Jcrpe-2018-0020.R2
Original Article

Under-recognized Hypoparathyroidism in Thalassemia

Tangngam H et al. Hypoparathyroidism in thalassemia

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Conflict of interest: None declared
Received: 17.01.2018
Accepted: 02.05.2018

Abstract
Objective: Symptomatic hypoparathyroidism [symptomatic hypocalcemia without elevated serum parathyroid hormone (PTH)] in patients with thalassemia is relatively rare. Asymptomatic mild hypocalcemia without elevated PTH which is considered hypoparathyroidism may be more common but under-recognized.
Methods: Sixty-six transfusion-dependent thalassemic patients, and 28 healthy controls were enrolled. Serum calcium (Ca), phosphate (P), creatinine (Cr), albumin, intact PTH, 25-hydroxyvitamin D (25-OHD), plasma intact fibroblast growth factor-23 (FGF-23), urine Ca, P and Cr were measured. Tubular reabsorption of phosphate was calculated.
Results: Thalassemic patients had significantly lower median serum Ca levels than the controls [8.7 (7.8-9.7) vs. 9.6 (8.7-10.1) mg/dL]. Hypoparathyroidism was found in 25 of 66 (38%) patients. Symptomatic hypoparathyroidism was not found. Thalassemic patients also had significantly lower median plasma FGF-23 levels than the controls [35.7 (2.1-242.8) vs. 53.2 (13.3-218.6) pg/mL]. In patients with hypoparathyroidism, median plasma FGF-23 level was significantly lower than that of patients with normoparathyroidism [34.8 (2.1-120.0) vs. 43.1 (3.2-242.8) pg/mL]. However, serum P, Cr, intact PTH and 25-OHD levels were not different.
Conclusion*:* Hypoparathyroidism was not uncommon in patients with transfusion-dependent thalassemia treated with suboptimal iron chelation. Plasma intact FGF-23 level in patients with hypoparathyroidism was lower than that of patients with normoparathyroidism.
Keywords: thalassemia, hypoparathyroidism, hypocalcemia, iron overload, FGF-23

What is already known on this topic?
Symptomatic hypoparathyroidism in patients with transfusion-dependent thalassemia is relatively rare. However, data on prevalence of asymptomatic hypoparathyroidism are scanty.
What this study adds?
Hypoparathyroidism in patients with thalassemia is not uncommon. Screening for asymptomatic mild hypocalcemia without elevation of parathyroid hormone should be considered in transfusion-dependent thalassemia for early detection and proper treatment. In comparison with patients with normoparathyroidism, plasma FGF-23 appeared to be lower in patients with hypoparathyroidism.

Introduction

Thalassemia is an inherited disease caused by abnormal hemoglobins, which results in ineffective erythropoiesis and increased peripheral hemolysis. Regular blood transfusion is inevitable in patients with moderate to severe thalassemia. Overt hypoparathyroidism in thalassemia is relatively rare (1). However, asymptomatic hypoparathyroidism was rarely reported; one study reported up to 42% (2). Previous studies showed that hypoparathyroidism was primarily associated with iron overload (3,4,5,6).
Children and adults with thalassemia had relatively high serum phosphate levels in several studies (7,8,9,10). Our previous study also demonstrated that serum phosphate levels in transfusion-dependent thalassemia had a trend toward higher than those in non-transfusion dependent thalassemia but not significant ($p = 0.081$) (11). In transfusion-dependent thalassemia, high phosphate loading due to regular blood transfusion, hemolysis and hypoparathyroidism, are factors contributing to elevated serum phosphate (12).

Fibroblast growth factor-23 (FGF-23), a phosphaturic hormone, is mainly synthesized and secreted by osteoblasts and osteocytes in response to hyperphosphatemia and elevated 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) level (13). FGF-23 acts at renal tubular cells to reduce phosphate reabsorption. In addition, FGF-23 inhibits 1α-hydroxylase, leading to a reduction in formation of 1,25-$(OH)_2$D (14). FGF-23 also reduces parathyroid hormone (PTH) secretion from the parathyroid glands, thereby attenuating PTH-mediated phosphaturic effect (15). However, FGF-23-phosphate axis control in thalassemia has not been elucidated.

Our previous histomorphometric study demonstrated that iron deposits in thalassemic bones impaired bone mineralization and reduced bone formation (16). In vitro studies demonstrated that excessive iron inhibited osteoblast proliferation and differentiation (17,18). Therefore, iron accumulation in thalassemic bones may compromise FGF-23 production by osteoblasts and osteocytes.

We therefore hypothesized that asymptomatic hypoparathyroidism might be common but under-recognized. In addition, impaired FGF-23 production secondary to iron deposits in bones might partly contribute to elevated serum phosphate in thalassemia. Our study aimed to determine serum calcium (Ca), phosphate (P) and 25-hydroxyvitamin D (25-OHD), PTH and plasma FGF-23 levels, in transfusion-dependent thalassemic patients.

Methods

Children and adolescents with transfusion-dependent thalassemia who have been attending the Hematology Clinic at the Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, were enrolled. Most transfusion-dependent thalassemic patients have received a standard regular blood transfusion therapy every 3-4 weeks to maintain their hemoglobin levels at 9-10.5 g/dL. Desferrioxamine injection has been used to be the only iron chelating agent used in thalassemic patients who had serum ferritin level of greater than 1,000 ng/mL. Additional oral iron chelators such as deferiprone and deferasirox have been used in the past 5 years. All patients received daily folic acid and multivitamin supplementation. Each tablet of multivitamin contains 400 IU of vitamin D_2. Patients with known underlying conditions including hypoparathyroidism, renal disease and acute hemolysis, and patients who had been taking other medications affecting Ca, P and vitamin D metabolism, were excluded. The controls were healthy children who attended the day camp regularly arranged by our hospital during the end of each school semester. All of them were hospital personnel's children. None of them had been taking medications which known to affect Ca, P and vitamin D metabolism.

Anthropometric measurements were determined at the time of enrollment. Z-scores of height and weight were calculated using the National Standard Growth Curve of the Ministry of Public Health, Thailand. The Z-score of body mass index (BMI) was calculated using the World Health Organization BMI for age and sex. Median serum ferritin was determined using serum ferritin levels periodically obtained during the routine clinic visits. Cumulative iron loading was estimated from cumulative volume of packed red cell (PRC) transfusion.

Cumulative iron loading (mg) = volume (mL) of PRC given x hematocrit of PRC x 1.16 (19).

Fasting blood samples were obtained for determination of serum Ca, P, Cr, albumin, intact PTH, 25-OHD and plasma intact FGF-23 in thalassemic patients and controls. In thalassemic patients, fasting blood samples were obtained on the day of transfusion just before the scheduled transfusion. Simultaneous spot morning urine samples for Ca, P and Cr in thalassemic patients were obtained. Serum PTH and 25-OHD were measured by chemiluminescence assay. Corrected serum Ca, tubular reabsorption of phosphate (TRP) and ratio of tubular phosphate reabsorption to the glomerular filtration rate (TP/GFR) were calculated using the following formulas:

Corrected serum Ca (mg/dL) = Total serum Ca (mg/dL) + 0.8 x [4 – serum albumin (g/dL)]

TRP (%) = {1- [(urine P/serum P) / (urine Cr / serum Cr)]} x 100

TP/GFR (mg/dL) = Serum P – (Urine P x Serum Cr/ Urine Cr) (20)

Definitions used in this study: hypocalcemia = corrected serum Ca <8.5 mg/dL; normocalcemia = corrected serum Ca 8.5-10.4 mg/dL; hypoparathyroidism = hypocalcemia without elevated intact PTH (reference range: 20-75 pg/mL).

Plasma intact FGF-23 was measured in duplicate by a commercial ELISA kit (2^{nd} generation human intact FGF-23 ELISA kit, Immutopics, Inc, San Clemente, CA). The intra-assay CV was 2.0-4.1% and the inter-assay CV was 3.5-9.1%. The lower limit of detection was 1.5 pg/mL.

The study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (Ethics Approval No. MURA2013/24 Np1). Informed consent was obtained from the patients and their legal guardians.

Statistical Analysis

Statistical analysis was performed using the software package SPSS 15.0 (SPSS, Inc., Chicago, USA). All parameters were not normally distributed; therefore, they were presented as median (range). Comparison between the patient and control groups was performed using Mann-Whitney *U* test. Pearson's correlation was used to determine the correlation between two variables. A *p* value of less than 0.05 was considered statistically significant.

Results

Sixty-six transfusion-dependent thalassemic patients, median age of 13.5 years (range 5.1-23.2), and 28 healthy controls, median age of 8.9 years (range 4.8-17.2) participated in the study. There were 60 patients with β-thalassemia/hemoglobin E (β-thal/E) disease and six with β-thalassemia homozygote (β-major) disease. Twelve patients were splenectomized. All patients have been receiving regular packed red cell transfusions (every month) and iron chelation therapy including desferrioxamine in combination with either deferiprone or deferasirox. In comparison with the controls, thalassemic patients were older, whereas the Z-scores of weight, height and BMI were lower (Table 1).

Biochemical data of thalassemic patients and controls

Thalassemic patients had significantly lower corrected serum Ca and plasma intact FGF-23 levels than those of the controls. No significant differences in serum P, PTH and 25-OHD levels were found between the 2 groups (Table 1). There were no significant differences in corrected serum Ca, P, 25-OHD, PTH and plasma intact FGF-23 levels between patients with β-thal/E and β-major. Hypoparathyroidism (corrected serum Ca 7.5-8.4 mg/dL and no elevation of PTH) was found in 25 of 66 (38%) thalassemic patients. None of the controls had hypocalcemia. Most thalassemic patients (94%) had normal vitamin D status (25-OHD ≥20 ng/mL). Only 4 of 66 (6%) patients (3 β-thal/E, 1 β-major) had mild vitamin D insufficiency (25-OHD, range 18.6-19.7 ng/mL). All but one controls (27 of 28) had normal vitamin D status (one child had mild vitamin D insufficiency, 25-OHD 16.8 ng/mL).

Comparison between thalassemic patients with hypoparathyroidism (N = 25) and normoparathyroidism (N = 41)

Twenty-five thalassemic patients had asymptomatic mild hypocalcemia. All of them had either inappropriately low or normal serum PTH which is considered hypoparathyroidism. The remaining 41 patients had normal serum Ca and PTH levels. There were no significant differences in gender, age, Z-scores of weight, height and BMI, and ages of onset of transfusion and chelation therapy between patients with hypoparathyroidism and those with normoparathyroidism. In the hypoparathyroid group, the lowest level of 25-OHD was 18.6 ng/mL; almost all patients had vitamin D sufficiency (median 27.6 ng/mL). In comparison with the patients with normoparathyroidism, median plasma intact FGF-23 was slightly lower in the hypoparathyroid group (43.1 vs. 34.8 pg/mL, $p = 0.048$), albeit no significant difference of serum phosphate. Cumulative iron loading was greater, while serum ferritin was lower in the hypoparathyroid group (Table 2). Serum Ca level had a negative correlation with duration of transfusions in hypoparathyroid patients ($r = -0.45$, $p = 0.022$), but had no correlation in the group with normoparathyroidism.

Discussion

The present study demonstrates asymptomatic hypoparathyroidism in transfusion dependent thalassemia. Previous studies reported low prevalences of hypoparathyroidism, ranging from 0.5 to 7.6% (5,12,21). Those prevalences primarily represented overt or symptomatic hypoparathyroidism. This cross-sectional study looked at calcium-phosphate metabolism in patients with transfusion-dependent thalassemia. All patients had neither symptoms of hypocalcemia nor history of fractures. The prevalence of hypoparathyroidism was 38% in this study, which suggests high prevalence of unrecognized asymptomatic hypoparathyroidism. This is in line with previous studies of Mostafavi et al (22) and Adil et al (23) that reported hypoparathyroidism in 22.7% and 35.3% of thalassemic patients, respectively. Previous studies demonstrated that hypoparathyroidism was associated with high serum ferritin (3,4,5,6). Serum ferritin of more than 2,500-3,000 ng/mL has been demonstrated to be associated with higher frequency of hypoparathyroidism (5,6). In addition, Belhoul et al (6) also reported that patients with a serum ferritin >2,500-3000 ng/mL had 3.27 times more likely to develop hypoparathyroidism. However, no relationship between hypoparathyroidism and serum ferritin had been reported (24,25). In the present study, the patients had modestly elevated median serum ferritin of 1,333 ng/mL and median serum ferritin in patients with hypoparathyroidism was lower than that of the patients with normoparathyroidism. This finding can be explained by recent additional iron chelation treatment with oral deferiprone and deferasirox. Therefore, iron chelation had been greatly improved, resulting in rapid reduction of serum ferritin. However, tissue iron accumulation might be relatively persistent. In fact, serum ferritin levels were greater than 3,000 ng/mL in our transfusion-dependent thalassemics during the past 5-10 years when only desferrioxamine injection had been used (11). Previous studies also demonstrated that serum ferritin may not be a reliable indicator of tissue iron overload (24,26). In patients with suboptimal iron chelation therapy, amount of iron from packed red cell transfusion may better reflect tissue iron accumulation (27). Hence, our patients with hypoparathyroidism had higher cumulative iron loading than patients with normoparathyroidism despite having less serum ferritin. The cause of hypoparathyroidism is likely due to iron deposition in parathyroid glands as demonstrated in previous reports (1,24,28,29).

With regards to FGF-23, it is a phosphaturic hormone secreted by osteoblasts and osteocytes in response to elevated serum phosphate. Elevated serum FGF-23 levels have been demonstrated in patients with hypoparathyroidism secondary to other causes such as post-parathyroidectomy, post-thyroidectomy with accidental parathyroidectomy and transient hypoparathyroidism in neonates who had maternal hyperparathyroidism (30,31,32). In contrast, our thalassemic patients who had hypoparathyroidism did not have elevated FGF-23 levels. A previous study reported that excessive iron disturbed metabolism of mouse osteoblastic cells (17). In addition, ferric iron inhibited osteoblast proliferation, differentiation and mineralization. Moreover, the inhibition of human osteoblast activity was concentration-dependent (18). Iron overload inevitably occurs in transfusion-dependent thalassemic patients and iron accumulation in thalassemic bones had also been demonstrated (16,33). Thus, FGF-23 production by osteoblasts and osteocytes could be compromised in thalassemic patients. Our study showed that plasma intact FGF-23 level was significantly higher in the controls as compared with the thalassemic group. The finding could reflect an impaired FGF-23 production among thalassemic patients. Median plasma FGF-23 level in the patients with hypoparathyroidism was significantly lower than that of the patients with normoparathyroidism (34.8 vs. 43.1 pg/mL, p = 0.048) although serum phosphate levels were not different and serum PTH levels were comparable. These findings suggest that FGF-23 response in patients with hypoparathyroidism might be impaired. One would expect to see elevated serum phosphate in these hypoparathyroid patients because of impaired both phosphaturic hormones, PTH and FGF-23. The reason for an absence of elevated serum phosphate in these patients is unclear.

Iron deficiency, an opposite condition to iron overload state, has been reported to be associated with elevated serum FGF-23 levels in patients with autosomal dominant hypophosphatemic rickets (ADHR), the elderly population and undernourished Gambian children (34,35,36). In addition, iron deficiency upregulated Fgf23 mRNA in bones of Fgf23 knock-in mice and consequently led to ADHR phenotype (37). Improvement of iron status following iron

supplementation was associated with a decrease in serum FGF-23 level in undernourished Gambian children and patients with ADHR (36,38). Moreover, the latter had a complete loss of biochemical ADHR phenotype following iron supplementation (38). The mechanism of iron status in influencing FGF-23 concentration remains to be elucidated. However, to our knowledge, the impact of iron overload state secondary to thalassemia or hereditary hemochromatosis on FGF-23 level has not been reported. One might speculate that iron overload lead to a decrease in FGF-23 production in an opposite direction to the effect of iron deficiency. Our previous study demonstrated "iron-associated focal osteomalacia" in bone histology of patients with thalassemia (16). Osteoblasts and osteocytes could be disturbed by iron accumulation in bones and thus resulting in impaired FGF-23 production. Further studies are required to assess the effects of iron overload on the synthesis, secretion and metabolism of FGF-23.

There were some limitations of this study. First, relatively small sample size was included. Second, unmatched age and pubertal maturation occurred between the control and thalassemic groups. However, previous studies reported no age- and puberty-associated changes in FGF-23 levels (39,40). Third, serum 1,25 $(OH)_2$ vitamin D was not measured. Thus, pathophysiological changes of serum 1,25 $(OH)_2$ vitamin D related to plasma FGF-23 during hypoparathyroidism or normoparathyroidism cannot be drawn.

In conclusion, hypoparathyroidism was not uncommon in patients with transfusion-dependent thalassemia treated with suboptimal iron chelation. Plasma FGF-23 level in patients with hypoparathyroidism was lower than that of patients with normoparathyroidism.

Acknowledgements
This study was supported by the research grant from the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Authorship contributions
Concept: P.M., H.T., P.P., A.C.; Data collection and analysis: P.M., H.T., P.P., A.C., N.S., L.C., P.K.; Writing: P.M., H.T., P.P., A.C.

Funding: research grant from the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

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Table 1. Characteristics and blood chemistries of thalassemic patients and controls

Parameters Median (range)	Thalassemia (n = 66)	Control (n = 28)	*p* value
Age (years)	13.5 (5.1-23.2)	8.9 (4.8-17.2)	0.004
Male, N (%)	35 (53)	10 (36)	NS
Z score of weight	-0.8 (-3.2 to 2.6)	+0.8 (-1.5 to 5.4)	<0.001
Z score of height	-0.7 (-4.0 to 2.0)	+0.2 (-1.4 to 0.8)	<0.001
Z score of BMI	-0.6 (-3.6 to 2.3)	+0.2 (-0.8 to 3.3)	0.016
Puberty, N (%)	39 (59)	11 (39)	<0.001
Corrected serum calcium (mg/dL)	8.7 (7.8-9.7)	9.6 (8.7-10.1)	<0.001
Serum phosphate (mg/dL)	4.9 (3.8-6.2)	4.9 (4.1-6.0)	NS
Serum creatinine (mg/dL)	0.4 (0.2-0.8)	0.4 (0.2-0.8)	NS
Serum PTH (pg/mL)	31.5 (12.5-74.6)	29.6 (20.5-65.8)	NS
Serum 25-OHD (ng/mL)	27.6 (18.6-57.3)	26.6 (16.8-38.7)	NS
Plasma FGF-23 (pg/mL)	35.7 (2.1-242.8)	53.2 (13.3-218.6)	0.010
Hemoglobin (g/dL)	8.6 (5.9-11.0)	-	-
Serum ferritin (ng/mL)	1,333 (372-6,752)	-	-

Data are presented as median (range), otherwise as indicated. BMI, body mass index; PTH, intact parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; FGF-23, intact fibroblast growth factor-23; NS, not significant different

Table 2. Biochemical parameters of thalassemic patients with hypoparathyroidism and normoparathyroidism

Parameters Median (range)	Hypoparathyroidism* (n = 25)	Normoparathyroidism** (n = 41)	*p* value
Male, N (%)	15 (60)	20 (49)	NS
Age (years)	14.2 (7.3 -18.9)	11.8 (5.1-23.2)	NS
Z score of weight	-0.8 (-1.9 to 2.3)	-0.7 (-3.2 to 2.6)	NS
Z score of height	-0.9 (-2.4 to 1.6)	-0.5 (-4.0 to 2.0)	NS
Z score of BMI	-0.8 (-2.9 to 2.3)	-0.5 (-3.6 to 1.4)	NS
Puberty, N (%)	18 (72)	20 (49)	0.030
Corrected serum calcium (mg/dL)	8.2 (7.8-8.4)	8.9 (8.5-9.7)	<0.001
Serum phosphate (mg/dL)	4.9 (4.0-5.8)	5.1 (3.8-6.1)	NS
Serum creatinine (mg/dL)	0.4 (0.2-0.8)	0.4 (0.2-0.7)	NS
Serum PTH (pg/mL)	31.8 (15.3-74.6)	31.3 (12.5-57.5)	NS
Serum 25-OHD (ng/mL)	28.9 (18.6-57.3)	27.4 (19.4-43.9)	NS
Plasma FGF-23 (pg/mL)	34.8 (2.1-120.0)	43.1 (3.2-242.8)	0.048
Urine Ca/Cr (mg/mg)	0.08 (0.01-0.28)	0.06 (0.01-0.51)	NS
TRP (%)	95.6 (88.6-99.1)	96.2 (87.8-98.6)	NS
TP/GFR (mg/dL)	4.7 (3.8-5.6)	4.9 (3.7-5.9)	NS
Serum ferritin (ng/mL)	1,087 (372-6,197)	1,957 (449-6,752)	0.001
Cumulative iron loading (g)	26.8 (3.7-60.8)	17.3 (3.6-62.9)	0.046

Data are presented as median (range), otherwise as indicated. *Corrected serum calcium <8.5 mg/dL; **Corrected serum calcium 8.5-10.4 mg/dL; PTH, intact parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; FGF-23, intact fibroblast growth factor-23; Urine Ca/Cr, urine calcium to creatinine ratio; TRP, tubular reabsorption of phosphate; TP/GFR, ratio tubular phosphate reabsorption to the glomerular filtration rate; NS, not significant different