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HIGH BURDEN OF VITAMIN D DEFICIENCY AND FERRITIN-LINKED IMPACT IN B-THALASSEMIA MAJOR

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ABSTRACT

Background: Background: Vitamin D plays an essential role in bone health and overall physiological function, and its deficiency is common in children and adolescents with β-thalassemia major (βTM). Iron overload, as reflected by elevated ferritin, may further influence vitamin D status. **Aim**: This study aimed to evaluate serum vitamin D levels in βTM patients and determine their association with ferritin levels. **Methods**: A total of 40 βTM patients and 20 age-matched healthy controls (aged 4–25 years) were enrolled between October 2024 and February 2025. Serum vitamin D, calcium, ferritin, and hemoglobin were measured. Statistical analysis, including correlation and logistic regression, was performed using SPSS v.26 to identify predictors of vitamin D deficiency. **Results**: Vitamin D deficiency was highly prevalent among βTM patients (70%) compared with controls. Patients showed significantly lower vitamin D levels (17.32±1.56) than controls (25.34±1.76). Vitamin D levels were positively correlated with age ($r = 0.788$), calcium ($r = 0.772$), and hemoglobin ($r = 0.771$), and negatively correlated with ferritin ($r = –0.517$). Logistic regression demonstrated that ferritin >1000 ng/mL strongly predicted vitamin D deficiency (OR = 17.875; 95% CI: 3.258–98.074; $p = 0.001$), while younger age (< 10 years) also increased the odds of deficiency (OR = 5.200; $p = 0.018$). **Discussion**: D deficiency is a prevalent and intrinsic metabolic disturbance in β-thalassemia major, closely linked to chronic iron overload and elevated ferritin levels. This interplay disrupts hepatic vitamin D hydroxylation, induces inflammation, and contributes to endocrine and skeletal complications, highlighting ferritin as a key predictor of deficiency in these patients. **Conclusion**: Vitamin D deficiency is highly prevalent in βTM and is strongly associated with elevated ferritin levels, suggesting that iron overload is a significant predictor. Integrating vitamin D assessment into routine monitoring may support better management of disease-related metabolic disturbances in patients with βTM.

Keywords:*Beta-thalassemia; Vitamin D deficiency; Serum ferritin; Iron overload; Ineffective erythropoiesis.*

RESUMO

Introdução: A vitamina D desempenha papel essencial na saúde óssea e na função fisiológica geral, e sua deficiência é comum em crianças e adolescentes com β-talassemia maior (βTM). A sobrecarga de ferro, refletida pela ferritina elevada, pode influenciar ainda mais o status da vitamina D. **Objetivo**: Este estudo teve como objetivo avaliar os níveis séricos de vitamina D em pacientes com βTM e determinar sua associação com os níveis de ferritina. **Métodos**: Um total de 40 pacientes com βTM e 20 controles saudáveis pareados por idade (com idades entre 4 e 25 anos) foram incluídos entre outubro de 2024 e fevereiro de 2025. Foram medidos vitamina D sérica, cálcio, ferritina e hemoglobina. A análise estatística, incluindo correlação e regressão logística, foi realizada usando SPSS v.26 para identificar preditores de deficiência de vitamina D. **Resultados**: A deficiência de vitamina D foi altamente prevalente entre pacientes com βTM (70%) em comparação com controles. Os pacientes apresentaram níveis de vitamina D significativamente menores (17,32±1,56) do que os controles

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(25,34±1,76). Os níveis de vitamina D correlacionaram-se positivamente com idade (r = 0,788), cálcio (r = 0,772) e hemoglobina (r = 0,771), e negativamente com ferritina (r = −0,517). A regressão logística demonstrou que ferritina >1000 ng/mL predisse fortemente a deficiência de vitamina D (OR = 17,875; IC 95%: 3,258–98,074; p = 0,001), enquanto idade mais jovem (< 10 anos) também aumentou as chances de deficiência (OR = 5,200; p = 0,018). Discussão: A deficiência de vitamina D é um distúrbio metabólico prevalente e intrínseco na β-talassemia maior, intimamente ligado à sobrecarga crônica de ferro e aos níveis elevados de ferritina. Essa interação interrompe a hidroxilação hepática da vitamina D, induz inflamação e contribui para complicações endócrinas e esqueléticas, destacando a ferritina como um preditor-chave de deficiência nesses pacientes. **Conclusão**: A deficiência de vitamina D é altamente prevalente na βTM e está fortemente associada a níveis elevados de ferritina, sugerindo que a sobrecarga de ferro é um preditor significativo. Integrar a avaliação da vitamina D no monitoramento de rotina pode apoiar melhor manejo dos distúrbios metabólicos relacionados à doença em pacientes com βTM.

Palavras-chave: *Beta-talassemia; Deficiência de vitamina D; Ferritina sérica; Sobrecarga de ferro; Eritropoiese ineficaz.*

1. INTRODUCTION

Beta-thalassemia major is a severe hereditary blood disorder characterized by defective hemoglobin synthesis, leading to chronic anemia and a lifelong dependence on regular blood transfusions (Bin et al., 2025). While transfusion therapy has dramatically improved survival, it also results in progressive iron overload, which can cause multi-organ dysfunction, particularly affecting the heart, liver, and endocrine system (Pinto & Forni, 2020; Pala *et al.,* 2023). Among the most prevalent and clinically significant complications in these patients is vitamin D deficiency, which has been increasingly recognized as a major contributor to morbidity (Abdelmotaleb *et al.,* 2021; Akram *et al*., 2025).

Recent studies have demonstrated that vitamin D deficiency is highly prevalent in children and adults with beta-thalassemia major, with reported rates ranging from 63% to over 90% (Bin *et al*., 2025; Meshram *et al*., 2025). This deficiency is multifactorial, resulting from decreased cutaneous synthesis, impaired hepatic hydroxylation due to iron-induced liver dysfunction, and possible nutritional inadequacies (Abdelmotaleb *et al.,* 2021; Majid and Sharba, 2025). Iron overload, as measured by elevated serum ferritin and direct organ iron quantification, has been shown to correlate negatively with vitamin D levels, suggesting that iron toxicity may directly impair vitamin D metabolism (Pala *et al.,* 2023; Verma *et al.,* 2020; Meloni *et al*., 2023).

The clinical consequences of vitamin D deficiency in beta-thalassemia major are profound. Patients are at increased risk for bone disease, including osteopenia and osteoporosis, as well as endocrine complications such as hypoparathyroidism and growth retardation (Mohammed *et al*., 2022; Meshram *et al.,* 2025). Furthermore, recent evidence indicates that low vitamin D status is associated with increased cardiac iron uptake and impaired cardiac function, highlighting the importance of regular monitoring and supplementation (Pala *et al.,* 2023; Meloni *et al*., 2023). Given these findings, integrated management strategies that address both iron overload and vitamin D deficiency are essential to improve long-term outcomes in this vulnerable population.

This study aims to determine the prevalence of vitamin D deficiency and its association with ferritin levels in patients with beta-thalassemia. The findings are expected to elucidate shared pathological mechanisms and provide a scientific basis for more effective interventional strategies to prevent and treat bone complications in this patient population, which may ultimately improve their quality of life.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

This case-control study was conducted between October 2024 and February 2025 and included 60 participants: 40 individuals with beta-thalassemia major (β-TM) and 20 age-matched healthy controls, all aged 4-25 years. Patients were recruited from the Hematology Diseases Center at Al-Zahraa Teaching Hospital in Al-Najaf, whereas controls were selected from healthy staff members of the Faculty of Science, University of Kufa. Written informed consent was obtained from adult participants and from parents or legal guardians of minors, with children's assent obtained when applicable. The study protocol was approved by the Institutional Review Board of the

University of Kufa, Faculty of Science, on September 5, 2024

2.2. Data Collection and Clinical Assessment

Clinical information for β-TM patients was collected through a structured questionnaire and a detailed review of medical records. Data included demographic characteristics (age, sex), disease-related variables (duration since diagnosis, age at first transfusion), current management plans (type and schedule of chelation therapy, transfusion frequency), and the presence of any systemic, metabolic, or immunological comorbidities.

2.3. Blood Sample Collection and Processing

Following an overnight fast of 8–12 hours, venous blood samples (approximately 3 mL) were drawn using standard aseptic procedures. Samples were divided into two tubes: a K_2EDTA tube (BD Vacutainer, USA) for hematological analysis and a serum-separator gel tube (BD Vacutainer, USA) for biochemical tests. Serum samples were allowed to clot for 15–20 minutes, centrifuged at 3000 × g for 10 minutes, then aliquoted into sterile microtubes and stored at –20 °C until batch analysis to minimize analytical variation.

2.4. Laboratory Assays

2.4.1. Hematological and Biochemical Assay

Hematological parameters were measured using an automated hematology analyzer (Abbott CELL-DYN Ruby, USA). Biochemical parameters were measured in serum using commercially supplied kits according to the manufacturers' protocols, with all measurements conducted in duplicate.

2.4.2. 25-Hydroxyvitamin D (Vit. D)

Serum 25(OH)D levels were determined using a competitive ELISA kit (Cat. No. E-EL-0012, MyBioSource, USA). Vitamin D status was classified according to widely accepted clinical cutoffs:

• Deficiency: < 20 ng/mL

• Insufficiency: 20–29 ng/mL

• Sufficiency: ≥30 ng/mL

This biomarker reflects total body vitamin D stores and is the standard indicator for assessing vitamin D status.

2.4.3. Ferritin

Serum ferritin concentration, used as a marker of iron stores and iron-overload status, was measured using a quantitative sandwich ELISA kit (Cat. No. MBS843444, MyBioSource, USA).

2.4.4. Calcium

Total serum calcium was quantified using a colorimetric assay (Cat. No. E-BC-K103-M, Elabscience, USA).

2.5. Statistical analysis

All data were analyzed using SPSS version 26. Comparisons between patients and control groups were performed using the Independent t-test for normally distributed variables or the Mann–Whitney U test for non-normally distributed variables. Comparisons among three groups were conducted using one-way ANOVA followed by Tukey's post hoc test. Nominal variables were presented as frequencies and percentages, and the Chi-square test was applied throughout the study. Pearson correlation coefficients were used for parametric variables and Spearman's rho for non-parametric variables. Binary logistic regression analysis was performed to evaluate the association between clinical variables and the outcome measures, and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were reported. Statistical significance was set at $p \leq 0.05$ and $p \leq 0.01$.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Distribution of demographic parameters in βTM patients and control groups.

The statistical analysis in Table 1. Documented the distribution of studied groups, which included 40 patients with beta-thalassemia major βMT whose mean age was about 10.78 (1.12), was compared with 20 healthy controls who matched in age 11.05 (1.23), non-significant ($p = 0.880$). No significant difference ($p = 0.850$) was observed between the two age groups of patients (62.5% age < 10 years and 37.5% age >10 years), compared with 65.0% and 35% in the age < 10 and >10 years, respectively, in the control groups. The distribution of sex in patients was males 52.5% and females 47.5%, compared with females/males 45.0%/55.0 % in the control group, with no significant difference between the two groups ($p = 0.584$). A significant difference in βTM thalassemia with vitamin D deficiency 70.0%, and insufficient 12.5% more than in patients with vitamin D sufficient 17.5% when compared with deficient, insufficient, and sufficient vitamin D 30.0%, 40.0%, and 30.0% in the control groups, $p = 0.009$. According to anemia status, the results showed that βTM with severe and moderate

anemia was about 35.0% and 37.5%, respectively, the highest among patients with mild anemia at 20.0% and nonanemic (normal) at 7.5%, compared with mild 15% and normal 50% in the control groups, respectively, p = 0.0001. In the βTM thalassemia group, 32 individuals (80.0%) had ferritin levels >1000 ng/dL, while only 8 (20.0%) were ≤1000 ng/dL. In contrast, all controls (20 individuals, 100.0%) fell within the ≤1000 ng/dL category, and none had elevated ferritin. This distribution yielded a highly significant association (χ^2 = 34.286, p = 0.0001),

3.1.2. Comparison of studied parameters in the βTM patients with control groups.

The results are in Table 2. Indicated a significant (p < 0.05) decrease in the serum vitamin D levels in the βTM patients, with a mean (SE) of 17.32 (1.56), as compared with the control groups 25.34 (1.76), p = 0.002, Figure 1. Serum Ca levels are significantly lower in the βTM patients (mean (SE) 6.97 (0.26)) compared with the control groups (9.01 (0.14), p = 0.0001, Figure 2. Highly significant elevated serum ferritin levels in the βTM patients as compared with the control group mean (SE) about (2080.76 (194.63) vs. 56.54 (5.08), p = 0.0001), figure 3. In similar results, the βTM patients had a significant (p < 0.05) decrease in Hb levels when compared with the control group mean (SE) about (9.21 (0.30) VS. 11.39 (0.27), P = 0.001)—Figure 4.

3.1.3 Comparison of studied parameters among vitamin D status in β-thalassemia major patients (βTM)

The statistical analysis is in Table 3. Show βTM patients divided into three groups according to vitamin D classification, which includes 7 patients with vitamin D Sufficient, with a mean (SE) of 35.27 (1.41), Insufficient (n = 5) with a mean (SE) of 23.82 (1.38), and 28 patients with Deficient 11.68 (0.64) ng/ml, these results showed significant differences p = 0.0001. Serum Ca levels in βTM patients indicated a significant (p < 0.05) decrease in patients with vitamin D Deficiency (6.36 (0.29)), compared with Insufficient (8.2 (0.35)) and Sufficient (8.54 (0.19)), p = 0.006. Figure 5. Highly significant increase in serum ferritin levels in patients with vitamin D deficiency, with a mean (SE) of 2365.1 (237.96), more than in patients with Insufficient 1529.11 (433.96), as compared with Sufficient patients' mean (SE) of 1260.77 (175.97), p = 0.003. Figure 6. Hb levels in βTM patients showed a significant (p < 0.05) decrease in patients with vitamin D Deficiency (8.59 (0.3) g/dl), compared with Insufficient (9.08 (0.69)) and Sufficient (11.78 (0.25)), p = 0.001. Figure 7. The β-thalassemia major group showed a significant association between ferritin status and vitamin D status, reflected by a Chi-square value of 14.464 and a p-value of 0.0001. Among those with ferritin >1000 ng/mL, 4 patients (80.0%) had insufficient vitamin D, 26 (92.9%) were vitamin D deficient, and 2 (28.6%) were sufficient. In contrast, within the ≤1000 ng/mL ferritin category, only 1 patient (20.0%) had insufficient vitamin D, 2 (7.1%) were deficient, while 5 (71.4%) were sufficient.

3.1.4.Correlation and logistic regression analysis of vitamin D with studied parameters in βTM patients.

The correlation results are shown in Table 4. Vitamin D showed significant positive correlations with age (r = 0.788), Ca (r = 0.772), and Hb (r = 0.771), but a negative correlation with ferritin (r = -0.517). Also, ferritin was inversely associated with Ca (r = -0.610) and Hb (r = -0.597), while a positive correlation was observed between the two last parameters (r = 0.866).

The logistic regression analysis compared vitamin D status across the studied categories in Table 4. For age groups, using individuals older than 10 years as the reference, those younger than 10 years with insufficient vitamin D showed a coefficient of 0.624, corresponding to an odds ratio (OR) of 1.867 with a 95% confidence interval (CI) of 0.392–8.894 and a p-value of 0.433, while those with deficient vitamin D had a coefficient of 1.649, an OR of 5.200 (95% CI: 1.322–20.460), and a p-value of 0.018. For sex, with females as the reference, males with insufficient vitamin D had a coefficient of –0.341, an OR of 0.711 (95% CI: 0.140–3.606), and a p-value of 0.681, whereas males with deficient vitamin D had a coefficient of –1.291, an OR of 0.275 (95% CI: 0.070–1.078), and a p-value of 0.064. Regarding anemia status, taking none–mild anemia as the reference group, participants with moderate to severe anemia and insufficient vitamin D had a coefficient of -0.811, producing an OR of 0.444 (95% CI: 0.137–1.443) with a p-value of 0.0001. In contrast, the deficient vitamin D category showed a coefficient of 1.540 and OR of 4.667 (95% CI: 1.932-11.270), For ferritin status, with < 1000 ng/mL as the reference group, individuals with ferritin >1000 ng/mL and insufficient vitamin D had a coefficient of 0.894, an OR of 2.444 (95% CI: 0.361–16.547), and a p-value of 0.360, whereas those with deficient vitamin D showed a coefficient of 2.883, an OR of 17.875 (95% CI: 3.258–98.074), and a p-value of 0.001.

3.2. Discussions

The present study adds to a growing consensus that vitamin D deficiency is a pervasive and clinically significant metabolic disturbance in individuals with β-thalassemia major (β-TM). Our findings, considered alongside previous reports, indicate that this deficiency is an intrinsic aspect of the disease's pathophysiology, rather than a consequence of external factors alone, such as sunlight exposure or dietary intake (Abdelmotaleb *et al.,* 2021; Al-Rubae *et al.,* 2023; Rothchild *et al.,* 2023).

A central feature of β-TM is chronic iron overload, primarily resulting from repeated red blood cell transfusions and from augmented intestinal iron absorption driven by ineffective erythropoiesis (Almousawi & Sharba, 2019). Excess circulating iron is stored in parenchymal tissues, particularly the liver, which is the principal

site for 25-hydroxylation of vitamin D. Iron-mediated hepatocellular injury disrupts the activity of key cytochrome P450 enzymes responsible for converting vitamin D into 25-hydroxyvitamin D, thereby diminishing circulating levels of the biomarker most commonly used to assess vitamin D status (Hamayun *et al.,* 2017; Mogire *et al*., 2020). This pathophysiological mechanism provides a biologically plausible explanation for the inverse association observed between ferritin and vitamin D in our cohort and in other thalassemia populations.

Beyond hepatic conversion, iron overload induces oxidative stress and inflammatory signaling, altering vitamin D metabolism at multiple regulatory points. Elevated ferritin reflects not only iron stores but also a state of chronic inflammation. Inflammatory mediators can suppress the synthesis of vitamin D–D-binding protein, alter receptor expression, and interfere with downstream signaling pathways critical for endocrine function (Urmi *et al.,* 2022). These effects may also modulate parathyroid hormone (PTH) activity, contributing to the secondary endocrine dysregulation observed in thalassemia, as hypothesized in experimental and clinical studies (Saki *et al.,* 2020).

Our logistic regression analysis reinforces the centrality of ferritin in predicting vitamin D deficiency. Elevated ferritin emerged as one of the strongest independent predictors of deficient vitamin D status in multivariable modeling. This analytic outcome aligns with mechanistic insights, indicating that iron burden is more than a correlate of deficiency—it has predictive value in identifying patients at the highest risk of metabolic derangement. Age was also a significant predictor in the model, suggesting that developmental and maturational factors may interact with iron-related metabolic stress to influence vitamin D homeostasis. These statistical associations provide quantitative support for the clinically observed interplay between iron overload and vitamin D deficiency.

The consequences of this metabolic interaction extend to skeletal health. Vitamin D plays an indispensable role in calcium absorption, skeletal mineralization, and bone turnover regulation. When its activation is compromised by iron-induced hepatic dysfunction, calcium homeostasis becomes destabilized. Concurrent iron toxicity in bone marrow and trabecular structures further impairs osteoblastic activity and enhances resorption, fostering an environment conducive to osteoporosis, deformities, and increased fracture risk (Pala *et al.,* 2023; Baird *et al.,* 2022). This synergistic deterioration of bone integrity underscores why bone disease remains a leading cause of morbidity in β-TM despite advances in supportive care.

Moreover, ineffective erythropoiesis and suppressed hepcidin levels create a persistent drive for dietary iron absorption, compounding systemic iron loading even in the context of chelation therapy. Low hepcidin facilitates continuous iron uptake from the gut and mobilization from macrophage stores, further elevating serum ferritin and perpetuating its deleterious metabolic effects (Saad *et al.,* 2021; Cazzola, 2022). Such alterations in iron physiology have implications not only for vitamin D metabolism but also for endocrine axes, glucose regulation, and immune competence.

Taken together, the present findings support a model in which iron overload and vitamin D deficiency are interdependent components of a broader metabolic dysregulation in β-TM. Rather than functioning as isolated abnormalities, they represent convergent pathways that exacerbate skeletal and systemic complications characteristic of this disorder.

4. CONCLUSIONS

This study confirms that vitamin D deficiency and elevated ferritin are intertwined metabolic entities in β-thalassemia major, with elevated ferritin serving as a robust predictor of deficient vitamin D status. These findings reinforce the need to incorporate routine monitoring of both parameters into standard care. Vitamin D supplementation should be individualized and closely supervised, particularly in the context of iron-mediated liver dysfunction. In addition, optimizing iron chelation therapy remains essential to mitigate the broader metabolic consequences of iron overload. Longitudinal and interventional studies are required to determine whether correcting vitamin D deficiency can improve bone outcomes and quality of life in patients with β-TM.

5. DECLARATIONS

5.1. Study Limitations

Case–control studies on vitamin D deficiency and iron overload in β-thalassemia major often face several limitations. Small sample sizes reduce statistical power and generalizability. Matching of cases and controls is sometimes inadequate, especially for age, sex, or disease severity. Variability in laboratory methods and in definitions of deficiency complicates comparisons across studies. Many studies lack detailed clinical or biochemical data, such as parathyroid hormone levels or chelation therapy regimens, limiting mechanistic insights. The absence of long-term follow-up also prevents evaluation of disease progression and intervention outcomes.

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5.4. Competing Interests

The authors declare no conflict of interest. More information about

5.5. Open Access

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The study protocol was reviewed and approved by the Institutional Review Board of the University of Kufa, Faculty of Science, on September 5, 2024. As the study used samples collected during patients' routine follow-up and pre-transfusion tests, which occur twice weekly, with no additional procedures performed on participants, informed consent was not required.

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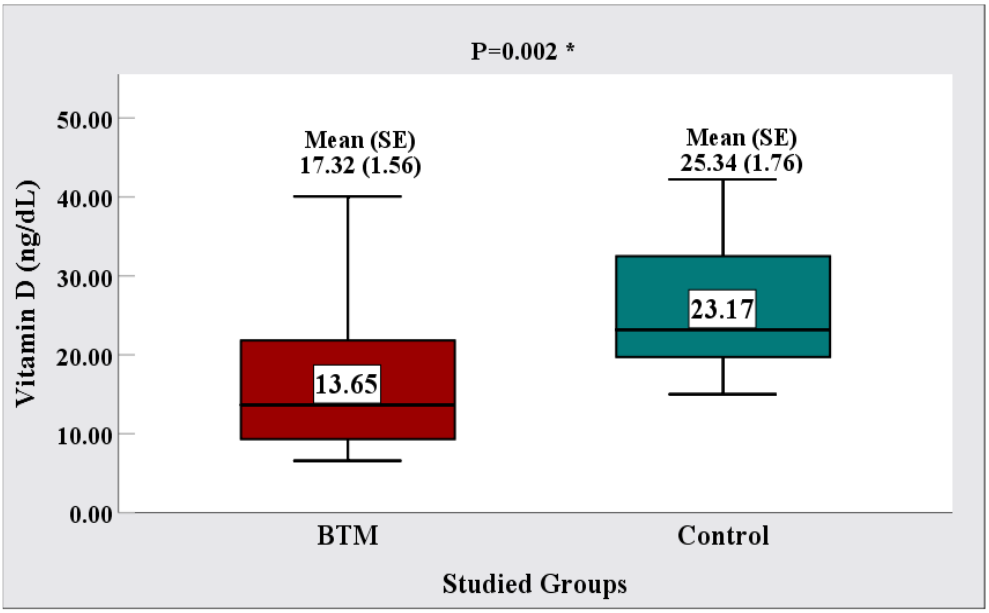

Figure 1. *Serum Vitamin D (ng/dL) levels in the βTM patients and control groups. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

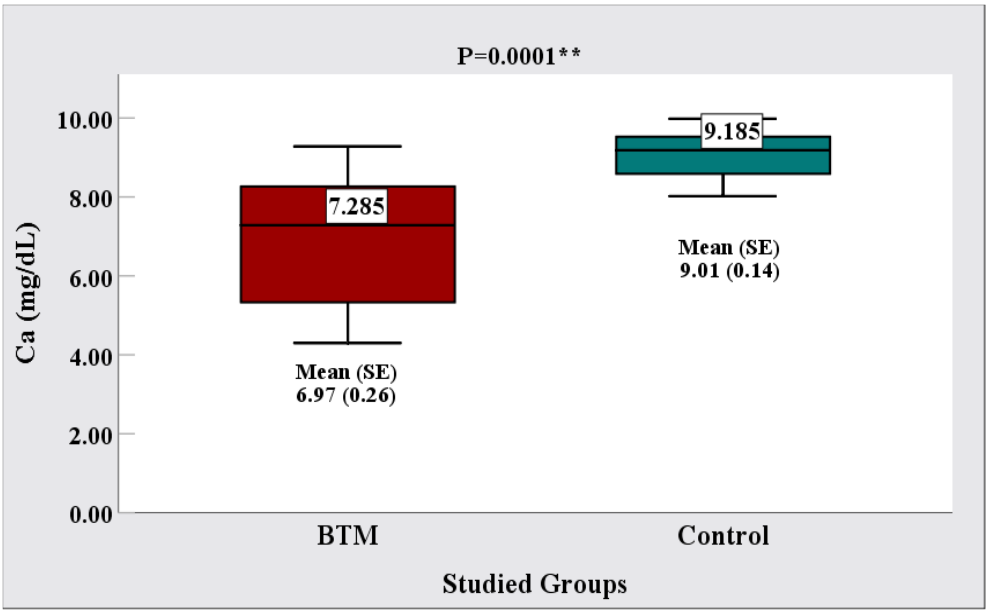

Figure 2. *Serum Ca (mg/dL) levels in the βTM patients and control groups. Significant differences at p-values * < 0.05, ** < 0.01. SEM: Standard error of the mean.*

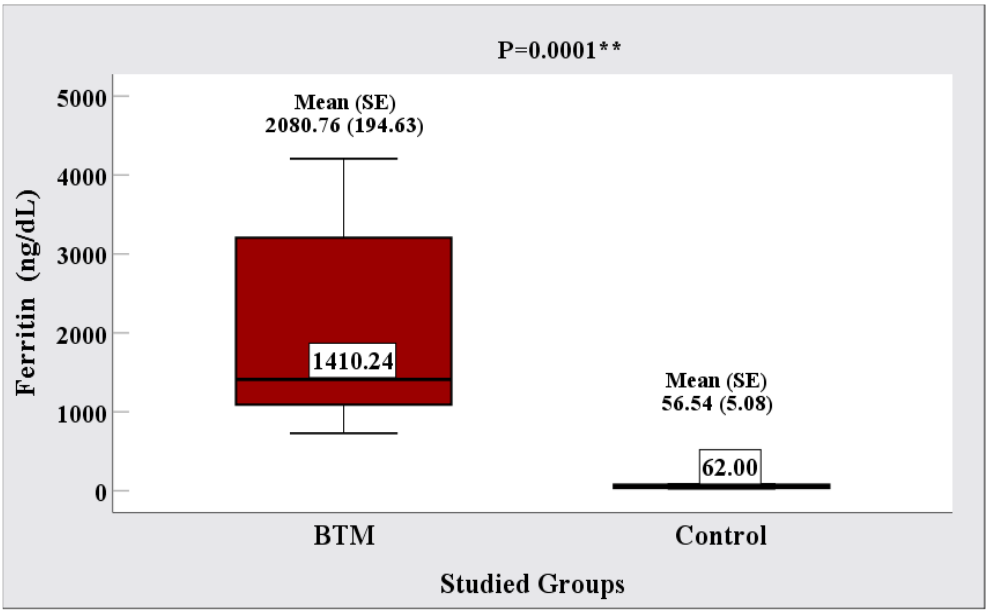

Figure 3. *Serum Ferritin (ng/dL) levels in the βTM patients and control groups. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

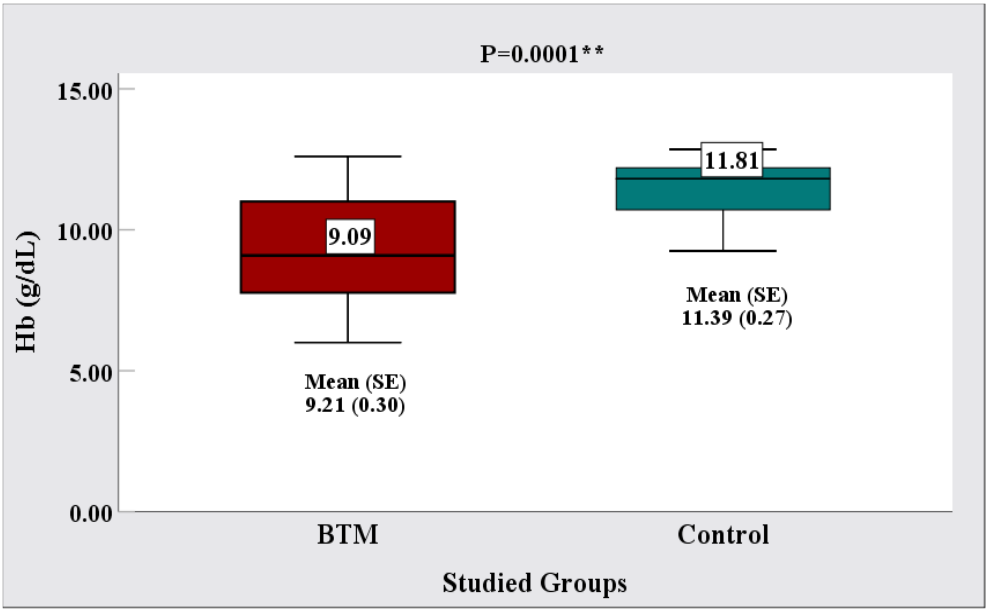

Figure 4. *Hb (g/dL) levels in the βTM patients and control groups. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

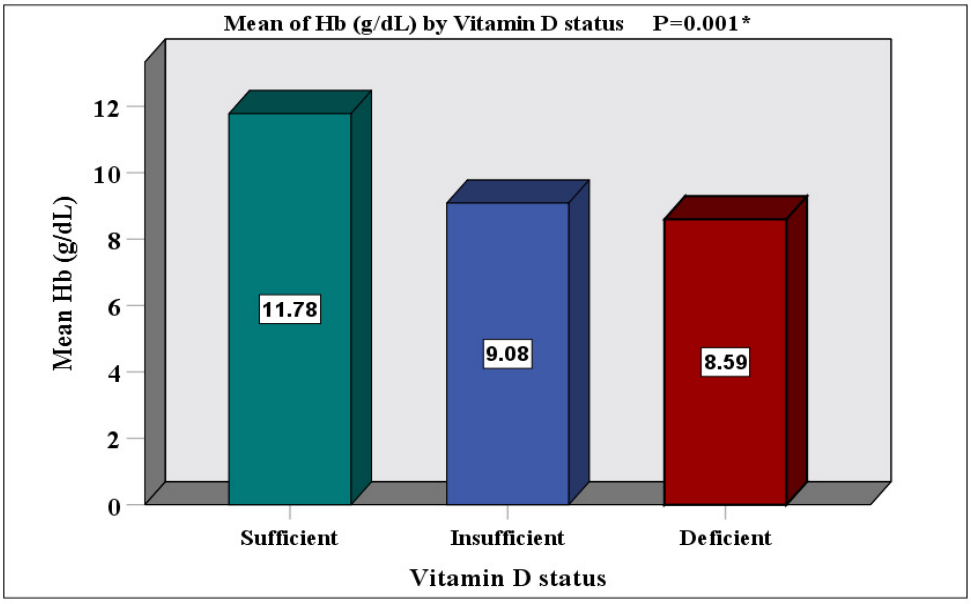

Figure 5. *Hb (g/dL) levels associated with vitamin D status in patients βTM. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

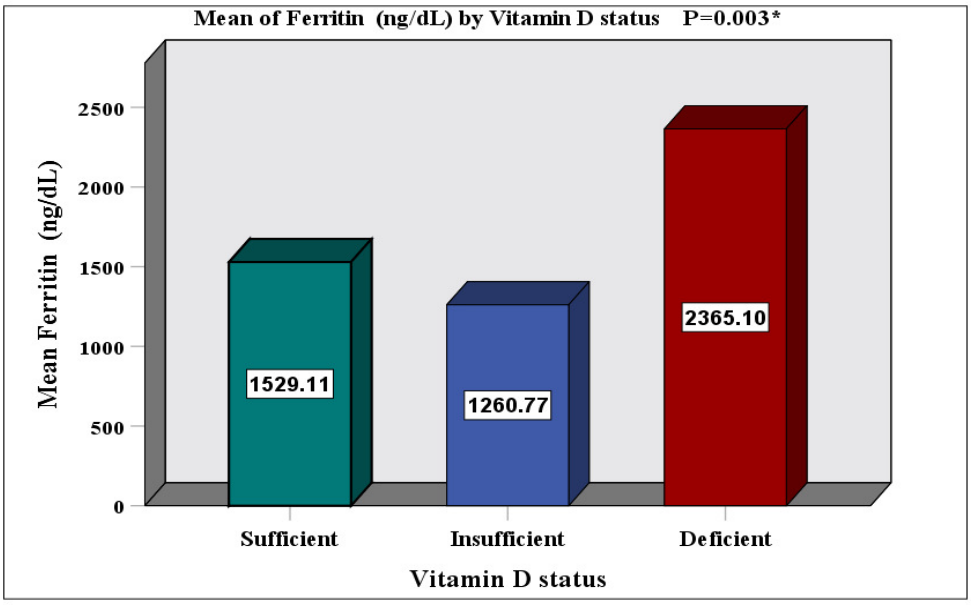

Figure 6. *Serum Ferritin (ng/dL) levels associated with vitamin D status in patients βTM. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

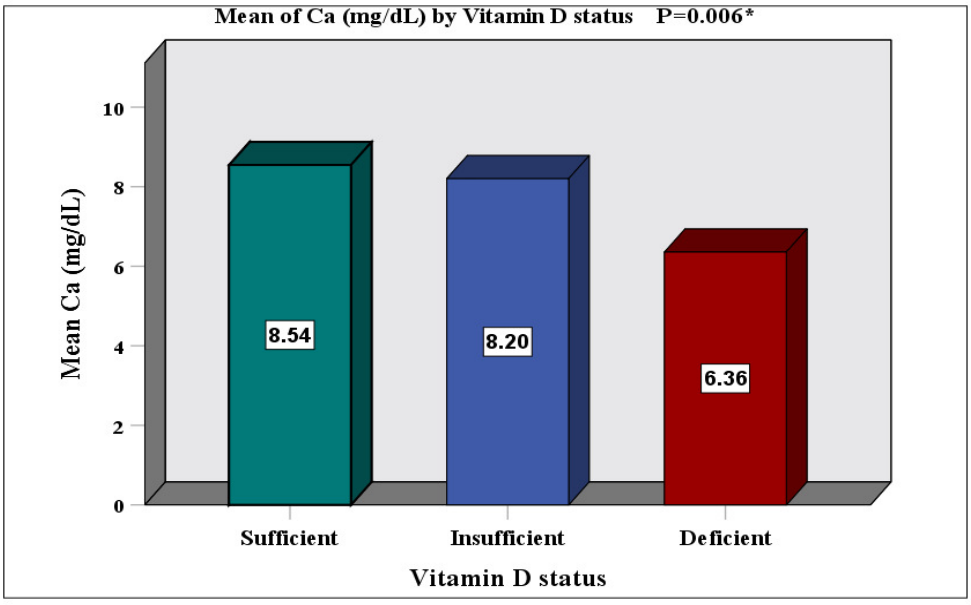

Figure7. *Serum Ca (mg/dL) levels associated with vitamin D status in patients βTM. Significant differences at p-values * < 0.05, ** < 0.01. SEM Standard error of the mean*

Table 1. Distribution of demographic parameters in βTM patients and control groups.

Variables	Categories	βTM	Control	Total	p-value
Age (year)	Mean (SE)	10.78(1.12)	11.05(1.23)		0.880 ns
	< 10 years	25	13	38	
		62.5%	65.0%	63.3%	0.036 [a]
	>10 years	15	7	22	0.850 ns
		37.5%	35.0%	36.7%	
Sex	Male	21	9	30	
		52.5%	45.0%	50.0%	0.300[a]
	Female	19	11	30	0.584 ns
		47.5%	55.0%	50.0%	
Vitamin D status	Deficient	28	6	34	
		70.0%	30.0%	56.7%	
	Insufficient	5	8	13	9.380[a]
		12.5%	40.0%	21.7%	0.009*
	Sufficient	7	6	13	
		17.5%	30.0%	21.7%	
Anemia status	Non-anemia	3	7	10	
		7.5%	35.0%	16.7%	
	Mild	8	10	18	
		20.0%	50.0%	30.0%	19.300[a]
	Moderate	15	3	18	0.0001**
		37.5%	15.0%	30.0%	
	Severe	14	0	14	
		35.0%	0.0%	23.3%	
Ferritin status	≤1000 (ng/dL)	8	20	28	
		20.0%	100.0%	46.7%	34.286[a]
	> 1000 (ng/dL)	32	0	32	0.0001**
		80.0%	0.0%	53.3%	
Total		40	20	60	
		100.0%	100.0%	100.0%	

Significant differences at p-value * < 0.05, ** < 0.01. a: Chi-Square test. Ns: non-significant.

Table 2. *Comparison of studied parameters in the βTM patients with control groups.*

Variables	Control n = 20	βTM n = 40	P-value
Age (year)	11.05 (1.23)	10.78 (1.12)	0.880 ns
Vitamin D (ng/dL)	25.34 (1.76)	17.32 (1.56)	0.002*
Ca (mg/dL)	9.01 (0.14)	6.97 (0.26)	0.0001**
Ferritin (ng/dL)	56.54 (5.08)	2080.76 (194.63)	0.0001**
Hb (g/dL)	11.39 (0.27)	9.21 (0.30)	0.0001**

Different letters have significant differences at p-values * < 0.05, ** < 0.01. SEM: Standard error of the mean. Independent T-test, or Mann-Whitney.

Table 3. *Comparison of studied parameters among vitamin D status in β-thalassemia major patients*

Variables	Sufficient N = 7	Insufficient N = 5	Deficient N = 28	p-value
Vitamin D (ng/dL)	35.27 (1.41) A	23.82 (1.38) B	11.68 (0.64) C	0.0001**
Ca (mg/dL)	8.54 (0.19) A	8.2 (0.35) A	6.36 (0.29) B	0.006*
Hb (g/dL)	11.78 (0.25) A	9.08 (0.69) B	8.59 (0.3) C	0.001*
Ferritin (ng/dL)	1529.11 (433.96) A	1260.77 (175.97) B	2365.1 (237.9) A	0.003*
>1000	2 (28.6%)	4 (80.0%)	26 (92.9%)	14.464[a]
≤1000	5 (71.4%)	1 (20.0%)	2 (7.1%)	0.0001**

Different letters have significant differences at p-values * < 0.05, ** < 0.01. SEM: Standard error of the mean. ANOVA test. a: Chi-Square test

Table 4. *Correlation of vitamin D with studied parameters in βTM patients.*

		Vitamin D	Ca	Ferritin	Hb
Age (year)	**r**	0.788**	0.493**	-0.293	0.463**
	p-value	0.0001	0.001	0.066	0.003
Vitamin D (ng/dL)	**r**	1	0.772**	-0.517**	0.772**
	p-value		0.0001	0.001	0.0001
Ca (mg/dL)	**r**			-0.610**	0.866**
	p-value			0.0002	0.0001
Ferritin (ng/dL)	**r**			1	-0.597**
	p-value				0.0001

Significant differences at p-value * < 0.05, ** < 0.01. r: Pearson correlation

Table 5: *Logistic Regression Analysis of Factors Associated with Vitamin D Status in β-Thalassemia Major Patients*

Variables	**Vitamin D Status**	**B**	**OR (95% CI)**	**P-value**
Age Group (Ref: >10 yr.)	Deficient	1.649	5.200 (1.322–20.460)	0.018*
	Insufficient	0.624	1.867 (0.392–8.894)	0.433
	Sufficient	0 (ref.)	—	—
Sex (Ref: Female)	Deficient	–1.291	0.275 (0.070–1.078)	0.064
	Insufficient	–0.341	0.711 (0.140–3.606)	0.681
	Sufficient	0 (ref.)	—	—
Anemia Status (Ref: None to Mild)	Deficient	1.540	4.667 (1.932-11.270)	0.001*
	Insufficient	-0.811	0.444 (0.137–1.443)	0.177
	Sufficient	0 (ref.)	—	—
Ferritin Status (Ref: < 1000 ng/mL)	Insufficient	0.894	2.444 (0.361–16.547)	0.360
	Deficient	2.883	17.875 (3.258–98.074)	0.001*
	Sufficient	0 (ref.)	—	—

OR: Odds Ratio; CI: Confidence Interval; B: Regression Coefficient; Ref. Reference Group. Asterisk (*) Statistical significance at P < 0.05.