Assessment of Vitamin D Levels in Patients with Beta Thalassemia Major

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Abstract: Objective: The aim of this study was to assess the status of vitamin-D in beta-thalassemia major (TM) patients as a probable and correctable factor for thalassemia bone disease. **Methods:** This case-control study took place in the El Hussein University Hospital, Cairo, Egypt, between March and December 2016. Forty Egyptian patients with TM (aged 11.9 ± 5) were compared with 20 sex- and age-matched healthy subjects serving as the control group. All patients were prescribed oral calcium (1 gm) and native vitamin-D (1,000 IU) as a combined daily supplementation for more than 2 years. Serum ferritin, ionized calcium, and 25-OH-vitamin-D were estimated for all patients and controls. **Results:** Vitamin-D levels in the patient group were significantly higher than for controls (p=0.0227), and were significantly negatively correlated with ferritin and age (p= <0.001, p=0.0028, respectively). Serum ionized calcium levels in patients were significantly lower than for controls (p=<0.001) and had a significant negative correlation with age (p =0.0042). In the patient group, the prevalence of hypocalcaemia was 87.5%. **Conclusion:** Although significant improvement occurred in the 25(OH)-vitamin-D levels of TM patients due to their daily supplementations with native vitamin-D (cholecalciferol), vitamin-D deficiency was still prevalent in older patients and in patients with high ferritin levels. In addition, TM patients still suffered from significant highly prevalent hypocalcaemia, which becomes worse with age. Further studies are required to assess the need for activated vitamin-D analogues.

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1. Introduction

Beta-thalassemia is one of the most common autosomal recessive disorders worldwide.[1] It results from a defect in beta-globin chain production and ranges from clinically silent heterogeneous thalassemia minor to severe transfusion-dependent thalassemia major.[2]

The current management of TM with regular blood transfusions and iron chelation therapy has successfully, significantly improved the life expectancy of patients. However, thalassemia bone disease has emerged as a major challenge and represents a prominent cause of morbidity in both genders.[3]

Thalassemic patients suffer from several bone disorders, including bone deformities, bone pain, rickets, scoliosis, pathologic fracture, osteopenia, and osteoporosis.[3,4] The estimated prevalence of osteoporosis even in well-treated TM patients ranges from 13.6% to 50%, with an additional 45% affected by osteopenia.[4]

The etiology of thalassemia bone disease is very complicated and multifactorial; factors including hormonal deficiency, especially hypogonadism; marrow expansion; iron overload; desferrioxamine toxicity; and calcium/vitamin D deficiency are suspected. All these factors have been described in TM, and all these factors have a serious impact on bone metabolism.[5,6]

Native (plain or natural) vitamin D, either synthetized in the skin or absorbed from the digestive tract, is firstly hydroxylated in the liver to become 25-OH-vitamin-D3, which represents the major circulating form.[7] Regulated by parathormone (PTH), 25-OH-vitamin-D3 is submitted to a second hydroxylation step in the kidney to form 1,25$(OH)_2$ vitamin D3 (calcitriol), which represents activated vitamin D.[7,8]

25-OH vitamin D3 is considered the best indicator of vitamin D status in the body because it is considered stable, having a half-life of two weeks, and has the highest concentration in relation to other vitamin D metabolites.[9]

The principal effect of vitamin D on skeletal health is indirect and mainly through enhanced intestinal calcium absorption making this ion available for bone mineralization.[10] Optimal intestinal calcium absorption is essential for protection of bone and prevention of osteoporosis.[11] Peak bone mass is usually achieved by age 30; maintaining sufficient levels of calcium and vitamin D during adolescence and early adulthood accompanied with physical activity can ensure adequate peak bone mass development.[12]

Also, vitamin D has a significant role in balance control and fall reduction.[13,14] This can be explained by the positive effect of $1,25(OH)_2$ vitamin D on muscle tissue through de novo protein synthesis and muscle cell proliferation and differentiation, leading to subsequent improvement in muscle strength and better balance control.[13,15] Also, the presence of vitamin D receptor (VDR) in some parts of the CNS, such as the cortex, subcortex and spinal motor zones, might indicate that vitamin D influences postural balance through direct effect on the CNS.[13]

Lastly, there is evidence that $1,25(OH)_2$ vitamin D can also drive a positive direct effect on mature osteoblasts with sufficient calcium supplementation.[10] Transgenic mice over expressing the VDR in osteoblastic demonstrated increased bone formation.[10,15] Also, an activated vitamin D analogue, eldecalcitol, appeared to inhibit osteoclastic activity and therefore prevented bone resorption.[14,16]

Vitamin D deficiency[17,18] and hypocalcaemia[19,20,21] have been reported to be highly prevalent in TM patients, and it is thought that this may contribute to their thalassemia bone disease.[5,6,18] As a result, vitamin D and Ca supplementations are routinely prescribed by several centers. However, there are few available studies about the effectiveness of these supplementations.

The current study assesses vitamin D status in TM patients after more than two years of daily supplementations by comparing the patients with age- and sex-matched healthy subjects.

2. Subjects and Methods

This case-control study took place in the El Hussein University Hospital, Cairo, Egypt, between March and December 2016. The study was conducted on 60 Egyptian subjects: 40 patients with TM as the patient group and 20 age- and sex-matched healthy subjects as the control group. The patient group include 22 males (55%) and 18 females (45%) with a mean age of 11.9 ± 5 years. All of the patients were on regular blood transfusions and iron chelation therapy. Of these patients, 42.5% had splenomegaly and 57.5% had undergone splenectomy. All patients were prescribed oral calcium (1 gm), and vitamin D (1,000 IU) as a combined daily supplementation for a duration of more than 2 years before the study began. The control group included 10 males (50%) and 10 females (50%) with a mean age of 12.7 ± 4.6. None of the controls received any supplementations.

The current study was approved by the Medical Research Ethical Committee of the Faculty of Medicine, Al-Azhar University. This study took the registration code: Cli-Path med._14Med. Research_Thalassemia. Major._0000014. All subjects were verbally informed about the procedures and the aim of the study, and an informed written consent was obtained from all adult participants and from the parents of minors.

The following laboratory tests were carried out for both groups:

1) Ferritin and 25-(OH)-Vitamin-D

Method: The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection) ELFA). Normal range for ferritin is: 20-280 ng/ml for males and 10-140 ng/ml for females. Concerning Vitamin D, levels < 20 ng/ml are considered deficient, while levels: 20-30ng/ml are considered insufficient.

2) Ionized calcium

Method: Ion selected electrode. Reference range: 4.5-5.2 (mg / dl).

Statistical Methods:

Independent t-test and Pearson correlation coefficient were used for the analysis of data; Microsoft Excel 2010 was used in drawing the correlation scatter graphs; p value of <0.05 was considered statistically significant.

3. Results

Compared to the control group, the patient group showed significantly higher levels of vitamin D and ferritin and significantly lower levels of ionized calcium **[Table 1].** The prevalence of Vitamin D deficiency was 40% in the patient group and 70% in the control group. The prevalence of Vitamin D insufficiency was 72.5% in the patient group and 100% in the controls. All the controls were normocalcemic; in contrast, 87.5% of the patient group was hypocalcemic.

Table1. Comparison between patients and controls groups.

	Patients group N=40	Control group N=20	P value
Age	11.9± 5	12.7± 4.6	0.5515
Vitamin D (ng/ml)	22,3± 10.9	16.3± 5.6	0.0245
Ionized Ca (mg / dl)	4.18±.35	4.66±.13	<0.001
Ferritin (ng/ml)	2804.6 ±1849.26	41.76±23.74	<0.001

In the patient group, analysis revealed a significant negative correlation between ferritin and vitamin D (r = −0.52, p = <0.001) **[Figure 1],** and a significant negative correlation between age and Vitamin D (r = −0.46, p = 0.0028) **[Figure 2].** The analysis also revealed a significant negative correlation between age and ionized calcium (r = −0.44, p = 0.0042) **[Figure 3].**

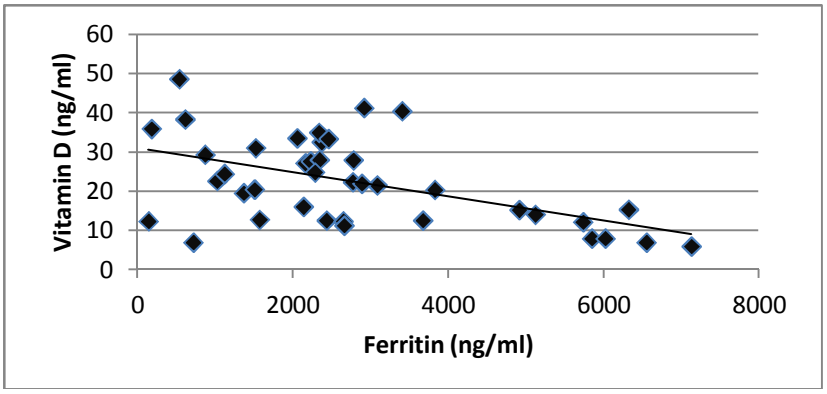

Figure 1. Ferritin and Vitamin D Correlation

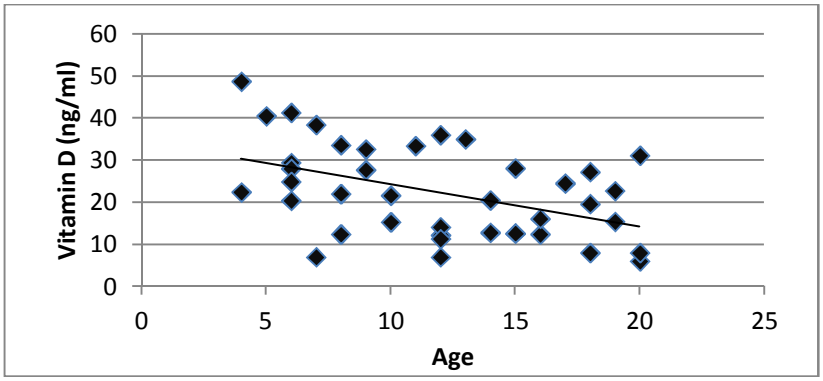

Figure 2. Age and Vitamin D Correlation

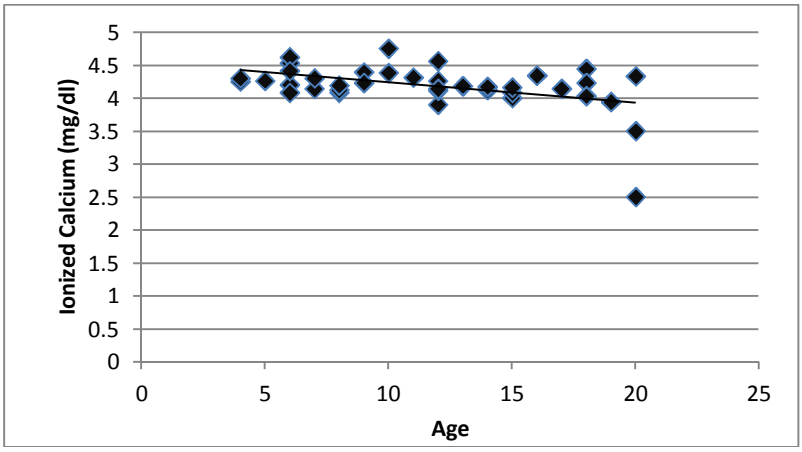

Figure 3. Age and Ionized Calcium Correlation.

4. Discussion

In contrast to *Fahim et al.*, *Pirinççioğlu et al.*, *and Agrawal et al.*,[22,23,24] the current study found that the level of 25-OH-vitamin-D was significantly higher in TM patients compared to the controls. This difference can be explained by the effect of the daily supplementations with vitamin D in the patient group; none of the controls received any supplementation.

In the current study, Vitamin D deficiency and insufficiency were observed in both groups, with a higher prevalence in the control group.

Concerning controls, this high percentage of vitamin D deficiency (70%) is nearly consistent with other broad studies that have reported the high prevalence of Vitamin D deficiency in the region of the current study.[25,26]The Middle Eastern and North African regions represent some of the highest rates of hypovitaminosis D worldwide. The prevalence of hypovitaminosis D in this region varies from 30–90%, considering a desirable 25-OH-vitamin-D level of 20 ng/ml.[26]These deficient levels might be attributed to decreased sun exposure (conservative dressing culture and/or avoidance of exposure to hot sunny weather).[27] In addition, food fortification policy is nearly absent in the majority of this region.[26] Another explanation might be related to the predisposition of the Arab population to 25-OH-vitamin-D3 deficiency as a result of certain intrinsic factors related to ethnicity.[25,27]

Concerning thalassemic patients, there are additional factors that might be implicated. Many authors, who have reported low vitamin D concentrations in thalassemia patients, attributed their results to hepatic dysfunction leading to defective hydroxylation of vitamin D and decreased serum levels.[22] In agree with Napoli et al., the current study found a significant negative correlation between vitamin D and ferritin and a significant negative correlation between vitamin D and age.[17]It is suspected that these two factors (ferritin, age), have a critical effect on hepatic hydroxylation, and hence vitamin D levels.

In the current study however, the significantly improved vitamin D levels were not associated with a parallel improvement in Ca levels; there was still significant, highly prevalent hypocalcaemia in the patient group, which becomes worse with age.

Hypocalcemia in TM patients is mainly attributed to hypoparathyrodism.[28]In the kidney, PTH normally stimulates the tubular reabsorption of calcium. Also, PTH normally stimulates the final renal hydroxylation step required for synthesis of activated vitamin D (1,25-$(OH)_2$ D). From this aspect, hypoparathyroidism can be described as a two-hormone deficiency state, i.e. parathormone deficiency plus 1, 25-$(OH)_2$ vitamin D deficiency.[29]

Several studieshave reported significantly reduced levels of PTH in patients with beta-thalassemia major.[30,31,21] Also, *Mohey El-Deen et al.*, observed that PTH levels were significantly lower in patients ≥6 years old compared with patients <6 years old, which could be explained by more blood transfusions and a subsequently greater iron overload.[32] However, PTH levels were not evaluated in the current study.

Unfortunately, few published studies have evaluated the effects of vitamin D supplementation on TM patients. *Fung et al.*'s study observed that vitamin D deficiency was still widespread among TM patients despite daily doses of 400–1,000 IU vitamin D, but there was significant improvement in vitamin D status after using higher doses (50,000 IU) of oral vitamin D2 every 3 weeks.[33] However, thalassemia bone disease is a very complicated disease; even if higher doses of supplementation successfully improved vitamin D levels and decreased the prevalence of the deficiency, failure to improve Ca levels is still probable, as occurred in the current study.

The lack of previous studies makes it difficult to confirm whether the native forms of vitamin D supplementations, with either ordinary routine doses of 400–1,000 IU or higher doses, are sufficient or if activated forms are necessary. However, several factors should be considered.

Although they cost more and have arisk of hypercalcemia,[34]activated vitamin D analogues, like calcitriol, need no further hydroxylation steps to be active and thereforewill not be affected by the decline of hepatic or renal functions.[35] Also, activated vitamin D analogues, such as calcitriol and eldecalcitol, appear to be associated with a positive impact on bone mineral density (BMD), successfully reduce the risk of fractures, and are approved for treatment of osteoporosis in Japan[14,16]; this is not the case for native forms.[16] Finally, the traditional therapy for hypoparathyroidism (if present) is the activated analogues, not native vitamin D.[29] All of these points are significantly important when dealing with thalassemia bone disease.

5. Conclusion

Although significant improvement occurred in the 25(OH)-vitamin D levels of TM patients due to their daily supplementations with native vitamin D (cholecalciferol), vitamin D deficiency was still prevalent in older patients and in patients with high ferritin levels. In addition, TM patients still suffered from significant highly prevalent hypocalcaemia, which becomes worse with age. Further studies are required to assess the need for activated vitamin D analogues with respect to calcium levels and bone mineral density outcomes. Further studies are also needed to assess the actual prevalence of vitamin D deficiency in the Egyptian populations.

Conflict of interest:

The authors declare no conflicts of interest.

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