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VITAMIN D, CALCIUM AND PHOSPHORUS LEVELS IN PATIENTS WITH β-THALASSEMIA MAJOR

Ade Hariza Harahap[1], Bidasari Lubis[2], Herman Hariman[1]

[1] Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/ Adam Malik Hospital Medan, Indonesia. E-mail: harnaz_dr@ymail.com
[2] Department of Pediatrics, Faculty of Medicine, University of North Sumatera/Adam Malik Hospital Medan, Indonesia.

ABSTRACT

There have been many reports that patients with β-thalassemia major have bone problems such as thinning of the bone, bone fragility and pathologic fractures. For so many years it was believed that the bone problems were mainly caused by bone marrow expansion due to its compensation to handle the chronic anemia and hypoxia in β-thalassemia major. Recently, there was evidence suggesting an occurrence of t hypocalcemia and hypovitaminosis D in β-thalassemia major. Therefore, this study aimed to clarify if hypovitaminosis D is truly the cause of the bone problem in thalassemia. 45 subjects were recruited in this study, 35 were β-thalassemia major patients and 10 were normal subjects as controls. 10 mL of venous blood was withdrawn from a median vein for measurement of total vitamin D [25(OH) vitamin D], total calcium and phosphorus levels using the Enzyme-Linked Fluorescent Assay (ELFA), metallochromic dye (Arsenazo III) and chemical reaction of inorganic phosphate with ammonium molybdate, respectively. Mean±SD of vitamin D in β-thalassemia major patients and control was 21.28±6.36 ng/mL and 34.85±3.50 ng/mL ($p<0.05$); total calcium in β-thalassemia major patients and control was 8.58±0.68 mg/dL and 9.22±0.35 mg/dL ($p<0.05$); and phosphor in β-thalassemia major patients and control was 3.98±0.53 mg/dL and 3.89±0.49 mg/dL ($p>0.1$), respectively. There was no significant correlation ($r = 0.17$, $p>0.05$) between vitamin D and calcium. This study demonstrated a state of hypovitaminosis D and hypocalcemia in β-thalassemia major but hypovitaminosis D was not the only causative factor of the decreased calcium levels. There should be another factor responsible for the decrease of calcium levels in β-thalassemia major and marrow expansion may remain the factors responsible for bone abnormalities.

Key words: Vitamin D, Calcium, phosphorus, β-thalassemia major

INTRODUCTION

Thalassemia patients will experience the most common disorders of the bones characterized by reduced bone mass, impaired bone architecture, increased risk of bone fragility and fractures, bone thinning as a result of bone marrow expansion due to ineffective erythropoiesis, impaired calcium and phosphorus metabolism, low PTH levels, level 1.25(OH)2 low D3 vitamin, growth hormone deficiency (GH/IGF-1), delayed puberty, hypogonadism, hypothyroidism, diabetes mellitus, increased osteoclast function and decreased function of osteoblasts, iron excess, and toxic effects from the treatment of desferrioxamine chelation.[1,2] Calcium and vitamin D deficiency are involved to cause a serious impact on bone metabolism alteration. Vitamin D deficiency is increasingly identified among β-thalassemia major patients.[1]

Hypoparathyroidism (HPT) is another endocrinopathy that is relatively common in thalassemia major. This condition is considered as a distribution factor for abnormal bone metabolism in thalassemia major. HPT is characterized by a decrease of serum calcium and an increase of serum phosphorus levels, followed by reduced levels of Parathyroid Hormone (PTH).[3] PTH and Vitamin D form a tightly controlled feedback cycle, with PTH being a major stimulator of vitamin D synthesis in the kidney while vitamin D exerts a negative feedback on PTH secretion. The major function of PTH and major physiologic regulator is to circulate ionized calcium. The effects of PTH on gut, kidney, and bone serve to maintain serum calcium within a tight range. PTH has a reciprocal effect on phosphate metabolism.[4]

The survival of thalassemia major patients is increasing with advances in therapy. However, osteoporosis and heart dysfunction still occur frequently. Vitamin D is very important for optimal bone health and reduction of the risk of fractures. It is correlated to bone disease including osteoporosis, rickets, scoliosis, fractures of spinal scar, and cardiac dysfunction. Vitamin D deficiency and insufficiency are reported to be high in thalassemia patients in

some countries despite the presence of good sunlight and routine prescription 400 – 1000 IU vitamin D per day. [5]

Osteopenia and severe osteoporosis are the main causes of morbidity in β-thalassemia major patients. The clinical manifestation of patients with β-thalassemia major is characterized by swelling and soft tissue pain in the area of ankle joints, and long bone fractures, especially those involving the femur because of high bone fragility. Bone abnormalities that are relatively common in β-thalassemia major patients include differences in the length of the limbs and the upper part due to a premature combination of epiphyseal lines, axial deviations of the limbs, osteochondrosis and short stature. Spinal involvement frequently occurs and potentially manifests as spinal abnormalities (such as scoliosis, kyphosis), vertebral fragility, cord compression or intervertebral disc degeneration.[6]

A study in Iran showed that patients with thalassemia major experienced 50.6% osteopenia and 27.3% osteoporosis in the lumbar region. Other data indicated that 42.09% of cases of osteopenia and 24.7% of those of osteoporosis occurred in the neck and femur regions. Also, thalassemia patients showed hypocalcemia, hyperphosphatemia, and deficiency to vitamin D.[7] In other studies, the overall prevalence of deformity was 12.1% with an almost equal distribution between males (12.7%) and females (11.5%). The deformity occurred more frequently in thalassemia major (16.6%) and thalassemia intermedia (12.2%), compared with α-thalassemia (2.3%).[8]

The frequency of osteoporosis and osteopenia in untreated thalassemia major patients was approximately 40-50% and 45%, respectively. In a study of children with thalassemia major in China found that 62% and 35% of BMD deficits were detected in the spine and in the hips, respectively. Peak bone mass was similarly affected. As a result, low bone mass could be seen in patients with thalassemia major, even in those younger than 12.[9]

In thalassemia major patients there is a correlation between vitamin D and calcium levels. However, vitamin D in beta major thalassemia is the only factor of calcium levels. So far, no one has reported vitamin D as the only one that affects calcium levels in beta major thalassemia patients.

METHODS

This research was a cross-sectional study of 45 Pediatric patients in the Clinical Pathology Installation of the Central General Hospital of Haj Adam Malik Medan from January to April 2018. This study was approved by the Ethics Committee of Health Research, Faculty of Medicine, University of North Sumatra, Indonesia with number No: 303/TGL/KEPK FK USU-RSUP HAM/2017. Of the obtained 45 patients, were 35 thalassemia major patients and 10 normal patients as control consisting of 17 males and 18 females.

Blood samples were withdrawn by venipuncture from the cubital vein. The venous puncture site was first swabbed aseptically with 70% alcohol, allowed to dry, and punctured using a venoject. Blood sampling was performed without excessive statis. Venous blood samples were taken as much as 5 mL and put into the vacutainer clot activator tube. The blood in the vacutainer tube clot activator was allowed to be kept for 20 minutes at room temperature, then centrifuged at 3,000 rpm for 20 minutes. The serum was separated and put into a 1 mL plastic tube (aliquot) for the measurement of vitamin D, calcium and phosphorus levels.

Vitamin D level was measured using a Mini Vidas Brahms with Enzyme-Linked Fluorescent Assay (ELFA) method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI). While the calcium and phosphorus levels were measured using the metallochromic dye method (Arsenazo III) assay and the reaction of inorganic phosphate with ammonium molybdate, respectively. The results of serum vitamin D measurement were then stated as deficiency (<20 ng/mL), insufficiency (20-29 ng/mL), normal (30 - 100 ng/mL), and toxic (> 100 ng/mL). The l serum calcium and phosphorus levels were 8.4 - 10.2 mg/dL and 2.3 - 4.7 mg/dL, respectively.

Statistical analysis and data were performed using SPSS version 22. Data were presented as number and percentages for categorical variables, and the mean±standard deviation; while continuous variables were compared using the Student t-test. Correlation between vitamin D and calcium, vitamin D and phosphor were determined by correlation coefficient tests.

RESULTS AND DISCUSSION

This study involved 35 β-thalassemia major patients with the characteristics of the study as shown in Table 1. In this study, the age of β-thalassemia major patients and control was between 2 to 18 years, divided into 2 parts: ages of 2-10 years with the total number of 19 patients (54.3%) and while ages of 11-18 years with total number of 16 patients (45.7%). The 35 subjects

consisted of 17 males (48.6%) and 18 females (51.4%); while patients as a control were 10 people consisting of 7 females (7%) and 3 males (3%). In another study, there were 14 (56%) females and 11 patients (44%) males. Study by Ilmi *et al*. reported that the proportion of male and female patients was equal.[10]

Table 1. Patients characteristics

Variable	β-Thalassemia Major f(%)	Control f(%)
Age	2 – 18	2 – 18
2 – 10 year	19 (54.3%)	7 (7%)
11 – 18 year	16 (45.7%)	3 (3%)
Gender		
Male	17 (48.6%)	7 (7%)
Female	18 (51.4%)	3 (3%)

From Table 2 it can be seen that the vitamin D levels of 12 (34.3%) β-thalassemia major patients were stated as deficiency, 20 (57.1%) were stated as insufficiency, and 3 (8.6%) were stated as normal. Calcium levels measurement in β-thalassemia patients showed 11 and 24 subjects with low and normal levels of 31.4%, and 68.6%, respectively. Phosphorus levels measurement in patients with β-thalassemia showed 100% normal result. 10 subjects as controls showed 100% normal levels of vitamin D, calcium and phosphorus. From various literatures, 41% of β-thalassemia major patients showed deficiency, 46% showed insufficiency and 13% showed normal vitamin D levels. There were no significant differences of phosphorus levels between patients and controls.

These results were consistent with studies suggesting that phosphorus levels were within the normal range among β-thalassemia major patients. Rashid *et al*. found that vitamin D deficiency was caused by nutritional deficiency and high serum ferritin levels in all children were caused by deficiency of vitamin D hydroxylation in the liver due to hemochromatosis. Vogiatzi *et al*. reported that 12% of thalassemia patients had vitamin D deficiency and 69.8% had insufficient levels.[11] The study of Al-Amir *et al.* reported that 26.3%, 5%, 7.5%, and 1.3% of thalassemia patients had deficiency, insufficiency, normal, and toxic levels of vitamin D.[12]

Our study showed low serum calcium levels in β-thalassemia major of 31.4%, while in normal controls there was no low serum calcium level. In the study of Meena *et al*. calcium levels <8 mg/dL were 31.3% of all cases, while none in the control group had serum values <8 mg/dL. There was a significant difference in serum calcium levels between patients and controls. This suggested other possible factors besides vitamin D deficiency which also played a role in causing hypocalcemia in thalassemia.[13]

In Pakistan, hypocalcemia in thalassemic patients was reported to be 35.3%. A study by Shah showed very low average serum calcium levels, leading to a possible effect of delay in starting chelation therapy and compliance therapy.[14]

Almost all the thalassemia patients who reported developed hypocalcemia were above the age of 10 years except two. Therefore, this complication in thalassemia mainly occurred in the second decade of life. It was obvious that although optimal chelation therapy had been given, complications such as hypoparathyroidism were prone to occur which in turn caused hypocalcemia.[15]

Table 3 showed statistical tests using unpaired T-test on the levels of vitamin D, calcium and phosphorus. Mean±SD of vitamin D, calcium and phosphorus levels in thalassemia major patients were 21.28±6.36 (ng/mL), 8.58±0.68 (mg/dL) and

Table 2. Results of Vitamin D, calcium and phosphorus levels measurement

Variable	β-Thalassemia Major (n=35) f(%)	Control (n=10) f(%)
Vitamin D		
Deficiency (< 20 ng/mL)	12 (34.3%)	
Insufficiency (20 – 29 ng/mL)	20 (57.1%)	
Sufficiency (30 – 100 ng/mL)	3 (8.6%)	10 (100%)
Calcium		
Low	11 (31.4%)	
Normal (8.4 – 10.2 mg/dL)	24 (68.6%)	10 (100%)
Phosphorus		
Normal (2.3 – 4.7 mg/dL)	35 (100%)	10 (100%)

Table 3. Mean±SD of vitamin D, calcium and phosphorus levels

Variable	β-Thalassemia Major (n=35) Mean±SD	Control (n=10) Mean±SD	p-value
Serum vitamin D (ng/mL)	21.28±6.36	34.85±3.50	<0.05
Serum calcium (mg/dL)	8.58±0.68	9.22±0.35	<0.05
Serum phosphorus (mg/dL)	3.98±0.53	3.89±0.49	>0.1

3.98±0.35 (mg/dL), respectively. While those of controls were 34.85±3.50 (ng/mL), 9.22±0.35 (mg/mL) and 3.89±0.49 (mg/dL), respectively. There were no significant difference between vitamin D and calcium levels with p <0.05, whereas the phosphorus levels showed no significant difference with p> 0.1.

The decreased level of serum vitamin D was statistically highly significant as compared to that of normal healthy children (control group) with (p<0.0001). The results of the present series of study resembled the findings of Fahim *et al*.[16]

High prevalences of vitamin D deficiency occur in thalassemic children and adolescents that may largely contribute to their bone diseases. Monitoring normal serum levels of 25-OH D through oral intake of vitamin D and early treatment of vitamin D deficiency by oral or parenteral use of vitamin D may significantly improve their bone mineral accretion and prevent bone disease.[5] Balanced nutrition, patient education, dietary counseling and supplementation therapy of calcium and vitamin D for high-risk groups of β-thalassemia is strongly recommended. Regular monitoring of serum calcium, alkaline phosphatase, and inorganic phosphorus is also recommended. Proper monitoring of parathyroid hormone may also improve skeletal status in these patients.[17]

Low serum vitamin D levels can be attributed to malabsorption of vitamin D as well as hepatic dysfunction, which also leads to defective hydroxylation of vitamin D resulting in its deficiency. Some researchers have attributed the etiology of vitamin D deficiency to hepatic iron overload. Multiple factors have been attributed to growth faltering in thalassemia, such as vitamin D deficiency which can be easily monitored and corrected.[18]

Vitamin D deficiency with increased S.ALP was more prevalent in >10 years of age group while Vitamin D insufficiency with hypocalcemia was prominently found in the 5-10 year age group. Vitamin D and calcium supplementation along with regular transfusion and chelation regimen can likely prevent or delay late complications like short stature, osteoporosis in thalassemia patients.[19]

CONCLUSION AND SUGGESTION

There were significant differences of vitamin D and calcium levels between β-thalassemia major and normal subjects but no differences found in phosphorus levels. However, there was no correlation between vitamin D and calcium levels. This showed that the reduction of calcium in β-thalassemia major case was not merely caused by the influence of vitamin D, Suggesting that the bone abnormalities in β-thalassemia major were not caused by hypovitaminosis D.

Calcium decrease in beta major thalassemia patients is not solely because vitamin D is low, but there are other factors that affect calcium in patients with thalassemia major. The need for vitamin D and calcium in thalassemia patients is important so, thalassemia patients do not have bone calcification, pathological fractures, thinning of bone due to ineffective erythropoiesis. This finding suggested that clinicians that treatment of bone disorder in β-thalassemia major should not only be based on vitamin D but to the improvement of bone marrow expansion.

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