

ORIGINAL ARTICLE

Evaluation of Calcium and Vitamin D Levels in Serum in A Sample of Thalassemia Patients from Baghdad

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ABSTRACT

Background: Beta thalassemia patients require lifelong blood transfusions that can lead to iron accumulation in tissues and a decreased vitamin D synthesis. This study determined vitamin D and calcium levels in thalassemia patients in Baghdad, Iraq.

Methods: In 2023, 100 adult patients of 15-25 years of both sexes who were regularly diagnosed with thalassemia in Center for Genetic Hematology at Ibn al-Baladi Hospital in Rusafa- Baghdad, Iraq were enrolled. Serum 25-OH-vitamin D and calcium were levels were assessed. Totally, 50 blood samples were collected from thalassemia patients together with 50 blood samples from healthy people as control for comparison.

Results: Vitamin D level was low in thalassemia patients. There was a significant difference in the decrease in vitamin D level between men and women ($p=0.008$). Females had a significantly higher calcium deficiency than males. The serum calcium level was low, and no significant difference was noticed between sexes ($p=0.28$). When comparing thalassemia rate, a significant difference was visible in vitamin D level ($p=0.002$). For calcium level, the difference between two groups was significant ($p=0.01$).

Conclusion: A significant difference was observed for vitamin D and calcium deficiency among thalassemia patients of both sexes when compared to healthy people.

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Introduction

Thalassemia and genetic hemoglobin disorders are common diseases enrolling about 5% of the global population who carry mutations in genes that regulate hemoglobin production (1, 2). Thalassemia is a heterogeneous inherited disorder of hemoglobin synthesis due to mutations of the globin gene, leading to ineffective erythropoiesis and anemia (1). Thalassemia as one of the most prominent hereditary blood disorders impairs the production of essential proteins of alpha and beta globin, which

are crucial in formation of functional hemoglobin. This can lead to an ineffective oxygen transport and cause chronic anemia from early childhood as a condition persisting throughout life (3, 4).

Thalassemia is categorized into various types based on the affected gene and the nature of the defect. Alpha thalassemia and beta thalassemia are the most common and clinically significant types. The disorder results from decreased synthesis of globin chains, distinguishing it from hemoglobinopathies that involve structural abnormalities in hemoglobin.

Beta-thalassemia occurs due to mutations in two genes located on chromosome 11, while alpha thalassemia arises from defects in four genes on chromosome 16 (5, 6). Beta-thalassemia is generally more severe and widespread, particularly in regions like the Mediterranean, the Middle East, and parts of Asia, where a high prevalence is observed. The disease spreads through migration and intermarriage within populations. In Iraq, for example, the prevalence of thalassemia is high, with approximately 12,000 patients reported by 2003, primarily due to consanguineous marriages and limited healthcare resources (7).

The disease manifests with severe anemia, often beginning between the ages of 3 to 6 months, accompanied by complications such as hepatosplenomegaly (enlarged liver and spleen), bone deformities, and growth retardation (8). Recurrent blood transfusions, essential for survival, often lead to iron overload, which deposits in organs such as the heart, liver, and endocrine glands (9), causing further complications, including heart failure, liver dysfunction, and endocrine disorders like hypoparathyroidism and growth failure. The excess iron also impairs calcium metabolism, leading to osteopenia and osteoporosis (10).

Diagnosing thalassemia involves a combination of clinical examination, patient history, and laboratory testing (11). In minor forms, patients may exhibit mild symptoms like slight pallor, while more severe forms, like beta-thalassemia major, cause significant symptoms such as characteristic facial bone deformities, delayed growth, and liver or spleen enlargement (8). Laboratory tests are critical for confirming the diagnosis, including blood tests for anemia and genetic testing to identify specific mutations (12).

Prevention of thalassemia includes several strategies, such as prenatal and pre-marital genetic testing, public health measures to discourage consanguineous marriages and embryo screening through genetic testing to reduce the transmission of severe forms of the disease (5).

Treatment for thalassemia revolves around managing anemia through regular blood transfusions and addressing iron overload with chelation therapy, such as the drug Exjade, which is often preferred over the painful Desferal injections. The disease disproportionately affects populations in lower-income regions, where access to medical care and expensive treatment options is limited, exacerbating the burden on affected families (7). Vitamin D is a fat-soluble vitamin derived from cholesterol and stored in body fat. Vitamin D deficiency is also a common complication in thalassemia patients, often due to

iron overload and liver dysfunction. Vitamin D is essential for calcium mineralization and homeostasis of the skeleton especially during infantile and pubertal growth periods which are periods of rapid growth. This deficiency, if left untreated, contributes to bone diseases like osteoporosis, further complicating the clinical management of the disease (13-15).

As thalassemia remains a global health challenge, particularly in areas with high rates of consanguineous marriages and limited access to healthcare; continued researches and advances in genetic therapies offer hope for improved management and potentially curative approaches in the future. Therefore, this study was undertaken to determine the vitamin D and calcium levels in thalassemia patients in Baghdad, Iraq.

Materials and Methods

In order to determine vitamin D and calcium levels in thalassemia patients, 50 blood samples were collected from thalassemia patients of both genders at Ibn Al- Baladi Center for Thalassemia Diseases. From each subject, 5 mL of blood were obtained by vein puncture using 5 mL disposable syringes. The liquid blood was dispensed in a plain tube and left for around an hour to clot at room temperature (22°C) and then separated by centrifugation at 3000 rpm for 10 minutes to collect serum to be used to assay vitamin D3 and calcium levels.

The remaining serum was divided into aliquots (250 micron) in Eppendorf tubes and stored in the freezer (-20°C) until use. In addition, 50 blood samples were provided from healthy people and considered as control group to be compared with the patients. To assess the serum level of Vit.D3, the samples were read using the ichroma™ II Advanced Compact Immuno-Analyzer/ made in India. The kits were used to measure the concentration of vitamin D (Boditech, ichroma vitamin D Neo, Korea).

To evaluate the calcium level, the samples were read using the semi-automated to be read at 610 nm (Mindray BA-88A, China). The kits were used to measure the concentration of calcium ions (Biosystems, made in Barcelona, Spain). For statistical analysis, SPSS software (Version 22, Chicago, IL, USA) was employed. For analysis, t-test was used to determine if there was a significant difference between the means of two groups.

Results

Table 1 shows a statistically significant difference ($p=0.008$) in calcium level between thalassemia patients by Gender revealing calcium deficiency in thalassemia patients. Females have a statistically significantly higher calcium deficiency than males.

Table 1: The difference in serum calcium level between thalassemia patients by gender.

Calcium (mg/dL)	Gender	
	Male	Female
Mean±SD	9.0±1.940	8.0±1.74
T test		3.340
P value		0.0086

Table 2: The difference in vitamin D level between thalassemia patients by gender.

Vitamin D (ng/mL)	Gender	
	Male	Female
Mean±SD	22.7±11.77	12.9±6.73
T test		1.34
P value		0.282

Table 3: Serum vitamin D level between thalassemia patients and the control group.

Vitamin D (ng/mL)	Group	
	Control	Patient
Mean±SD	46.89±7.83	18.16±5.88
T test		3.47
P value		0.002

Table 4: The difference in serum calcium level between thalassemia patients and control group.

Calcium (mg/dL)	Group	
	Control	Patient
Mean±SD	8.86±0.64	7.41±1.74
T test		2.68
P value		0.01

Hypocalcemia was shown to be a common finding in thalassemia patients. Table 2 shows the data of thalassemia patients without any significant difference regarding vitamin D level by gender.

Table 3 presents the level of vitamin D as 18.16±5.88 ng/mL in thalassemia patients, whereas in control group, it was 46.89±7.83 ng/mL and the difference was statistically significant ($p=0.002$) between two groups. Table 4 demonstrates the mean serum calcium level in thalassemia patients (7.41±1.74) in comparison to those of control group (8.86±0.64), while a significant difference was seen between control and thalassemia patients ($p=0.01$).

Discussion

A high prevalence of vitamin D deficiency as well as calcium deficiency was found in our study among thalassemia patients. There was a significant difference in vitamin D deficiency between thalassemia patients and healthy people.

When measuring calcium level, a significant decrease was found in thalassemia patients when compared to healthy people. When comparing calcium level between men and women, there was a significant difference between both genders among

thalassemia patients and the decrease was greater in women than men. So based on high prevalence of vitamin D and calcium deficiencies in thalassemia patients, frequent monitoring and appropriate therapeutic interventions are recommended to reach normal levels of vitamin D and calcium and to improve bone health and quality of life for these patients.

It was shown that vitamin D deficiency was high in thalassemia patients in some countries. Merchant *et al.* reported vitamin D deficiency in 62% of children in India (16). Gomber *et al.* found that serum vitamin D level was significantly low in children with thalassemia (17). Fujiazzi *et al.* demonstrated that 12% of thalassemia patients were vitamin D deficient and 69.8% suffered from low levels of vitamin D (18). The findings indicate that exposure to sunlight alone is not sufficient for vitamin D synthesis in children with thalassemia (19).

There are many factors such as low intake, skin anatomy, and poor absorption in people that make them susceptible to the deficiency (20-24). Our findings are also consistent with these previous studies who found that vitamin D deficiency occurred in patients with iron overload as thalassemia patients

are exposed to continuous blood transfusion that can lead to iron accumulation and a deficiency of both calcium and vitamin D (25). This study showed a higher prevalence of vitamin D deficiency and lower total blood calcium levels in thalassemia patients with a significantly lower prevalence than the healthy group. Hypocalcemia and vitamin D deficiency may be the result of the negative effect of frequent blood transfusions and malnutrition (26-28). There was other research similar to our result that was conducted in Baghdad in 2023. This study revealed a high prevalence (77%) of vitamin D deficiency in thalassemia patients who received multiple blood transfusions (29).

Conclusion

Vitamin D and calcium deficiency were prevalent among thalassemia patients and were more among females. So frequent monitoring and appropriate therapeutic interventions required to maintain normal levels of vitamin D and calcium in the blood to improve bone health and quality of life for these patients.

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Authors' Contribution

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Conflict of Interest

The authors declare no conflict of interest.

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