J Musculoskelet Neuronal Interact 2014; 14(3):398-400

Hylonome

Clinical Quiz

Changes of fibroblast growth factor 23 (FGF23) levels following calcitriol treatment in a vitamin D deficient patient

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Keywords: Fibroblast Growth Factor 23, Vitamin D Deficiency, Calcitriol, Secondary Hyperparathyroidism

Clinical case

We report a case of a 62 year-old woman complaining of leg weakness, diffuse musculoskeletal pain, with difficulty in climbing stairs and standing up from the sitting position during the last six months. Her past medical history includes hypothyroidism and hypertension. Secondary hyperparathyroidism was diagnosed on the basis of biochemical tests [low-normal serum calcium and 25OHD levels and elevated PTH (Table 1)] due to low dietary calcium intake (160 mg per day) and to reduced sun exposure.

We aimed to investigate the effect of calcitriol and calcium administration on serum fibroblast growth factor - 23 (FGF23) levels in a vitamin D deficient woman.

The patient was evaluated before initiating treatment (baseline) and after one and three months. Assessment of bone metabolism at baseline [calcium, albumin, phosphate, creatinine, urinary excretion of calcium and phosphate, bone turnover markers, 25OHD, PTH, $1,25(OH)_2D$ and FGF23] was indicative of secondary hyperparathyroidism due to vitamin D deficiency (PTH: 86,1 pg/ml, 25OHD: 10,9 ng/ml). We opted to start treatment with 1 μg calcitriol and 1 gr of calcium carbonate ($CaCO_3$) per day, to investigate the effect of calcitriol and calcium administration on serum fibroblast growth factor-23 (FGF23) concentrations. Plasma intact FGF23 protein concentrations were measured by a commercial ELISA kit (Immutopics, Inc., San Clemente, CA, USA). The lowest concentration of human intact FGF-23 measurable was 1.0 pg/mL (assay sensitivity) and the highest concentration of human intact FGF23 measurable without dilution is the value of the highest standard (~650 pg/ml) and the intra-assay coefficient of variation was 4.4%.

One month later PTH had declined to high-normal levels, calcium and phosphate levels had increased, whereas FGF23 levels had not changed. Three months after the start of treatment, the patient reported a significant improvement of musculoskeletal pain. Her serum calcium and 24h urine calcium had normalised, while PTH levels declined further, whereas 25OHD levels increased and were just below the normal range of IOM, probably due to the seasonal variation (the last clinical evaluation and laboratory tests of the patient took place in summer). Serum concentrations of FGF23 and $1,25(OH)_2D$ had increased significantly from baseline (52% and 72% respectively) three months after initiation of treatment (Table 1).

Commentary

Fibroblast growth factor 23 (FGF23) was discovered as the causal factor in the pathogenesis of rare forms of hypophosphatemic rickets two decades ago. FGF23 is a 251 amino-acid protein that is proteolytically processed to N-terminal and C-terminal fragments, with only intact FGF23 protein having full biological activity. FGF23 is a phosphaturic protein that is produced mainly by osteocytes and mature osteoblasts. The major target tissue is the renal proximal tubule. High levels of FGF23 result in impaired renal tubular phosphate reabsorption due to suppression of expression of NaPi-IIa/c cotransporters that lead to urinary phosphate wasting, and low serum phosphate levels, which in the long term is associated with poor bone mineralization, osteomalacia and rickets, and is often accompanied by the presence of myopathy. In addition, FGF23 suppresses the expression of 1-α hydroxylase that converts 25OHD to $1,25(OH)_2D$, resulting in decreased intestinal phosphate and calcium absorption. At the same time, FGF23 enhances the expression of 24-hydroxylase which converts $1,25(OH)_2D$ to more hydrophilic metabolites, further aggravating the low serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. The biological

The authors have no conflict of interest.

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Edited by: P. Makras
Accepted 29 July 2014

Parameters / Time	Baseline	1 mo	3 mo	Reference ranges	D 0-1 (%)	D 0-3 (%)	D 1-3 (%)
Ca (mg/dl)	8,7	9,3	9,4	8,5-10,3	7	8	1
P (mg/dl)	2,7	3,4	3,7	2,7-4,5	26	37	9
Creat (mg/dl)	0,7	0,9	0,9	0,4-1,1	29	29	0
ALP (IU/L)	72	62	48	40-129	-14	-33	-23
BASP (μg/L)	19	19,3	11,4	<22.4	2	-40	-41
BGP (ng/ml)	24,3	26	24,6	20-48	7	1	-5
PTH (pg/ml)	86,1	54,9	38,1	15-65	-36	-56	-31
25OHD (ng/ml)	10,9	18,8	19,3	20-100	73	77	3
Cau24h (mg)	54	74	222	100-300	37	311	200
Pu24h (mg)	485	459	785	400-1300	-5	62	71
Creat u24h (mg)	890	814	1224	800-1800	-9	38	50
TMP/GFR (mg/dl)	2,3	2,9	3,2	2.6-4.4	26	39	11
$1,25(OH)_2D$ (pg/ml)	22,5	28,4	38,8	18-62	26	72	37
FGF23 (ng/ml)	17,6	15,8	26,8	8-50	-10	52	70

Conversion in International Unit: Ca: mg/dl * 0,25= mmol/L, P: mg/dl * 0,323= mmol/l, creat: mg/dl * 88,4= μmol/l, PTH: pg/ml * 0,1061= pmol/L, 25OHD: ng/ml * 2,5= nmol/L, Cau24h: mg/24h * 0.025= mmol/d, Pu24h: mg/dl * 0.032= mmol/d, Creat u24h: mg/dl* 0.0088= mmol/d, TMP/GFR: mg/dl *0,31= mmol/L, $1,25(OH)_2D$: pg/mL * 2,4= pmol/L.

Table 1. Biochemical test prior treatment, one and three months after treatment.

functions of FGF23 are mediated through the activation of FGF receptors, in the presence of klotho protein, as an obligate co-receptor. Klotho protein forms a complex with FGF receptors, the FGFR-Klotho receptor complex, required to activate FGF23 downstream signaling events. Recent studies have demonstrated that the parathyroid glands express the FGFR-Klotho receptor complex, suggesting that the parathyroid gland is a target for FGF23. FGF23 acts on the parathyroid glands and activates the MAPK pathway resulting in a decrease in gene expression and PTH secretion[1,2] (Figure 1).

Both experimental and clinical data indicate that calcitriol, phosphorus and PTH independently increase circulating FGF23 levels. The effect of calcitriol is probably mediated by the VDR, since there is a VDRE upstream to the promoter of the FGF23 gene, at least in the mouse and rat. The effect of phosphate on circulating FGF23 seems to be delayed by a couple of days and probably involves a yet unidentified phosphate sensor resulting in increased FGF23 expression or altered processing. PTH or activation of the PTH signaling pathway is associated with increased FGF23 levels. Finally calcium seems to modulate circulating FGF23 since hypocalcemia is associated with reduced FGF23 levels, independent of PTH or vitamin D. Given the close association between calcitriol, PTH, phosphorus and calcium, it is possible that the hierarchy and the magnitude of their effect on circulating FGF23 differs between normal individuals and patients with metabolic bone disease such as secondary hyperparathyroidism[2,3].

In our patient one month after the start of treatment with calcitriol and calcium carbonate, PTH levels had returned to normal, whereas FGF23 levels remained relatively constant, despite the administration of calcitriol. It is possible that vitamin D deficiency resulting in low-normal calcium and phosphate levels blunted the ability of calcitriol to increase FGF23 levels even in the presence of high PTH.

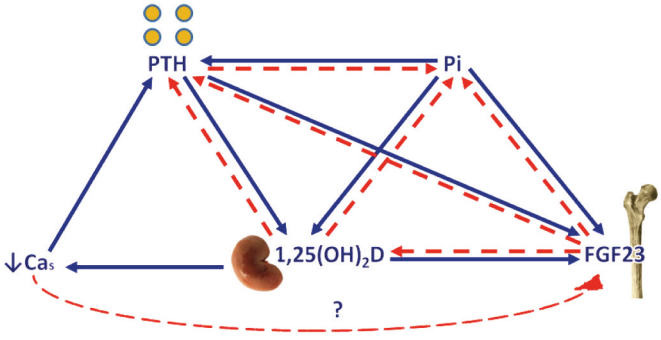

Figure 1. The principal biological mechanisms that influence phosphate homeostasis and FGF23 secretion. PTH and $1,25(OH)_2D$ are also regulated by serum calcium. Solid lines indicate stimulation of hormonal production [PTH, $1,25(OH)_2D$] or increase in serum level (Ca, Pi, FGF23). Broken lines indicate inhibition of hormonal production or decrease in serum level.

Secondary hyperparathyroidism due to vitamin D insufficiency is associated with increased urinary phosphate excretion that can lead to hypophosphatemia or low normal serum phosphate levels. Hypophosphatemia inhibits FGF23 secretion from osteoblasts - osteocytes and enhances expression of 1-a hydroxylase. Thus reduced levels of FGF23 in patients with secondary hyperparathyroidism may be attributed to vitamin D deficiency, hypophosphatemia and low normal serum calcium levels. The decreased FGF23 levels may have a favorable action on bone mineralization.

Indeed an experimental study in rats with normal renal function fed a diet low in calcium and vitamin D, resulting in hypocalcemia, was associated with low FGF23 despite high parathyroid hormone (PTH) and calcitriol levels. The serum levels of FGF23 were lower in parathyroidectomized rats, which had low serum of calcium and calcitriol levels despite high serum phosphate concentrations, than in wild- type rats in another study. Moreover, the decrease of PTH levels following calcitriol and calcium administration might have contributed to the blunted FGF23 response, given the positive effects of PTH on circulating FGF23[4,5].

In our patient three months after initiating treatment serum calcium, phosphate and PTH levels had normalized while serum concentrations of FGF23 had increased by 69% (Table 1). This finding supports previous data on the stimulating effect of calcitriol on circulating FGF23 levels. However, we cannot exclude the possibility that the effect of calcitriol administration might have been indirect, given the concurrent calcium administration and the increase in calcium and phosphate levels. Experimental studies in parathyroidectomized rats, suggest that the administration of elemental calcium (*per os* or *i.v.*) is associated with an increase in serum FGF23 levels. Also in parathyroidectomized rats the replacement with calcitriol is sufficient to normalize serum calcitriol and calcium levels resulting in elevation of FGF23 levels.

These results suggest that the presence of secondary hyperparathyroidism results in a blunted FGF23 response following the concurrent administration of calcitriol and calcium, in presence of low normal calcium and phosphorus levels. This response might be a protective mechanism to prevent further decrease in phosphate and calcitriol levels, which could aggravate hypocalcemia and hypophosphatemia and possibly bone disease. The increase in FGF23 levels following correction of secondary hyperparathyroidism supports the existence of bone-kidney endocrine axis.

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Questions

1. FGF23 protein is mainly produced by
A. Parathyroid glands
B. Proximal tubule of the kidney
C. Osteocytes and osteoblasts

Critique

FGF-23 protein is a key regulator of phosphorus and vitamin D metabolism. This phosphaturic factor is produced primarily in bone, specifically by the osteocytes and osteoblasts in mineralized bone.
The correct answer is C.

2. High FGF23 serum concentrations inhibit
A. 25-hydroxyvitamin D 1-alpha-hydroxylase (CYP27B1)
B. 25-hydroxyvitamin D-24-hydroxylase (CYP24A1)
C.Vitamin D 25-hydroxylase (CYP2R1)

Critique

FGF23 acts in the proximal tubule of the kidney suppressing 25-hydroxyvitamin D 1-alpha-hydroxylase (CYP27B1) expression and at the same time, enhances the expression of 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) which converts $1,25(OH)_2D$ to more hydrophilic metabolites. These effects result in low serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$].
The correct answer is A.