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Vitamin D and calcium status in children with thalassemia beta major: A cross sectional study in Banda Aceh

Status vitamin D dan kalsium pada anak dengan talasemia beta mayor: Studi potong lintang di Banda Aceh

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Abstract

Frequent blood transfusions in children with beta-thalassemia major result in iron overload, leading to hemosiderosis in various body tissues, and impair the function of these organs, disrupting vitamin D metabolism, which contributes to osteoporosis and other morbidities, which are believed to be associated with hypocalcemia, which significantly affects growth. This study aimed to investigate the relationship between vitamin D and calcium status in children with β-thalassemia major at dr. Zainoel Abidin Public Hospital in Banda Aceh, Indonesia. Methods**:** An analytical observational study employed a cross-sectional design involving 40 children aged 2–18 years with a beta-thalassemia major attending the Children's Thalassemia Clinic at dr. Zainoel Abidin Public Hospital in Banda Aceh, Indonesia. from July to November, 2024. Categorical data were analyzed using the Spearman test, with a p-value of <0,05. Results, of the 40 subjects, 55% subjects were male, in 45% of subjects, there was Vitamin D deficiency, 25% was vitamin D insufficiency, hypocalcemia in 77,5% subjects. Among subjects with vitamin D deficiency, 94,4% also had hypocalcemia (r = 0,037, p = 0,017). In conclusion, there was a significant association between vitamin D and calcium status in children with beta-thalassemia major.

Keywords: Beta thalassemia major, hypocalcemia, pediatric patients, vitamin D deficiency

Abstrak

Transfusi darah yang sering pada anak dengan talasemia beta mayor mengakibatkan kelebihan zat besi, yang menyebabkan hemosiderosis pada berbagai jaringan tubuh, dan mengganggu fungsi organ-organ ini, mengganggu metabolisme vitamin D, yang berkontribusi terhadap osteoporosis dan morbiditas lain, yang diyakini terkait dengan hipokalsemia, yang secara signifikan mempengaruhi pertumbuhan. Penelitian bertujuan untuk menilai hubungan antara vitamin D dan status kalsium pada anak-anak dengan talasemia beta mayor di RSUD dr. Zainoel Abidin di Banda Aceh, Indonesia. Metode, penelitian observasional analitik ini menggunakan desain potong lintang yang melibatkan 40 anak berusia 2–18 tahun dengan talasemia beta mayor yang di Poliklinik Thalassemia Anak di RSUD dr. Zainoel Abidin di Banda Aceh, Indonesia. dari Juli hingga November 2024. Data kategorikal dianalisis menggunakan uji Spearman dengan nilai p <0,05. Hasil, dari 40 subjek, 55% subjek adalah laki-laki, pada 45% subjek terdapat defisiensi vitamin D, 25% mengalami insufisiensi vitamin D dan hipokalsemia pada 77,5% subjek. Pada subjek dengan defisiensi vitamin D, 94,4% juga mengalami hipokalsemia (r = 0,037, nilai p = 0,017). Kesimpulan, terdapat hubungan yang signifikan antara vitamin D dan status kalsium pada anak dengan talasemia beta mayor.

Kata Kunci: Hipokalsemia, kekurangan vitamin D, pasien anak-anak, talasemia beta mayor

Introduction

Thalassemia is a chronic, inherited disorder characterized by quantitative and qualitative deficits in hemoglobin, resulting from mutations that impair the synthesis of alpha or beta globulin chains and shorten the erythrocyte lifespan. This condition is associated with a range of endocrine and metabolic abnormalities (Tharwat et al., 2019). Thalassemia caused by red blood cell abnormalities necessitates lifelong blood transfusions in affected individuals. According to the World Bank, 7% of the global population has thalassemia. Indonesia has a high prevalence of thalassemia carriers, with epidemiological studies showing that the frequency of the β-thalassemia gene ranges from 3% to 10%. Aceh province has the highest prevalence of thalassemia in Indonesia, at 13,4% (Paloma, 2023).

Despite advances in transfusion and iron chelation protocols, thalassemia patients continue to face challenges, including a heightened risk of osteoporosis (Herawati et al., 2021). Ineffective erythropoiesis in thalassemia necessitates frequent blood transfusions, which leads to iron overload. This overload affects multiple organs, including the endocrine glands, skin, and kidneys, all of which influence the vitamin D levels. Vitamin D is crucial for calcium homeostasis and bone mineralization, particularly during rapid growth, such as infancy and puberty. Deficiency in vitamin D results in conditions like rickets and osteomalacia (Ahmed et al., 2022; Soliman, 2013).

A systematic review 2021 reported that the prevalence of vitamin D deficiency in individuals with thalassemia ranges from 24,8% to 80,6%, indicating that nearly all thalassemia patients are at risk of deficiency regardless of age, gender, race, or geographic location (Ahmed et al., 2022). This risk increases with age, as older patients with thalassemia exhibit significantly lower vitamin D levels than healthy age-matched controls. Despite awareness and routine vitamin D supplementation, addressing vitamin D deficiency in thalassemia remains challenging (Ali et al., 2023). Consequently, research on vitamin D status and its relationship with thalassemia continues worldwide.

The Thalassemia Clinical Research Network in North America reported 2009 that 12,8% of 361 children with thalassemia had vitamin D deficiency (Vogiatzi et al., 2009). A 2022 study in Egypt found that 49% of thalassemia patients were vitamin D deficient (Abdelmotaleb et al., 2021), while a 2016 study in India reported an alarming 98% deficiency prevalence among children with thalassemia (Agrawal et al., 2016). Additionally, 73% of pediatric patients with beta-thalassemia major in the United States and 90% in Thailand were vitamin D deficient (Herawati et al., 2021). Low serum calcium levels have also been reported in patients with thalassemia. A 2022 study in Egypt and a 2016 study in Bangladesh revealed significantly lower serum calcium levels in children with beta-thalassemia major (Abdelmotaleb et al., 2021; Karim et al., 2016). Similarly, significantly reduced ionized calcium levels have been reported among children with beta-thalassemia major in Pakistan (2013) and West Sumatra (2017). These findings underscore the critical role of iron overload and hemosiderosis in causing hypocalcemia (Saboor, 2014; Sultan et al., 2016).

No previous studies have been conducted on the vitamin D and calcium status of children with β-thalassemia major in Aceh. Owing to the high prevalence of thalassemia in Aceh, valid data are necessary for optimal future management. This study explores the relationship between vitamin D and calcium status in children with β-thalassemia major in Aceh, and this topic has not been widely researched in this region, as it provides new insights into the importance of nutritional monitoring for patients with thalassemia to improve their quality of life.

Vitamin D deficiency in thalassemia leads to impaired bone mineralization, significantly reducing bone mineral density (Ahmed et al., 2022; De Sanctis et al., 2018; Herawati et al., 2021; Soliman, 2013). At the thalassemia center of Dr. Zainoel Abidin Public Hospital (RSUDZA), Banda Aceh, Indonesia, three beta-thalassemia major patients were found to have pathological fractures. This study investigated the relationship between vitamin D and calcium status in children with beta-thalassemia major in RSUDZA Banda Aceh.

Methods

This study employed an observational analytical design, using a cross-sectional approach. The accessible population comprised all pediatric patients diagnosed with β-thalassemia major at the Pediatric Thalassemia Polyclinic of Dr. Zainoel Abidin Public Hospital, Banda Aceh, Indonesia.

The study was conducted from July to November 2024 and included 40 pediatric subjects aged 2–18 years who had received regular repeated blood transfusions and iron chelation therapy. Exclusion criteria included children with beta-thalassemia major who also experienced malnutrition (either undernutrition or obesity), newly diagnosed infectious diseases within the preceding two weeks, and those with a history of consuming vitamin D supplements within one month before the examination. Ethical approval for this research was obtained from the Ethics Committee of Dr. Zainoel Abidin Public Hospital (approval number 145/ETIK-RSUDZA/2024), issued on June 10, 2024. The samples were selected using a purposive sampling technique, a non-probability method in which samples were sequentially selected by the researcher with minimal variation.

Data collection was conducted through laboratory examinations, where vitamin D and calcium levels were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) and spectrophotometry methods. For data processing and analysis, SPSS software was used, with statistical tests including Pearson correlation to evaluate the relationship between vitamin D and calcium status, as well as t-tests to compare the means between groups. The significance level was set at $p < 0,05$, which was chosen to determine whether the obtained results could be considered statistically significant, given the importance of ensuring the validity of the findings in the context of pediatric patients with thalassemia.

Result and Discussion

As shown in Table 1, the study population was predominantly male, comprising 22 males (55%). This aligns with research by Abdelmotaleb et al. (2021) which reported that major cases of beta-thalassemia were predominantly male (65,45%) compared to female (34,55%). Similarly, in Barua et al.'s research, found male predominance (62,9%) over females (37,1%) (Barua et al., 2020). However, Chin et al. reported an equal distribution, with 50% of cases in boys and 50% in girls (Chin et al., 2019). Meanwhile, Ridha et al.'s. study at Wahidin Sudirohusodo Hospital Makassar found a slightly higher proportion of females (53,3%) than males (46,7%) (Ridha et al., 2022). These differing findings confirm no established relationship between sex and β-thalassemia major, consistent with the theory that the thalassemia gene is inherited in an autosomal recessive manner according to Mendel's laws, which do not depend on sex.

In this study, the age distribution included 6 toddlers (15%), 15 children (37,5%), and 19 adolescents (47,5%), with the average age of beta thalassemia major patients being 10,06 ± 4,24 years, ranging from 2,5 to 17 years. These results align with the research of Ridha et al. who reported an average age of 10,1 years (1,6 – 17,8 years) (Ridha et al., 2022). Other studies show variations; for example, Arjmandi et al. found that the average age of pediatric patients with beta-thalassemia major was 14 ± 6,5 years (Arjmandi et al., 2009). Similarly, Chin et al. reported that the 7-12 age group comprised 65 subjects (60,2%), while the 13–17-year group included 43 subjects (39,8%), with an average age of 11,9 ± 2,9 years (Chin et al., 2019). Such variability suggests no specific relationship between age group and the incidence of beta-thalassemia major. However, based on our study, children with beta-thalassemia major typically do not exhibit symptoms until around 2–3 months of age, when beta chains are required to pair with alpha chains to form HbA, following the cessation of gamma chain production. In some cases, the condition may remain undiagnosed until 3–5 years of age due to delayed cessation of HbF production (Trehan et al., 2015). Consequently, beta thalassemia may be more prevalent in older children as the condition becomes more apparent.

In our study, 24 subjects (60%) with beta-thalassemia major had a good nutritional status, while 16 subjects (40%) experienced malnutrition. This finding aligns with research by Ridha et al. (2022) conducted in 2022, which reported that 80,9% of children with beta-thalassemia had good nutritional status. However, the results slightly differ from the study by Nasir et al. (2018) where 41% subjects of children with beta-thalassemia major had good nutritional status, 49% subjects were malnourished, and 4% were overweight. Several factors, including routine transfusions, duration of illness, and adherence to iron chelation therapy, within the study population may have influenced these variations. Multifactorial growth failure in thalassemia is often associated with chronic anemia, chelation toxicity, and endocrinopathies such as hypogonadism,

hypothyroidism, and growth hormone deficiency. Researchers in the United States also highlight the role of optimal nutritional status in growth failure and pubertal development (Arjmandi et al., 2009).

In our study, 20 subjects (50%) with beta-thalassemia major had hepatomegaly. This is consistent with the findings of Hagag et al. (2015) who reported that 48 subjects (80%) with beta-thalassemia major experienced hepatomegaly. Hepatomegaly is strongly associated with extramedullary hematopoiesis, and patients undergoing blood transfusions may develop hepatomegaly or chronic hepatitis owing to iron overload from transfusions. As the primary storage site for iron reserves, the liver is particularly susceptible to severe damage caused by the accumulation of hemosiderin, which leads to the swelling of phagocytic cells and parenchymal tissue. Liver involvement typically occurs early in the course of the disease (Ahmed et al., 2022).

Similarly, 20 patients (50%) in the present study had splenomegaly. Trehan et al. reported a higher prevalence, with 92,1% subjects of children with beta-thalassemia major experiencing clinical splenomegaly (Trehan et al., 2015). In β-thalassemia, the absence of β-chains results in an excess of unbound free α-chains. These free alpha chains cause hemolysis of erythrocytes owing to morphological changes during extravascular hemolysis within the reticuloendothelial system, particularly in the spleen. This leads to splenic hypertrophy and splenomegaly. Splenomegaly can be identified through patient-reported abdominal enlargement or physical examination during abdominal assessment (Kapila et al., 2024).

Table 1. Basic characteristics of children with beta thalassemia major subjects

Characteristics	n	%
Gender		
Male	22	55
Female	18	45
Age Group		
Toddler	6	15
Child	15	37,5
Adolescent	19	47,5
Nutritional status		
Mild Malnutrition	16	40
Good Nutrition	24	60
Liver		
Normal	20	50
Hepatomegaly	20	50
Spleen		
Normal	20	50
Splenomegaly	20	50
Anemia		
Mild	7	17,5
Moderate	23	57,5
Severe	10	25
Ferritin		
<2000 ng/mL	12	30
≥2000 ng/mL	28	70
Vitamin D Status		
Deficiency	18	45
Insufficiency	10	25
Normal	12	30
Calcium Status		
Hypocalcemia	31	77,5
Normal	9	22,5
Iron chelation		
DFX	17	42,5
DFP	23	57,5

The results of our study showed an average hemoglobin (Hb) level of 7,95 ± 1,222 g/dL, with the prevalent moderate anemia group (57,5%). Our findings are consistent with research conducted by Hagag et al. which reported an average Hb level of 7,61 ± 1,27 g/dL in children with beta-thalassemia major compared to 11,79 ± 0,59 g/dL in healthy children, a statistically significant difference ($p < 0,001$) (Hagag et al., 2015). Similarly, in a study by Aslamy et al., 69,23% of children with beta-thalassemia major were in the moderate anemia category during pre-transfusion conditions. Hemoglobin levels and anemia status were measured before the blood transfusion. As highlighted by the aforementioned studies, most children with thalassemia are in a state of moderate anemia before transfusion. This finding underscores the importance of parental awareness and adherence to routine transfusion schedules (Aslamy et al., 2023).

Our study observed ferritin levels of > 2000 ng/mL in 28 subjects (70,0%). Comparable findings were reported by Yuksel et al. in 2016, where 51% of thalassemia patients had ferritin levels >2000 ng/mL (Yuksel et al., 2016). The Indonesian Thalassemia Foundation, Banyumas Branch, in 2012 revealed that 90,4% of patients had ferritin levels ≥2000 ng/mL. This condition is due to routine blood transfusions in thalassemia patients, as transfused erythrocytes contribute to iron overload (Rejeki et al., 2012).

Ferritin levels are an important measure of reticuloendothelial iron stores, and are useful for diagnosing iron deficiency or overload. Increased serum ferritin levels ≥2000 ng/mL are associated with a nearly sixfold increased risk of myocardial hemosiderosis in thalassemia patients (Yuksel et al., 2016).

Regarding iron chelation therapy, our study found that DFP (Deferiprone) was the most commonly used chelator (57,5%). This aligns with findings from Nurbahiyah et al. conducted at X Jatinegara Hospital between 2022 and 2023, which reported that 55,56% of children with thalassemia used DFP, while 44,4% used DFX (Nurbahiyah & Maulina, 2023). Similarly, Agouzal et al. in 2010 found that DFP was used by 52% of patients, deferoxamine by 37%, and a combination of DFP and deferoxamine by 8% (Agouzal et al., 2010). A systematic review and meta-analysis by Saleem et al., which included five studies with 607 patients, concluded no significant difference in the efficacy of DFX and DFP in managing iron overload in pediatric patients with thalassemia (Saleem et al., 2023).

In our study, 45% of the subjects had vitamin D deficiency, with an average vitamin D level of 17,03 ± 6,622 ng/mL. This finding is consistent with research by Ridha et al. which reported vitamin D deficiency in 86,6% subjects of children with beta-thalassemia major (Ridha et al., 2022). Similarly, Buyuksimsek et al. found an average vitamin D level of 15,70 ± 11,19 ng/mL in children with beta-thalassemia major (Büyükşimşek & Başlamışlı, 2020). In contrast, a study by Caroline et al. conducted in Bali, reported higher average vitamin D levels of 25,96 ng/mL in the thalassemia group and 27,54 ng/mL in the non-thalassemia group, with no significant difference between the two groups ($p = 0,45$) (Caroline et al., 2021). Our findings revealed a significant prevalence of vitamin D deficiency and insufficiency (70%) among beta-thalassemia major patients, aligning with previous research by Fadilah et al. which reported vitamin D deficiency in 85,5% of children with beta-thalassemia major at Hasan Sadikin Public Hospital (Fadilah et al., 2016).

The etiology of vitamin D deficiency in thalassemia is thought to be due to increased iron concentration in the liver as a result of repeated transfusions. Iron accumulation disrupts the activity of 25-hydroxylase in the liver, which is essential for the hydroxylation of vitamin D to 25-hydroxy-vitamin D. Hyperpigmentation is also common in patients with thalassemia. Impaired vitamin D absorption is another contributing factor to the deficiency and insufficiency in up to 90% of patients with thalassemia major and intermedia. This finding suggests that exposure to sunlight alone is insufficient to synthesize adequate vitamin D in children with thalassemia. Vitamin D deficiency is generally rare in children who live in countries with abundant exposure to sunlight (Singh et al., 2012).

Our study identified hypocalcemia in 31 subjects (77,5%), with an average calcium level of 8,87 ± 0,366 mg/dL. This aligns with the study by Manzoor et al., which found that 43 (53,8%) children with beta-thalassemia major had hypocalcemia (Manzoor et al., 2022). Similarly, Yu et al. reported an average calcium level of 8,61 ± 1,2 mg/dL (Yu et al., 2019). Karim et al. also found that the average calcium level in children with beta-thalassemia major was 7,9 ± 0,6 mg/dL, significantly lower than that of healthy children (8,5 ± 1,1 mg/dL, $p < 0,05$) (Karim et al., 2016).

Vitamin D deficiency was more prevalent among male subjects (50%) than among females (38,9%). The highest prevalence of vitamin D deficiency was observed in the child age group (53,3%) and among those with a good nutritional status (50%). Subjects with hepatomegaly or splenomegaly (50%), severe anemia (60%), ferritin levels <2000 ng/mL (58,3%), and those consuming DFP (52,2%) also demonstrated a high prevalence of vitamin D deficiency in Table 2.

Among the male subjects in our study, 50% had vitamin D deficiency and 22,7% had vitamin D insufficiency. Among the female subjects, 38.9% had vitamin D deficiency and 27,8% had insufficiency. This finding is consistent with the study by (AlQuaiz et al., 2018), which found that 89% of male and 83% of female subjects experienced vitamin D deficiency (Nasir et al., 2018). Similarly, Fadilah, et al. reported that vitamin D deficiency affects 55% of male and 54% of female children with beta-thalassemia major. These findings indicate no significant gender differences in vitamin D status among children with beta-thalassemia major (Fadilah et al., 2016).

Table 2. Distribution of vitamin D status by subject baseline characteristics

Basic Characteristics	Vitamin D status					
	Deficiency (n= 18)		Insufficiency (n= 10)		Normal (n= 12)	
	n	%	n	%	n	%
Gender						
Male	11	50	5	22,7	6	27,3
Female	7	38,9	5	27,8	6	33,3
Age Group						
Toddler	3	50	1	16,7	2	33,3
Child	8	53,3	3	20	4	26,6
Adolescent	7	36,8	6	31,5	6	31,5
Nutritional status						
Mild malnutrition	6	37,5	5	31,3	5	31,3
Good Nutrition	12	50	5	20,8	7	29,2
Liver						
Normal	8	40	5	25	7	35
Hepatomegaly	10	50	5	25	5	25
Spleen						
Normal	8	40	5	25	7	35
Splenomegaly	10	50	5	25	5	25
Anemia						
Mild	3	42,8	2	28,6	2	28,6
Moderate	9	39,1	6	26,1	8	34,8
Severe	6	60	2	20	2	20
Ferritin						
< 2000	7	58,3	2	16,7	3	25
≥ 2000	11	39,3	8	28,6	9	32,1
Iron chelation						
DFX	6	35,3	6	35,3	5	29,4
DFP	12	52,2	4	17,4	7	30,4

In our study, 53,3% of subjects over 5 years of age had vitamin D deficiency (53,3%), making this the largest affected age group. This finding is consistent with a study by Yu et al., which showed that the average serum vitamin D concentration was significantly higher in toddlers than in older children. Additionally, the mean serum vitamin D levels remained within the normal range in younger children but fell below the normal range in older children. No significant age-related differences were observed in this study in children with beta-thalassemia major (Yu et al., 2019).

A good nutritional status was more commonly observed among children with vitamin D deficiency in our study (50%). This is consistent with the findings of Ridha et al., who reported that 23 subjects (76,6%) with a good nutritional status also had vitamin D deficiency. However, these results differ from those of other studies that indicate that inadequate food intake is prevalent among children with thalassemia. Poor nutritional support can lead to suboptimal growth and development, reduced immune function, and a lower quality of life for children with thalassemia (Ridha et al., 2022).

Our study results indicate that 50% subjects of children with beta-thalassemia major and clinical hepatomegaly had vitamin D deficiency, while 40% of subjects without hepatomegaly also exhibited vitamin D deficiency. Yu et al. in their study, demonstrated that excess iron in the liver, characterized by hepatomegaly, can be associated with low serum vitamin D concentrations, which can indicate vitamin D deficiency and alterations in bone metabolism (Yu et al., 2019). Iron accumulation has also been linked to higher ALT concentration, a sensitive marker of liver damage. Patients with thalassemia progressively develop iron overload and deficiency in the hydroxylation of vitamin D in the liver. Iron accumulation in the liver and skin of beta-thalassemia major patients disrupts the hydroxylation and synthesis of vitamin D,

resulting in most patients experiencing vitamin D deficiency (Angastiniotis et al., 2021).

Vitamin D deficiency was also observed in 50% of subjects with splenomegaly, while 25% exhibited vitamin D insufficiency. Among subjects without splenomegaly, only 35% had a normal vitamin D status. This finding aligns with that of Abdelmotaleb et al., who reported that 65% of subjects with beta-thalassemia major experienced splenomegaly, and 75% of subjects were associated with vitamin D deficiency. Long-term ineffective erythropoiesis can lead to splenomegaly (Abdelmotaleb et al., 2021). It is important to note that inadequate blood transfusion may cause splenomegaly. This condition can be mitigated by providing adequate blood transfusions and intensive iron chelation therapy over several months (Kothimira et al., 2020).

Our study also found that among subjects with ferritin levels ≥2000 ng/mL, 39,3% had vitamin D deficiency and 28,6% had vitamin D insufficiency. These proportions were higher than those with a normal vitamin D status (32,1%). This result is consistent with the findings of Pala et al., who observed that 13% of children with beta-thalassemia major and ferritin levels >2500 ng/mL had vitamin D deficiency, while 31,4% had vitamin D insufficiency, accounting for a total of 44,3%. In contrast, 55,7% of subjects of children in the same ferritin group had normal vitamin D status (Pala et al., 2023). Similarly, Fadilah et al. reported that 94% of subjects had serum ferritin levels >1,000 ng/mL, and 86% had vitamin D deficiency.(Fadilah et al., 2016)

Several studies have reported an association between increased ferritin levels and decreased vitamin D concentrations. High ferritin levels in thalassemia patients can lead to the failure of sexual development, growth defects, and skin pigmentation. Most thalassemia patients at RSUDZA exhibit black or gray skin, indicating pigmentation due to iron accumulation. This condition interferes with endogenous vitamin D synthesis in the epidermis, where vitamin D3 (cholecalciferol) is synthesized from 7-dehydrocholesterol under sunlight and UVB radiation. Furthermore, ferritin accumulation in the liver, kidneys, and parathyroid glands adversely affects the vitamin D metabolism. Excessive iron in the liver disrupts the hydroxylation of vitamin D.21 The hydroxylation process, wherein cholecalciferol (D3) is converted to 25-hydroxycholecalciferol {25(OH)D} in the liver and subsequently to the active form, calcitriol, in the kidneys, is impaired due to disruption of the catalytic activity of the microsomal enzyme vitamin D 25-hydroxylase produced by hepatocytes (Fadilah et al., 2016).

In this study, 81,8% of the male subjects and 72,2% of the female subjects exhibited hypocalcemia. These results are consistent with research by Nama et al. which showed no significant gender-based differences in calcium status among children with beta-thalassemia major (p= 0,472).(Ali Rahman Nama et al., 2023) This finding is also in line with the research of Waheeb et al. which revealed no significant difference in vitamin D levels between females (15,6 ng/mL) and males (15,5 ng/mL). Similarly, no significant differences in calcium levels were observed between genders (Qasim & Aziz, 2019).

Our findings revealed that 86,6% of children, 78,9% of adolescents, and 50% of toddlers with β-thalassemia major exhibited hypocalcemia. This finding aligns with the Bulgurcu et al. study, which reported hypocalcemia in 17,2% of cases and found no significant differences across age groups.(Cevher Bulğurcu et al., 2021) However, this result contrasts slightly with Ansaf et al. who observed that 55,7% of hypocalcemic children with beta-thalassemia major were adolescents. This association is linked to parathyroid hormone levels, which decrease with age, thereby contributing to hypocalcemia (Ansaf et al., 2024).

Among the subjects with clinical hepatomegaly and splenomegaly, 80% were found to have hypocalcemia. This differs from the study by Aslamy et al. (2023) which reported that 100% of thalassemia children with a normal calcium status had hepatosplenomegaly (64%) or splenomegaly (100%). Currently, limited research has directly linked hepatomegaly and splenomegaly to hypocalcemia. Hepatomegaly in beta-thalassemia patients may result from extramedullary hematopoiesis and excessive iron accumulation, while splenomegaly is caused by splenic hyperplasia as a compensatory response to increased erythrocyte destruction, leading to chronic hemolytic anemia.

Hypocalcemia was more common in males (81,8%) compared to females (72,2%). The highest prevalence of hypocalcemia was observed in the child age group (86,6%), among those with good

nutritional status (79,2%), hepatomegaly (80%), splenomegaly (80%), severe anemia (80%), ferritin levels ≥2000 ng/mL (22 subjects, 78,5%), and in subjects consuming DFP (78,2%) (Table 3).

Table 3. Distribution of subject baseline characteristics by calcium status

Basic Characteristics	Calcium Status			
	Hypocalcemia (n=31)		Normal (n=9)	
	n	%	n	%
Gender				
Male	18	81,8	4	18,2
Female	13	72,2	5	27,8
Age group				
Toddler	3	50,0	3	50
Child	13	86,6	2	13,3
Adolescent	15	78,9	4	21,1
Nutritional status				
Mild malnutrition	12	75	4	25
Good Nutrition	19	79,1	5	20,9
Liver				
Normal	15	75	5	25
Hepatomegaly	16	80	4	20
Spleen				
Normal	15	75	5	25
Splenomegaly	16	80	4	20
Anemia				
Mild	5	71,4	2	28,6
Moderate	18	78,3	5	21,7
Severe	8	80	2	20
Ferritin				
< 2000	9	75	3	25
≥ 2000	22	78,5	6	21,4
Iron Chelation				
DFX	13	76,5	4	23,5
DFP	18	78,2	5	21,7

Table 4. Relationship between vitamin D status and calcium status in children with beta thalassemia major

Vitamin D Status	Calcium Status				p-value	Rs
	Hypocalcemia (n=31)		Normal (n=9)			
	n	%	n	%		
Deficiency	17	94,4	1	5,6		
Insufficiency	8	80,0	2	20,0	0,017	0,377
Normal	6	50,0	6	50.0		

Note : Rs = Rank Spearman

The results in Table 4 indicate that participants with vitamin D deficiency were more likely to experience hypocalcemia (94,4%) than those with vitamin D insufficiency (80%) or normal vitamin D levels (50%). Conversely, a normal calcium status was more prevalent among subjects with normal vitamin D levels (50%) than among those with insufficiency (20%) or deficiency (5,6%). Statistical analysis revealed a significant relationship between vitamin D and calcium status in children with β-thalassemia major (p=0,017). Spearman's correlation coefficient indicated a weak but positive relationship (r=0,037), suggesting that lower vitamin D levels are associated with lower calcium levels.

These findings were statistically significant (p = 0,017), indicating a true association between vitamin D deficiency and hypocalcemia among children with beta-

thalassemia major. Clinically, this underscores the critical need for routine screening and correction of vitamin D deficiency in thalassemia patients, as inadequate vitamin D levels may contribute substantially to disturbances in calcium homeostasis and subsequent bone metabolism complications.

In this study, 94,4% of the subjects with hypocalcemia had vitamin D deficiency, 80% had vitamin D insufficiency, and 50% had normal vitamin D status. Using the Spearman test, a significant correlation was identified between vitamin D level and calcium status ($p = 0,017$, $r=0,377$), indicating a positive but weak relationship. Lower vitamin D levels correspond to lower calcium levels. Average calcium levels among children with vitamin D deficiency, insufficiency, and normal status were 8,76 ± 0,31 mg/dL, 8,79 ± 0,39 mg/dL, and 9,1 ± 0.34 mg/dL, respectively. Similarly, Jabbar et al. observed that 100% of children with hypocalcemia had vitamin D deficiency, while normal calcium levels were more frequent among those with normal vitamin D status.(Jabbar et al., 2023) This trend supports the findings of Shah et al. which identified a correlation between vitamin D synthesis and calcium status in beta-thalassemia major patients (Shah, 2015).

Geographic, age-related, and socioeconomic factors, including inadequate sun exposure and dietary vitamin D and calcium intakes, influence vitamin D deficiency and hypocalcemia. In Aceh Province, cultural practices such as conservative dress and sun avoidance exacerbate these issues, compounded by limited food fortification policies. In pediatric thalassemia patients, liver dysfunction from ferritin accumulation further impairs vitamin D hydroxylation (Mustafa & Shekhar, 2021).

In the gastrointestinal tract, calcitriol, the active form of vitamin D, increases the active absorption of calcium by stimulating the synthesis of calcium-binding and phosphorus-binding proteins in the small intestinal mucosa. In bones, calcitriol, together with parathyroid hormone, stimulates calcium release from the bone surface into the blood. Calcitriol stimulates calcium and phosphorus reabsorption in the kidneys. Vitamin D is involved in calcium metabolism, where serum vitamin D levels <30 ng/mL are associated with a significant decrease in intestinal calcium absorption.(Jabbar et al., 2023; Soliman, 2013) Without vitamin D, only 10-15% of dietary calcium is absorbed.(Zoga et al., 2014) Studies by Zoga et al. and Hamayun et al. have demonstrated significant positive correlations between vitamin D and calcium levels in thalassemia patients (Hamayun, 2024; Zoga et al., 2014). Low ionized calcium levels in vitamin D deficiency stimulate parathyroid hormone (PTH) secretion, increasing renal calcium reabsorption and vitamin D synthesis (Abbassy et al., 2019; Gaudio et al., 2019).

Vitamin D influences bone mineralization directly through genomic mechanisms via the VDR, and indirectly by stimulating the absorption of calcium and phosphorus in the intestine. Vitamin D is a critical factor in calcium and bone metabolism and, together with calcium, plays an essential role in bone development and maintenance (Saboor, 2014). Previous studies have reported that patients with thalassemia undergoing multiple blood transfusions exhibit a significant decrease in vitamin D levels of approximately 90%. In these patients, increased iron absorption significantly impaired calcium absorption. However, several factors beyond vitamin D deficiency can contribute to hypocalcemia in thalassemia patients, including hypoparathyroidism, inadequate vitamin D and calcium intake, impaired absorption of these nutrients, and iron overload (De Sanctis et al., 2018). Nevertheless, vitamin D deficiency remains the primary cause of bone disease in patients with beta-thalassemia major, which is further exacerbated by reduced outdoor activity due to anemia and bone damage.

Research by Abbasy et al. found no significant difference in serum vitamin D levels between pediatric beta thalassemia major patients and controls, likely because thalassemia patients received vitamin D supplementation as part of their routine treatment (Abbassy et al., 2019). In their study, none of the subjects had vitamin D deficiency, 22,5% had vitamin D insufficiency, and 77,5% had normal vitamin D levels.

Overall, the findings of this study demonstrate a relationship between vitamin D and calcium status in children with beta-thalassemia major. Children with vitamin D

deficiency were more likely to experience hypocalcemia (94,4%) than those with normal vitamin D status (50%). Statistical analysis revealed a significant positive correlation between vitamin D and calcium levels. These findings are expected to guide appropriate management strategies, including optimizing blood transfusions, iron chelation therapy, and vitamin D and calcium supplementation, for children with beta-thalassemia major. These measures can assist clinicians in making timely and informed decisions to prevent further complications.

Conclusion

This study concluded that children with β-thalassemia major were predominantly male, adolescents, well-nourished, and commonly presented with hepatomegaly, splenomegaly, moderate anemia, ferritin levels >2000 ng/mL, vitamin D deficiency, insufficiency, and hypocalcemia. A statistically significant positive correlation was observed between vitamin D and calcium levels, indicating that children with vitamin D deficiency were more likely to have hypocalcemia.

These findings highlight the clinical importance of monitoring vitamin D levels in children with thalassemia, as deficiency may exacerbate calcium imbalance and lead to complications in bone metabolism. Routine screening for vitamin D and calcium status is recommended for children with beta-thalassemia major. Where deficiency is found, vitamin D and calcium supplementation should be initiated as part of the standard care to prevent long-term skeletal and endocrine complications.

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