Association of vitamin D and FGF23 with serum Ferritin in hypoparathyroid thalassemia, a case control study

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Research article

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Abstract

Background:FGF23 controls serum l,25(OH)2D3 levels and phosphate homeostasis. This study evaluates the effects of Ferritin on intact PTH, FGF23 and l,25(OH)2D3 in patients with major thalassemia. It evaluates FGF23 changes in patients with hypoparathyroidism to clarify the interaction between FGF23 and PTH in the absence of proper PTH function in human. Methods:In this case-control study,25 patients with major-beta thalassemia with hypoparathyroidism and their age- and gender-matched patients with major-beta thalassemia having normal parathyroid function were enrolled. Biochemical studies assessed serum calcium, albumin phosphorus, alkaline phosphatase, PTH, FGF23, 25(OH)D, 1,25(OH)2D3, Ferritin and Fractional excretion of phosphorous. Results:FGF23 was higher in the patients with hypoparathyroidism compared to controls(p=0.002). Fractional excretion of phosphorous was lower in patients with hypoparathyroidism, despite of high FGF23(p=0.001). There was a correlation between serum1,25(OH)2D3 and FGF23 with ferritin in the controls(P=<0.001and P=<0.001,respectively). Conclusions: The present study suggested that rise in FGF23 in patients with thalassemia, may be due to either stimulating effect of PTH and 1,25(OH)2D3 on FGF23 production, or might be direct stimulating effect of ferritin. It seems that in hypoparathyroid patients with insufficient PTH action, the FGF23 is not able to exert its full function in reducing serum phosphorus level by its phosphaturic action.

Background

Thalassemia is an inheritable disease caused by abnormal hemoglobin production, resulting in ineffective erythropoiesis and increased peripheral hemolysis. The clinical outcomes of iron overload vary and reflect the key location of iron deposition. The concentration of ferritin in serum provide a quantitative measure for iron storage (1). In patients with major thalassemia, frequent blood transfusion and iron overload, despite intensive chelation therapy, become prone to many endocrine complications, such as hypogonadotropic hypogonadism, diabetes mellitus, hypothyroidism, and hypoparathyroidism (2, 3).

PTH is a potent stimulator in producing l, 25$(OH)_2D_3$ by increasing 1-alfa-hydroxylase system activity in the proximal renal tubules. Reduced PTH secretion, results in hypocalcemia and hyperphosphatemia (4, 5). PTH and Fibroblast growth factor 23 (FGF23) are the primary hormones that regulate phosphate and calcium homeostasis (6). FGF23 is a member of FGF19 subfamily, produced by osteocytes in response to high serum phosphate and high l,25$(OH)_2D_3$ levels (7,8). FGF23 acts throughout FGFR-klotho co-receptors in the kidneys to provoke phosphaturia, and diminish 1-alfa-hydroxylase activity and controls production of l,25$(OH)_2D_3$ (9-12).

Previous studies have shown the effects of dietary phosphate and serum phosphate on the release of FGF23 (13,14). The interaction between PTH and FGF23 on regulation of serum phosphate is not clearly understood. Recent studies have shown that both iron deficiency and iron transfusion have some effect on serum FGF23 (15-18). But there is lack of sufficient data evaluating the association or interaction between high serum ferritin and serum l,25$(OH)_2D_3$, FGF23 and PTH in patients with thalassemia. The

objective of this study was to examine the association of Ferritin and intact PTH, FGF23, and $l,25(OH)_2D_3$ in patients with major thalassemia having normal parathyroid function and hypoparathyroidism. We also performed this study to clarify the interaction between FGF23 and PTH in hypoparathyroidism, which is not clearly understood, yet.

Methods

We studied 25 patients with major-beta thalassemia having hypoparathyroidism from October 2017 through March 2018 at Shiraz University of Medical Sciences affiliated thalassemia clinics in Fars province, southern Iran. Total of 25 age and gender matched participants were selected as the control who had major-beta thalassemia with normal parathyroid function. Hypoparathyroidism was diagnosed on the basis of hypocalcemia (Serum calcium less than 8.5 mg/dl) accompanied with a low or undetectable serum levels of intact parathyroid hormone (iPTH), and high serum phosphate level. All patients with hypoparathyroidism had routine follow up by an expert Endocrinologist, and received proper dose of calcium carbonate (500mg tablet, manufactured Toliddaru pharmaceutical, Tehran, Iran), plus calcitriol (0.25µg capsule, manufactured Zahravi pharmaceutical, Tehran, Iran). The range of daily dose of calcitriol was 0.5 - 2.5 µg/day to maintain albumin-corrected serum calcium level in the low-normal range of 8–9 mg/dl (5). Majority of transfusion-dependent thalassemia patients received routine blood transfusion therapy every 3-4 weeks to maintain their hemoglobin levels at 9-10.5 g/dL. In these patients', Iron chelating agents, such as Oral chelators (deferasirox and deferiprone) and Deferrioxamine injection were used. Deferoxamine subcutaneous injection, was used in patients with thalassemia who had serum ferritin level of greater than 1000 ng/mL with a dose of 20-40 mg/kg/day. The Exclusion criteria in both groups were renal failure (Glomerular filtration rate less than 60 ml/min), liver failure, and other metabolic bone disease (e.g., rickets), hyperthyroidism, and diabetes mellitus.

Blood samples were obtained from all participants for a minimum of 15 days after transfusion and overnight fasting. All blood samples were centrifuged for 15 min at *3000 rpm* (1500 × *g)* and serums were separated and stored at -70°C until further analysis. All the biochemical studies were performed at the endocrinology and metabolism research center laboratory of Shiraz University of Medical Sciences. Colorimetric assays were used to analyze calcium (mg/dL), phosphorus (mg/dL), albumin (g/dL) and alkaline phosphatase (IU/L) levels, using Biosystem SA auto-analyzer, made in Spain. Electrochemiluminescence methods were used to measure serum parathyroid hormone (PTH) (pg/ml) and 25(OH)D (ng/ml) levels using Cobas E411, Roche, Germany. Sensitivity, intra- and inter-assay CVs for 25(OH)D were 2 ng/ml, 3.3 % and 5.1%, respectively. ELISA method was used to determine the serum intact FGF23 (pg/ ml) and 1,25(OH)2D3 (pg/ ml) using Bioassay technology laboratory kit, Spain. Sensitivity, intra- and inter-assay CVs for 1,25(OH)2D3 were 3.14 (pg/ ml), <8 % and <10%, respectively. Sensitivity, intra- and inter-assay CVs for FGF23 were 2.4 pg/ml, <8 % and <10%, respectively. Serum

ferritin levels were recorded on Roche Diagnostic E 170 analyzer (Roche Diagnostics 1010/2010, Mannheim, Germany) by Chemiluminescence's Immunoassay (ECLIA) method.

Ethical statement: All authors declare that they have no conflict of interest. All the patients signed a written informed consent form after explaining the study objective and method. Shiraz University of Medical Sciences Local Ethics Committee and Vice-Chancellor of research at SUMS approved this study with number 1396-01-01-15805.

Statistics

Statistical analysis was performed using SPSS statistical software (SPSS 22, IBM SPSS software, Armonk, NY). Data are shown as mean ± SD. Sample size formula was used to compare the two independent groups ($\alpha = 0.05$, $\beta = 0.2$); it was calculated at 25 participants in each group. Kolmogorov–Smirnov test was used to evaluate the normality of data distribution. Normally distributed data were compared using Student's *t*-test, and the Mann–Whitney test was used to compare non-normally distributed ones. Pearson's test and Spearman's ranking test were used to evaluate the correlations between normally distributed parameters and non-normal distributed ones, respectively. *P*-values less than 0.05 were considered to be statically significant. Multiple linear regression model was used to determine the independent factors influencing FGF23 in both case and control groups. We used pearson chi-square test to compare different groups of Iron chelating agent. We also used multiple linear regression analysis to explore factors determining the serum FGF23 and 1,25(OH)2D3 levels in control group and patients with hypoparathyroidism.

Results

In the present study, 50 patients with beta thalassemia including 25 cases with hypoparathyroidism and 25 controls with normal parathyroid function were enrolled. Patients' mean age in the case and control groups was 26.9±3.09 years and 25.7±5.1 years, respectively. Hypoparathyroid group included 59.3% male. General characteristic and biochemical parameters of patients are summarized in table1. The mean serum calcium and PTH level was lower in patients with hypoparathyroidism in comparison with the controls (P=0.001 and $P < 0.001$, respectively). Serum phosphorus and FGF-23 level was significantly higher in patients with hypoparathyroidism compared to the control group (P=0.002 and P=0.005 respectively). The mean FE phosphorous was lower in the case group (5.1±3.1) in comparison with the controls (9.2±5 pg/ml) (P=0.001). There was no significant difference in serum alkaline phosphatase, 25(OH)D, ferritin, 1,25(OH)2D3, and Urine Ca/Cr ratio between the case and control groups (p= 0.65, p=0.22, p=0.08, p=0.51 and p=0.92, respectively).

In the control group, there was a positive strong correlation between serum ferritin and FGF23 (P <0.001, CC:0.801) and also between serum ferritin and 1,25(OH)2D3 (P <0.001, CC:0.754). However, serum ferritin level in patients with hypoparathyroidism did not correlated with those of serum FGF23, calcium, phosphorous, PTH, 25(OH)D, 1,25(OH)2D3, and FEPh (P>0.05). Table2 shows Multiple linear regression analysis of covariates of FGF23 and 1,25(OH)2D3 in both case and control groups. It shows that association of ferritin with FGF23 or 1,25(OH)2D3 was persist after considering other contributing factors such as serum Ca, Ph, and PTH.

Based on the received Iron chelating agents, patients were divided into four groups according the daily dose of deferrioxamin and deferasirox (group1 <500 mg/day, group2 >500-1000 mg/day, group3 >1000-1500 and group4 >1500-2000). There was no significant difference in dosage and kind of receiving iron chelating agents between two groups.

Discussion

In the present study, we observed high serum level of FGF23, 1,25OH2D and high normal PTH level in normo-parathyroid controls. We also detect strong positive correlation between 1,25(OH)2D3, FGF23 and ferritin level in control group. Expectedly in this study, low serum calcium in association with low serum PTH in patients with hypoparathyroidism were detected. In contrast, high normal serum calcium and PTH in control group was observed. That suggested other factors might be involved in the stimulation of parathyroid secretion. It seems that high ferritin levels in patients with thalassemia might have had possible stimulatory effect on PTH secretion in intact parathyroid function, resulting in high normal serum PTH and calcium.

Pawlotsky et al. revealed a positive correlation between serum ferritin and high Serum PTH in patients with iron overload syndrome (19). Kurtoglu et al. showed a high PTH level in major thalassemia patients more in first two decades (2). Also, another study on 90 patients with thalassemia showed that more than 25% of them had high normal levels of PTH and calcium. They also found a significant correlation between ferritin and PTH in these patients (20). On the other hand, some patients with thalassemia may develop parathyroid dysfunction at older age because of iron overload and iron deposition on parathyroid glands (2). The underlying mechanism might be that iron overload could induce lysosomal and sarcolemmal membrane damage through free radical formation and lipid peroxidation and causes the destruction of parathyroid glands (21). And, cell surface transferrin receptors could able to play a role in protecting parathyroid glands against inorganic iron (22).

In present study we have showed that both case and controls had insufficient 25(OH)D serum level. Napoli et al. reported that serum 25(OH)D deficiency in adult patients with beta thalassemia (23). In this study, we observed a high normal 1,25(OH)2D3 serum level, in spite of 25(OH)D deficiency in the control group. High normal serum PTH might be a potent factor to enhance alfa-1-hydroxilase activity in these patients. Also this study showed a strong positive correlation between 1,25(OH)2D3 and ferritin level in the control group, which was not observed in patients with hypoparathyroidism. Therefore, it is possible

that in state of intact parathyroid function, high ferritin level might enhance 1,25OH2D production through direct stimulation of alfa-1-hydroxilase or indirectly through parathyroid hormone action. Some previous reports showed a significant low level of vitamin D in patients with thalassemia, but few of them evaluated serum 1,25(OH)2D3 in patients with thalassemia (1,23). Wood et al. showed high serum level of 1,25(OH)2D3 in patients with thalassemia. He suggested that it could occur even through primary hyperparathyroidism or upregulation of extra-renal alfa-1 hydroxylase activity (24).

This study revealed a normal serum phosphate level, in spite of high FGF23 and high urinary phosphate loss in the control group. It was suggested that high serum level of 1,25(OH)2D3 in patients with thalassemia could enhance intestinal phosphate absorption, which leads to a normal serum phosphate even with high urinary phosphate loss (25). Another finding of the present study was high level of serum FGF23 in patients with thalassemia. Two mechanisms could be put forward to explain the increase of serum FGF23 in these patients. The first is the stimulatory effect of PTH or 1,25(OH)2D3 on FGF23 production (26,27). Also, Moshayoff et al. showed that serum FGF23 levels were increased by PTH administration in both *in vivo* and *in vitro* (28). In additional, one study revealed that PTH has direct and indirect effect through 1,25(OH)2D3 on FGF23 secretion (29). The second mechanism of FGF23 increasing, might be due to ferritin. There are controversies about the effects of iron deficiency or iron overload on serum level of FGF23. Recent studies have shown that iron deficiency could increases FGF23 degradation and administration of parenteral iron products, such as ferric carboxymaltose increases FGF23 level (15,16,18). On the other hand, some studies have shown the association between Iron deficiencies with increase serum FGF23 and conversely iron transfusion resulted in decline FGF23 level (17). However, the effect of ferritin on FGF23 has not been studied in patients with thalassemia. As result of strong positive correlation between ferritin and FGF23 in our patients with thalassemia, it is possible that there is a direct stimulatory effect of ferritin on FGF23 secretion, which should be more investigated in future studies.

Another finding of the present study is that, in our patients with thalassemia affected by hypoparathyroidism and hyperphosphatemia, in spite of increase in FGF23 serum level, there was no rise in urinary phosphate excretion. Recently, limited numbers of studies were performed to evaluate the effect of PTH on FGF23 function in phosphate homeostasis (30). Yamashita et al. showed that FGF23 was increased in hypoparathyroidism and hyperphosphatemia, which was normalized along with serum phosphate normalization after parathyroid function improvement (31). The present study might suggest that FGF23 was not able to exert its full function in reducing serum phosphate in the absence of PTH. This could be due to PTH role in regulating FGF23 function.

In spite many interesting finding in this study we have some limitations, the first one was that the present study is a descriptive with small number of patient and not a clinical trial. Future clinical trials is suggested in patients with hypoparathyroidism after PTH treatment to investigate the FGF23 functions more accurately. Also, investigating FGF23 and PTH gene expression could lead to having more detailed about the physiology, cause and effects. Further studies are suggested to evaluate the role of ferritin on PTH, FGF23 in normal population with and without hypoparathyroidism.

Conclusions

The present study suggested that rise in FGF23 in patients with thalassemia, may be due to either stimulating effect of PTH and 1,25(OH)2D3 on FGF23 production, or might be due to direct stimulating effect of ferritin. In addition, it showed that in state of intact parathyroid function, it is possible that high ferritin level might enhance 1,25OH2D production through direct stimulation of alfa-1-hydroxilase or indirectly by increasing parathyroid hormone. Also, it seems that in patients with hypoparathyroidism due to lack of PTH action, the FGF23 is not able to exert its full function in reducing serum phosphorus level by its phosphaturic action. Future clinical trials should be conducted on patients with hypoparathyroidism after PTH treatment to investigate the FGF23 functions more accurately.

Declarations

Ethical approval

Shiraz University of Medical Sciences local ethic committee and vice-chancellor of research at SUMS approved this study with number 1396-01-01-15805. All the patients signed a written informed consent form after a session explaining the aim, method and goal of the study for each participant.

Consent for publication: All the patients signed a written informed consent form for publication of any data from their results after a session explaining the aim, method and goal of the study for each participant.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: Gholamhossein Ranjbar Omrani, Azita Salehifar, Seyed Reza Kassaee and Forough Saki declare that they have no conflict of interest.

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Authors' contributions

1. **Forough Saki:** concept, design, data gathering, data analysis, preparing the manuscript
2. **Azita Salehifar,** design, data gathering, preparing the manuscript
3. **Seyed Reza Kassaee,** data gathering, preparing the manuscript
4. **Gholam hosein Ranjbar Omrani**: Concept, data gathering, preparing the manuscript and the correspondence

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Disclosure Summary

1. Saki, A. Salehifar, SR.Kassaee and GHR. Omrani have nothing to declare.

Tables

Table 1. General characteristics and biochemical studies in both case and control groups and the related comparisons.

Variable	control	case	P value
Age (y)	25.7±5.1	26.9 ±3	0.32
Weight(Kg)	50.5±9.3	54.04±10.2	0.213
Height(cm)	157.5±8.8	162.4±10.6	0.089
BMI(Kg/m^2)	21.4±7.5	20.3±2.4	0.479
PTH(pg/ml)	55.6±15.7	13.9±4.6	<0.001
Ca(mg/dl)	10.1±0.9	8.7±1.6	0.001
Ph(mg/dl)	4.8±0.8	5.9±1.6	0.005
Alk(IU/L)	279.3±149	260,9±121.5	0.65
1,25(OH)2D3(pg/ml)	105.4±39,7	98.7±32,9	0.51
25 OHD(ng/ml)	21.6±4.5	23,8±7,8	0.22
FGF23(pg/L)	241,2±121	381,9±175	0.002
ferritin(ng/ml)	1690±548	1396±638	0.86
FE phosphorous	9.26±5	5.1±2.9	0.001
Urine Ca/Cr ratio	0.16±0.2	0.17±0.1	0.925

FGF_{23}: Fibroblast Growth Factor 23, Ph: phosphorus, Ca: Calcium, PTH: Parathyroid Hormone, FEph : fraction excretion of phosphorus, P = predictive value

Table 2a. Multiple linear regression analysis of covariates of $1,25(OH)_2D_3$ in both case and control groups; performed with method of enter

Group	Associated factor	Beta	P value
Control (R square = 0.534)	ferritin(ng/ml)	0.716	0.016
	FGF23(pg/L)	-0.012	0.96
	PTH (Pg/ml)	0.215	0.207
	Ca(mg/dl)	0.033	0.84
Case (R square = -0.081)	ferritin(ng/ml)	0.087	0.68
	FGF23(pg/L)	0.278	0.201
	PTH (Pg/ml)	0.036	0.88
	Ca(mg/dl)	0.168	0.503

FGF_{23}: Fibroblast Growth Factor 23, Ca: Calcium, PTH: Parathyroid Hormone

Table 2b. Multiple linear regression analysis of covariates of FGF23 in both case and control groups; performed with method of enter

Group	Associated factor	Beta	P value
Control (R square = 0.753)	ferritin(ng/ml)	0.722	0.002
	$1,25(OH)_2D_3$	0.076	0.72
	PTH (Pg/ml)	0.25	0.08
	ph(mg/dl)	-0.061	0.65
Case (R square = 0.137)	ferritin(ng/ml)	-0.197	0.38
	$1,25(OH)_2D_3$	0.264	0.21
	PTH (Pg/ml)	-0.144	0.51
	ph(mg/dl)	0.159	0.46

PTH: Parathyroid Hormone , ph:phosphorous

Figures

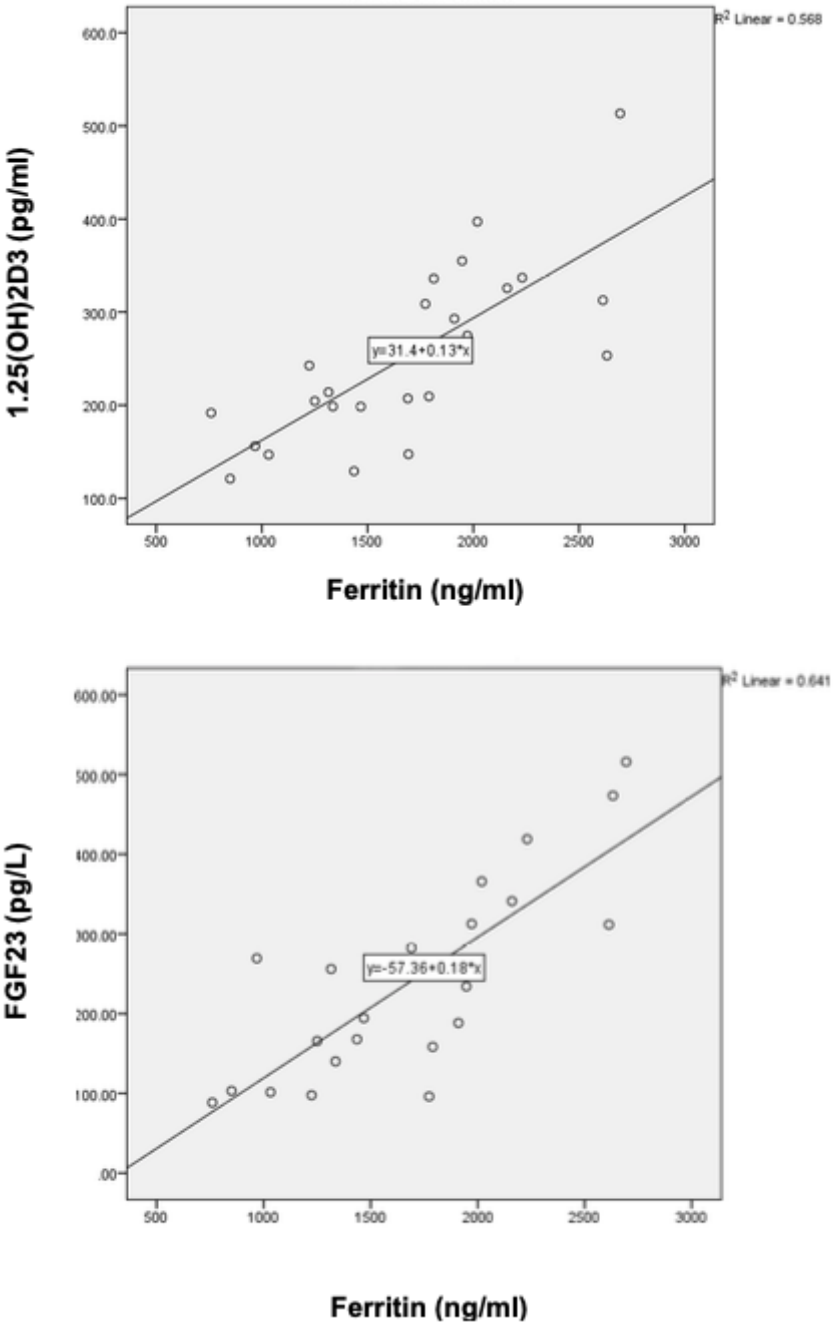

Figure 1

1a: The correlation between values of serum 1.25(OH)2D3 and ferritin in control group. 1b: The correlation between values of serum FGF23 and ferritin in control group.