of anemia that requires lifelong blood transfusion.

Several studies have shown that calcium homeostasis is altered in patients with β-thalassemia major, leading to high prevalence of vitamin D deficiency in patients with β-Thalassemia as compared to the healthy controls (9). However, there is limited information regarding the association of serum vitamin D level with duration and number of blood transfusions in patients with β-Thalassemia patients.

In our study which included 65 patients with β-thalassemia major, 64 (98%) had vitamin D levels below the lower limit of reference interval, out of which 36 (55%) were found to have vitamin D deficiency (<10 ng/mL), 28 (43%) were found to have insufficient levels of vitamin D (10-30

ng/mL), with a mean concentration of serum vitamin D of 11.65 ng/ml. These findings are similar with the findings in several studies including Agrawal *et al*, [8] Ali *et al* [9] Ridha [10] *et al* that demonstrated decreased serum levels of vitamin D (<30 ng/mL) in 98%, 94% and 87% of patients with β-Thalassemia respectively, with a mean serum vitamin D concentration of 8.85, 11.11 and 13.54 ng/mL respectively. This indicates that serum concentration of vitamin D is decreased in majority of the patients with β-Thalassemia.

Concentrations of serum VitD between male and female patients revealed no significant difference (p = 0.32). While Agrawal *et al* [8], found no significant difference in serum VitD levels between males and females, Singh *et al* [12], on the other hand, found serum VitD concentration to be significantly higher in males (Mean = 13.54 mg/dL) than in females (Mean = 6.32 mg/dL), (p = 0.026) [11]. We also compared the concentrations of serum VitD between two different age groups; 5-10 years and 11-15 years, which revealed a statistically significant (p=0.03) higher median values of serum VitD among patients belonging to 5-10 years age group when compared to 11-15 years age group. On analysing serum VitD level in patients who had undergone BT for 1-5 years, 6-10 years, and >10 years, we found out that the median values of VitD levels were significantly higher in patients who had undergone BT for 1-5 years as compared to those who underwent BT for 6-10 years as well as >10 years (p = 0.02 and 0.02 respectively). We infer that serum VitD decreases significantly in patients with β-Thalassemia after 5 years of BT.

Conclusion

After analysing the results of the study, we can conclude that patients with β-Thalassemia major undergoing multiple blood transfusion (BT) have high prevalence of vitamin D deficiency. Vitamin D deficiency worsens with increased duration and number of blood transfusions. Specifically, serum VitD levels decrease significantly after 5 years of BT and continue to decline with greater numbers of transfusions (>200 BT). There is major impact of chronic transfusions on VitD status in these patients. Age-based analysis revealed higher median VitD levels in younger patients (5-10 years) compared to older ones (11-15 years). Thus, we suggest regular monitoring of VitD level in β-Thalassemia patients, particularly with increasing duration and frequency of blood transfusions, to mitigate the risk of related complications.

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References

1. Cao A, Galanello R. Beta-thalassemia. Genetics in Medicine. 2010;12(2):61-76.
2. Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR. Complications of β-thalassemia major in North America. Blood. 2004 Jul 1;104(1):34-39.
3. Prati D. Benefits and complications of regular blood transfusion in patients with beta-thalassemia major [Internet]. Vox Sanguinis. 2000;79(2):129-137. Available from: www.karger.com/journals/vox
4. Nakavachara P, Viprakasit V. Children with hemoglobin E/β-thalassemia have a high risk of being vitamin D deficient even if they get abundant sun exposure: A study from Thailand. Pediatric Blood & Cancer. 2013 Oct;60(10):1683-1688.
5. Lertsuwan K, Wongdee K, Teerapornpuntakit J, Charoenphandhu N. Intestinal calcium transport and its regulation in thalassemia: Interaction between calcium and iron metabolism. Journal of Physiological Sciences. 2018;68(3):221-232.
6. Rao LV, Yu H. Laboratory Tests (Calcium, Total). In: Dubey S, editor. Wallach's Interpretation of Diagnostic Tests. South Asian Edition. New Delhi: Wolters Kluwer (India); 2021. p. 98-101.
7. Sultana A, Akhter QS. Serum calcium and serum phosphate levels in transfusion-dependent beta-thalassemia. Journal of Bangladesh Society of Physiology. 2018 Dec;13(2):54-58.
8. Agrawal A, Garg M, Singh J, Mathur P, Khan K. A comparative study of 25-hydroxy vitamin D levels in patients of thalassemia and healthy children. Pediatric Review: International Journal of Pediatric Research. 2016 Sep;3(9):652-656.
9. Ali GS, Najeeb MK, Jubrail AMS, Selamoğlu Z. Ridhae. Turkish Journal of Agriculture-Food Science and Technology. 2019 Nov 23;7(11):1924-1927.
10. Ridha NR, Gautama J, Ganda IJ. Analysis of vitamin D levels in children with thalassemia beta. International Journal of Health Science & Medical Research. 2022 Feb;1(1):XX-XX.
11. Sultan S, Irfan SM, Ahmed SI. Biochemical markers of bone turnover in patients with β-thalassemia major: A single-center study from Southern Pakistan. Advances in Hematology. 2016;2016:1-5.
12. Singh K, Kumar R, Shukla A, Phadke SR, Agarwal S. Status of 25-hydroxyvitamin D deficiency and effect of vitamin D receptor gene polymorphisms on bone mineral density in thalassemia patients of North India. Hematology. 2012 Sep;17(5):291-296.