



STUDY OF CALCIUM AND ALKALINE PHOSPHATASE AS BIOCHEMICAL MARKERS OF CALCIUM HOMEOSTASIS IN PATIENTS OF BETA THALASSEMIA MAJOR AT TERTIARY CARE HOSPITAL

Biochemistry

Dr Onesh Ch Marak

Department Of Biochemistry, Pg Resident, M.g.m. Medical College, Indore

Dr Purnima Dey Sarkar

Department Of Biochemistry, Professor And Head, M.g.m. Medical College, Indore

Dr Rajeev Lohokare

Department Of Biochemistry, Associate Professor, M.g.m. Medical College, Indore

Dr Bhavana Tiwari*

Department Of Biochemistry, Assistant Professor, M.g.m. Medical College, Indore
*Corresponding Author

Dr Tshetij Rai

Department Of Biochemistry, Pg Resident, Maulana Azad Medical College, Delhi

Shifa Patel

Student, M.g.m. Medical College, Indore

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. calcium is an important factor in bone turnover. Their levels are typically below normal in thalassemia patients. **Methods:** A cross-sectional survey was carried out on 65 beta-thalassemia major patients aged 5-15 years. In this study, the intention was to estimate the serum levels of calcium, and alkaline phosphatase (ALP) in patients with beta-thalassemia major and their correlation with the duration and frequency of transfusion **Results:** A high prevalence of hypocalcaemia (98%) was found among the beta-thalassemia patients. The levels of calcium decreased with a significant rise in transfusion duration and frequency. Transfusion parameters did not demonstrate any significant correlation with serum calcium levels. Most of the patients ALP levels were found within normal limits. **Conclusion:** Since prolonged transfusion is one of the criteria in this study among the patients with beta-thalassemia, assessment of calcium levels should be checked routinely among these patients, and interventions to improve calcium homeostasis may be necessary to avoid bone complications.

KEYWORDS

Beta-thalassemia major, Calcium, alkaline phosphatase and Transfusion.

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).

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 (4). In thalassemia, calcium metabolism is hindered due to Iron overload,

Alkaline phosphatase is an enzyme present with significant levels in liver, bone, kidney, placenta and intestine. pre- β ALP is involved in bone formation and mineralization, α 1 ALP and α 2 ALP is essential for metabolism of lipids and bile acids, γ -ALP is responsible for digestion and absorption of nutrients. ALP levels in thalassemia are influenced

by bone abnormalities, liver dysfunction and treatment effects.

Ferritin is a globular protein that serves as a primary iron storage, in thalassemia, ferritin level provide an important insights into iron metabolism. Elevated ferritin level is a marker of inflammation, infection, chronic disease and iron overload, as ferritin is an acute-phase reactant.

A study by Ali et. al demonstrated a low total serum calcium level in thalassemia patients which was significantly lower than the healthy group (5). Another study by Sultana et al. concludes that transfusion dependent beta thalassemia patients have low calcium levels and high serum alkaline phosphatase level (6). Still, paucity of information regarding effects of multiple blood transfusion on serum levels of calcium and alkaline phosphate amongst patients with β -thalassemia exists.

This study aims to address this knowledge gap by determining the serum levels of calcium and alkaline phosphatase as biochemical markers of calcium homeostasis and attempts 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 calcium, alkaline phosphatase and ferritin 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 calcium was measured through a colorimetric reaction with Arsenazo III, analyzed via spectrophotometry. Alkaline phosphatase levels were quantified using the DGKC and SCE method with enzyme kinetics on the ELITech clinical system Selectra Analysers. For ferritin measurement, a chemiluminescent immunoassay (CLIA) was employed.

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.

OBSERVATION & RESULTS;

Demographic Profile

Table 1: Status of Calcium and ALP levels in serum of patients

Serum levels	Number of patients (n=65)	Percentage	Mean concentration
Calcium			
• hypocalcaemia (<8.7 mg/dl)	64	98%	7.28 mg/dl
• normal calcium levels (8.7-10.7 mg/dl)	1	2%	
ALP			
• below limit (<30 U/L)	5	8%	62.77 U/L
• within limit (30-300 U/L)	60	92%	

64 (98%) patients out of the total 65 were found to have serum calcium levels <8.7 mg/dL (hypocalcemia), while only 1 (2%) patient had normal serum calcium levels. The mean concentration of serum calcium among our study subjects was 7.28 mg/dL.

60 (92%) patients out of the total 65 were found to have serum ALP levels within biological reference limits for their age (30–300 U/L), while 5 (8%) patients had serum ALP levels below the Lower Reference Limit (<30 U/L). The mean level of serum ALP among β -Thalassemia patients in our study was 62.77 U/L.

Comparison of concentration of serum calcium and ALP in male

and female subjects

Table 2: Median, IQR, Mean, SD AND p-value for serum calcium and ALP across both the gender

Gender	Male (n=45)	Female (n=20)	p-value
Parameters	Median		
Calcium	7.5 (IQR 6.44 – 8.39)	7.51 (IQR 6.55 – 8.02)	0.78
ALP	Mean (SD) 61.67 $\pm$ 23.82	65.25 $\pm$ 25.35	0.58

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 serum calcium concentrations between males (Median = 7.5) and females (Median = 7.51); $p = 0.78$ and serum ALP levels between males ($M = 61.67$, $SD = 23.82$) and females ($M = 65.25$, $SD = 25.35$); $t(63) = -0.55$, $p = 0.58$.

Comparison of concentration of serum calcium and ALP in different age groups

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, Mean, SD AND p-value for serum calcium and ALP across both the age-groups

Age-groups	5-10 years (n=31)	11-15 years (n=34)	p-value
Parameters	Median (IQR)	Median (IQR)	
Calcium (mg/dl)	7.5 (5.96-8.19)	7.51 (6.58-8.38)	0.64
ALP (U/L)	Mean (SD) 64.55 $\pm$ 27.75	Mean (SD) 61.15 $\pm$ 20.65	0.57

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

No significant difference was observed in the concentration of serum calcium between patients of age-group 5–10 years (Median = 7.5) and patients of age-group 11–15 years (Median = 7.51); $p = 0.64$.

Parametric test – unpaired t-test, was performed to compare the mean level of ALP between the two age-groups. No significant difference was observed in serum ALP levels between patients of age-group 5–10 years ($M = 64.55$, $SD = 27.75$) and 11–15 years ($M = 61.15$, $SD = 20.65$); $t(63) = 0.56$, $p = 0.57$.

Comparison of concentration of serum calcium and ALP levels 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, Mean, SD and p-value for serum calcium and ALP 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)	
Calcium (mg/dl)	7.66 (5.89-8.19)	7.5 (6.5-8.37)	7.49 (6.53-8.18)	0.99
ALP(U/L)	Mean (SD) 66.74 $\pm$ 30.1	Mean (SD) 60.44 $\pm$ 19.26	Mean (SD) 61.95 $\pm$ 24.18	0.69

There was no statistically significant difference in serum calcium concentrations between the different groups, $2(2) = 0.03$, $p = 0.99$ with a median serum calcium concentration of 7.66 mg/dL for patients with 1–5 years of BT, 7.5 mg/dL for patients with 6–10 years of BT, and 7.49 mg/dL for patients with >10 years of BT.

Parametric test – One-way ANOVA, was performed to compare the mean level of ALP between the three groups. There was no statistically significant difference in ALP levels between the groups, $F(2, 62) = 0.377$, $p = 0.69$.

Comparison of concentration of serum calcium and ALP levels with number of blood transfusions in β -thalassemia patients

Table 5: Median, IQR, Mean, SD and p-value for serum calcium and ALP 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
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Parameters	Median (IQR)	Median (IQR)	Median (IQR)	0.78
Calcium (mg/dl)	7.5 (5.96-8.16)	7.8 (6.46-8.4)	7.38 (6.46-8.06)	

There was no statistically significant difference in serum calcium concentrations between the different groups, $2(2) = 0.489$, $p = 0.78$

Results of the ANOVA indicated that there was no statistically significant difference in serum ALP levels between the groups, $F(2, 62) = 0.589$, $p = 0.56$.

Association between serum calcium and ALP 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 Calcium and ALP 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 6: Association between serum calcium and ALP 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
Calcium (mg/dl)	-0.12	0.63	+0.02	0.93	-0.58	0.005	-0.006	0.63
ALP (U/L)	-0.22	0.37	+0.10	0.62	-0.30	0.19	-0.11	0.38

Overall, patients with β -thalassemia undergoing BT were found to have statistically insignificant, poor negative correlation $r(63) = -.06$, $p = 0.63$, between their serum calcium concentration and poor negative correlation $r(63) = -.11$, $p = 0.38$, between their serum ALP level and the duration of BT in years.

Table 7: Association between serum calcium and ALP 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
Calcium	0.17	0.54	-0.50	0.008	-0.25	0.26	-0.14	0.27
ALP	-0.09	0.76	-0.34	0.08	-0.07	0.76	-0.15	0.25

Overall, patients with β -thalassemia undergoing BT were found to have statistically insignificant, poor negative correlation $r(63) = -.14$, $p = 0.27$, between their serum calcium concentration and poor negative correlation $r(63) = -.15$, $p = 0.25$, between their serum ALP level and the total number of BT they underwent.

Comparison of concentration of serum calcium and ALP in patients with serum ferritin concentration of <1000 ng/ml vs >1000 ng/ml

Non-parametric test – Mann-Whitney U test, was performed to compare the median of these parameters between the two groups

Table 8: Median, IQR and p-value for serum calcium and ALP in patients with serum ferritin concentration of <1000 ng/ml vs >1000 ng/ml

Serum ferritin	<1000 ng/ml (n=8)	>1000 ng/ml	p-value
Parameters	Median (IQR)	Median (IQR)	
Calcium (mg/dl)	7.10 (6.00-8.14)	7.53 (6.46-8.37)	0.42
ALP (U/L)	56.50 (47.25-84.50)	60.0 (46.0-79.0)	0.87

There was no significant difference in serum calcium concentration ($p=0.42$) and ALP concentration ($p=0.87$) in patients with serum ferritin concentration of <1000 ng/mL when compared to patients with serum ferritin concentration of >1000 ng/mL

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 hypocalcaemia in patients with β -Thalassemia as compared to the healthy controls (5). However, there is limited information regarding the association of serum calcium levels 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%) patients were found to have serum calcium levels <8.7 mg/dL (hypocalcaemia), while only 1 (2%) patient had normal serum calcium levels. The mean concentration of serum calcium among our study subjects was 7.28 mg/dL. These findings are similar with the findings in several studies including Sultan et al (10) Ali et al (8) Sultana et al (6) that demonstrated hypocalcaemia in 66%, 44.73% and 67.5% of patients with β -Thalassemia respectively, with a mean serum calcium concentration of 8.1, 7.9, and 6.9 mg/dL respectively. This indicates that serum concentration of calcium is decreased in majority of the patients with β -Thalassemia.

Concentrations of serum calcium and ALP between male and female patients revealed no significant difference ($p = 0.78$ and 0.58 for calcium and ALP respectively). We also compared the concentrations of serum calcium and ALP between two different age groups; 5–10 years and 11–15 years. No significant difference was observed in the concentration of serum calcium between patients of age-group 5–10 years (Median = 7.5) and patients of age-group 11–15 years (Median = 7.51); $p = 0.64$. No significant difference was observed in serum ALP levels between patients of age-group 5–10 years ($M = 64.55$, $SD = 27.75$) and 11–15 years ($M = 61.15$, $SD = 20.65$); $p = 0.57$.

On analysing serum calcium and ALP levels in patients who had undergone BT for 1–5 years, 6–10 years, and >10 years, There was no statistically significant difference in serum calcium concentrations between the different groups, $p = 0.99$ with a median serum calcium concentration of 7.66 mg/dL for patients with 1–5 years of BT, 7.5 mg/dL for patients with 6–10 years of BT, and 7.49 mg/dL for patients with >10 years of BT and There was no statistically significant difference in ALP levels between this groups, $p = 0.69$.

There was no significant difference in serum calcium concentration ($p=0.42$) and ALP concentration ($p=0.87$) in patients with serum ferritin concentration of <1000 ng/mL when compared to patients with serum ferritin concentration of >1000 ng/mL.

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 hypocalcaemia. there was no significant correlation between serum calcium levels and duration or number of BT within the initial 10 years of transfusion. A significant negative correlation was observed after 10 years of BT suggest that Hypocalcaemia becomes more pronounced with prolonged transfusion therapy.

Serum ALP levels did not show significant variations across different durations or numbers of BT, indicating it may not be a sensitive marker in this context though Age-based analysis revealed no significant differences in calcium and ALP levels between younger patients (5–10 years) compared to older ones (11–15 years),

Thus, we suggest regular monitoring of calcium levels in β -Thalassemia patients, particularly with increasing duration and frequency of blood transfusions, to mitigate the risk of hypocalcaemia-related complications.

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