CLINICAL STUDY

Fibroblast growth factor (FGF)-23 in patients with primary hyperparathyroidism

Hiroyuki Yamashita, Takeyoshi Yamashita¹, Masashi Miyamoto¹, Takashi Shigematsu², Junichiro James Kazama³, Takashi Shimada¹, Yuji Yamazaki¹, Seiji Fukumoto⁴, Masafumi Fukagawa⁵ and Shiro Noguchi

Noguchi Thyroid Clinic and Hospital Foundation, Oita, Japan, ¹Pharmaceutical Research Labs, KIRIN Brewery Co. Ltd, Takasaki, Japan,

²Division of Nephrology, Tokyo-Jikeikai Medical School Aoto Hospital, Katsushika, Japan, ³Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medicine and Dental Research, Niigata, Japan, ⁴Division of Laboratory Medicine, Tokyo University Hospital, Bunkyo, Japan and ⁵Division of Dialysis and Metabolism, Kobe, Japan

(Correspondence should be addressed to H Yamashita, Noguchi Thyroid Clinic and Hospital Foundation, 6-33 Noguchi-Nakamachi, Beppu Oita 874-0932, Japan; Email: yama@noguchi-med.or.jp)

Abstract

Objective: We aimed to determine the serum level of fibroblast growth factor-23 (FGF-23) in patients with primary hyperparathyroidism (pHPT) to understand its physiological role in the disorder.

Patients and methods: Ninety-eight patients with pHPT who underwent parathyroidectomy formed the study group. We also measured serum FGF-23 in 11 of these patients on postoperative day 6.

Results: Serum FGF-23 levels were significantly higher in pHPT patients than in healthy controls (35.6 ± 17.8 ng/l vs 28.9 ± 11.2 ng/l (mean $\pm$ s.d.); $P < 0.001$ (Pearson's correlation coefficient)), but there was no significant difference in the serum FGF-23 level between pHPT patients with normal renal function (creatinine clearance (Ccr) of ≥ 70 ml/min) and healthy controls. Serum FGF-23 correlated positively with serum calcium ($P < 0.0001$) and intact parathyroid hormone (PTH) ($P < 0.01$), and negatively with Ccr ($P < 0.001$), serum phosphate ($P < 0.05$), and serum 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) ($P < 0.05$). Multiple linear regression analysis of factors potentially determining serum FGF-23 levels in pHPT patients showed serum calcium ($P < 0.01$) and Ccr ($P < 0.001$) to be significant predictors. The serum levels of FGF-23 did not change after parathyroidectomy despite the normalization of serum calcium values. Multiple linear regression analysis revealed that serum FGF-23 was not a significant predictor of serum phosphate or $1,25(\text{OH})_2\text{D}$ in pHPT patients.

Conclusions: FGF-23 may not play a significant role in regulating phosphate or $1,25(\text{OH})_2\text{D}$ in pHPT patients, especially in those with normal renal function. Further studies are warranted to determine the role of FGF-23 in renal insufficiency or failure.

European Journal of Endocrinology 151 55–60

Introduction

The kidney plays a major role in maintaining the normal level of serum phosphate, which is essential for many biologic processes, including mineralization of cartilage and bone matrix. Renal phosphate-wasting disorders cause hypophosphatemic rickets, or osteomalacia. Of these, X-linked hypophosphatemia (XLH) (1, 2), autosomal dominant hypophosphatemic rickets (ADHR) (3, 4), and tumor-induced osteomalacia (TIO) (4–6) show very similar clinical features: hypophosphatemia secondary to reduced proximal renal tubular phosphate reabsorption, low or inappropriately normal serum $1,25(\text{OH})_2\text{D}$ concentrations, normal serum levels of calcium and parathyroid hormone, and defective bone mineralization induced by a putative active hormone 'phosphatonin' (7, 8). Phosphatonin regulates phosphate homeostasis via a parathyroid hormone (PTH)-independent mechanism (9). Recent

studies showed that phosphatonin may in fact be fibroblast growth factor (FGF)-23 (7, 8), although other candidate factors have been identified, including matrix extracellular phosphoglycoprotein (10) and secreted frizzled-related protein (11). Overproduction of FGF-23 by tumors (as in TIO), mutations that resist FGF-23 cleavage (as in ADHR), and loss-of-function mutations in the responsible gene for XLH potentially increase serum levels of FGF-23. Riminucci *et al.* (12), Yamamoto *et al.* (13), and Bloch *et al.* (14) recently reported increased circulation levels of FGF-23 in patients with McCune–Albright syndrome, which is sometimes complicated by hypophosphatemic rickets.

Primary hyperparathyroidism (pHPT) is associated with hypophosphatemia and osteoporosis induced by elevated PTH; however, pHPT is characterized by higher levels of serum calcium and $1,25(\text{OH})_2\text{D}$ than those in TIO, ADHR, or XLH. Singh and Kumar (15) recently reported elevated concentrations of the

C-terminal FGF-23 in patients with pHPT, but the physiological role of the elevated C-terminal fragment is unclear. We previously developed a sandwich enzyme-linked immunosorbent assay (ELISA) for human FGF-23 that uses two monoclonal antibodies to FGF-23 to measure biologically active full-length FGF-23 and found elevated FGF-23 levels in patients with TIO and XLH (16). The aim of the present study was to determine the serum FGF-23 levels in patients with pHPT to understand its physiological role in the disorder.

Materials and methods

Patients

One hundred forty-nine Japanese patients with pHPT underwent parathyroidectomy at Noguchi Thyroid Clinic and Hospital Foundation between January 2000 and June 2002. Frozen serum samples obtained on admission were available for 102 of these patients. Written informed consent for measurement of FGF-23 was obtained from 87 of these patients. In addition, we prospectively studied sequential changes in serum FGF-23 concentrations in 11 patients after parathyroidectomy. These patients also provided informed consent. Therefore, our study group comprised 98 pHPT patients. The study protocol was approved by the Noguchi Thyroid Clinic Ethical Committee.

Laboratory tests

Blood and urine samples were collected after an overnight fast on the day after admission, on postoperative days 1 and 6. Serum levels of alkaline phosphatase, total calcium, albumin and inorganic phosphate were measured by routine automated procedures. Serum intact parathyroid hormone (iPTH) was measured by the ECLusys 2010 (Roche Diagnostics, Mannheim, Germany). Serum 25-hydroxyvitamin D (25OHD) was measured by a competitive protein-binding assay involving the use of high-performance liquid chromatography purification (17); intra- and interassay coefficients of variation (CVs) were 5.8% and 12.6% respectively. Serum 1,25(OH)₂D levels were measured by a receptor-binding assay with bovine mammary gland receptor (18); intra- and interassay CVs were 8.1% and 8.3% respectively. Serum bone gla protein (BGP) was measured with a Mitsubishi Yuka BGP IRMA kit (Tokyo, Japan) that used a mouse monoclonal antibody to human BGP. Serum FGF-23 levels (reference range, 10–50 ng/l) were determined by sandwich ELISA, as previously reported (16); intra- and interassay CVs for this assay were less than 5.0%. The serum FGF-23 level in 104 healthy controls ranged from 8.2 to 54.3 ng/l (mean $\pm$ s.d.; 28.9 $\pm$ 11.2) (16).

Urinary calcium and phosphorus levels were assessed by atomic absorption spectrometry, and the excretion rates were determined by 24-h urine collection.

Creatinine clearance (Ccr) was calculated on the basis of ml/min per 1.48 m² of body surface area. Normal renal function was assumed to be a Ccr of $\geq$ 70 ml/min (19). ELISA was used to assess bone resorption by measuring urinary excretion of type 1 collagen cross-linked N-telopeptides (NTx) (Osteomark, Ostex International, Seattle, WA, USA); results were normalized for the urinary creatinine concentrations assessed by a standard colorimetric method.

Statistical analysis

Data are expressed as mean $\pm$ s.d. unless otherwise indicated. Student's *t*-test was used to compare laboratory data. Categorical data were analyzed with Pearson's chi-square test. Correlation was determined by calculating Pearson's correlation coefficient. *P* < 0.05 was considered significant. Statistical analyses were performed with SAS-JMP, version 4.0.2, software (SAS Institute, Cary, NC, USA).

Results

The clinical and biochemical patient data are summarized in Table 1. Of the 83 women patients, 65 were postmenopausal, but none were undergoing estrogen therapy. Patient histories included nephrolithiasis (*n* = 28), bone fracture (*n* = 8), and complaints of severe fatigue (*n* = 3). Sixty-four (65%) had asymptomatic pHPT. The mean serum calcium level was 2.75 $\pm$ 0.21 mmol/l, and the mean weight of the resected parathyroid was 954 $\pm$ 1139 mg.

The serum FGF-23 level in 98 patients with pHPT was significantly higher than in 104 healthy controls (35.6 $\pm$ 17.8 ng/l vs 28.9 $\pm$ 11.2 ng/l; *P* < 0.001).

Table 1 Preoperative clinical and biochemical data for pHPT patients.

	Value	Reference range
Age (years)	57 $\pm$ 13	
Sex (F/M)	83/15	
Serum calcium (mmol/l)	2.76 $\pm$ 0.21	2.20–2.54
Serum phosphate (mmol/l)	0.96 $\pm$ 0.16	0.81–1.45
Serum creatinine (μ mol/l)	62 $\pm$ 18	44–100
Serum BUN (mmol/l)	4.9 $\pm$ 1.3	3.2–6.8
Serum BGP (ng/ml)	19 $\pm$ 13	2.5–13
Serum alkaline phosphatase (IU/l)	143 $\pm$ 70	43–155
iPTH (pmol/l)	18 $\pm$ 12	1.1–7.4
25OHD (nmol/l)	36 $\pm$ 13	25–137
1,25-(OH) ₂ D (pmol/l)	173 $\pm$ 65	48–144
Urinary NTX (nmol BCE/mmol creatinine)	145 $\pm$ 109	14–100
Parathyroid gland weight (mg)	954 $\pm$ 1139	

Mean $\pm$ s.d. data are shown.

BUN, blood urine nitrogen; BGP, bone GLA protein; iPTH, intact parathyroid hormone; 25OHD, 25-hydroxyvitamin D; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; NTx, N-telopeptide.

Table 2 Correlation between FGF-23 and other variables in patients with pHPT.

Clinical factor	<i>r</i>	<i>P</i> value
Age	0.168	0.10
Serum calcium	0.401	<0.0001
Serum phosphate	-0.228	<0.05
Serum ALP	-0.116	0.27
Serum creatinine	0.381	<0.0001
Serum BUN	0.226	<0.05
Serum intact PTH	0.316	<0.01
1,25(OH) ₂ D	-0.227	<0.05
TRP (%)	-0.578	<0.0001
TmP/GFR	-0.356	<0.001
Phosphate excretion rate (%)	0.578	<0.0001
Creatinine clearance	-0.411	<0.001
Parathyroid weight	0.003	0.98

pHPT, primary hyperparathyroidism; BUN, blood urine nitrogen; PTH, parathyroid hormone; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; TRP, tubular reabsorption of phosphate; TmP/GFR, maximum tubular reabsorption of phosphate per liter of glomerular filtrate.

To investigate the factors determining serum FGF-23 levels in pHPT patients, associations between specific variables and serum FGF-23 levels were tested with Spearman's coefficient (Table 2). Multiple linear regression analysis showed serum calcium ($P < 0.01$) and Ccr ($P < 0.001$) to be significant predictors of serum FGF-23 levels (Fig. 1 and Table 3). Of the three significant factors relating to renal function (BUN, serum creatinine and Ccr), only Ccr was used for the multiple linear regression analysis; 1,25(OH)₂D, tubular reabsorption of phosphate (TRP) (%) and maximum TRP per liter of glomerular filtrate (TmP/GFR) were also excluded because these are considered physiological sequences of FGF-23. The 98 patients were assigned to one of two groups based on Ccr level: a renal insufficiency group ($n = 23$) in which Ccr of patients was < 70 ml/min and a normal renal function group ($n = 75$) in which Ccr was ≥ 70 ml/min. There were 12 patients (12%) with a FGF-23 level above the reference range: five (7%) in the normal renal function group and seven (30%) in the renal insufficiency group. Table 4 shows clinical and biochemical data of pHPT patients per group. Serum levels of FGF-23 and calcium were significantly higher in the renal insufficiency group than in the normal renal function group; however, serum level of 1,25(OH)₂D, 24-h urinary phosphate excretion and TRP (%) were significantly lower in the renal insufficiency group than in the normal function group. There was no significant difference in the serum FGF-23 level between the healthy controls and the pHPT patients with normal renal function (28.9 ± 11.2 ng/l vs 32.1 ± 10.5 ng/l).

Because calcium was a significant determinant of FGF-23, we also measured serum FGF-23 after parathyroidectomy. Serum FGF-23 levels before surgery and at postoperative day 6 were 24.7 ± 11.3 ng/l and 22.2 ± 7.7 ng/l (NS) respectively. The serum

Figure 1 Correlations between serum fibroblast growth factor (FGF)-23 and serum calcium (A), serum phosphate (B), serum 1,25-dihydroxyvitamin D (C), creatinine clearance (D), and intact PTH (E) in patients with primary hyperparathyroidism.

calcium level decreased from 2.79 ± 0.14 mmol/l to 2.23 ± 0.14 mmol/l on postoperative day 6.

The factors determining serum levels of phosphate and 1,25(OH)₂D in patients with pHPT are shown on Tables 5 and 6. Multiple linear regression analysis showed serum iPTH to be a significant predictor of the serum phosphate, and serum iPTH and Ccr to be significant predictors of the 1,25(OH)₂D level. TRP, phosphate excretion rate, and TmP/GFR were excluded

Table 3 Multivariate analysis of biochemical factors FGF-23 in patients with pHPT.

Biochemical factor	<i>P</i> value
Serum calcium	<0.01
Serum phosphate	0.265
Serum intact PTH	0.881
Creatinine clearance	<0.001

pHPT, primary hyperparathyroidism; PTH, parathyroid hormone.

Table 4 Clinical and biochemical data of pHPT patients per group.

Clinical factor	Renal insufficiency group (n = 23)	Normal renal function group (n = 75)	P value
Age (years)	64±11	55±13	<0.005
FGF-23	48±29	32±11	<0.001
Serum calcium (mmol/l)	2.84±0.27	2.73±0.18	<0.05
Serum phosphate (mmol/l)	0.94±0.19	0.97±0.15	0.444
Serum creatinine (μmol/l)	79±26	57±11	<0.0001
Creatinine clearance	57±12	98±20	<0.0001
iPTH (pmol/l)	22±16	17±10	0.057
1,25(OH) ₂ D (pmol/l)	144±59	182±64	<0.05
24 h urinary phosphate excretion (mmol)	18±4	21±4	<0.0001
TRP (%)	77±9	84±5	<0.0001
TmP/GFR	2.3±0.7	2.5±0.5	0.059

FGF-23, fibroblast growth factor-23; pHPT, primary hyperparathyroidism; BUN, blood urine nitrogen; PTH, parathyroid hormone; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; TRP, tubular reabsorption of phosphate; TmP/GFR, maximum tubular reabsorption of phosphate per liter of glomerular filtrate. Mean±S.D. data are shown. Statistical significance of between group difference determined by Student's *t*-test.

Table 5 Correlation between serum phosphate levels and other variables in patients with pHPT.

Clinical factor	<i>r</i>	P value
FGF-23	-0.228	<0.05
Serum calcium	-0.382	<0.0001
Serum intact PTH	-0.464	<0.0001
1,25(OH) ₂ D	-0.064	0.531
TRP (%)	0.611	<0.0001
TmP/GFR	0.967	<0.0001
Creatinine clearance	0.077	0.456

FGF-23, fibroblast growth factor-23; pHPT, primary hyperparathyroidism; PTH, parathyroid hormone; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; TRP, tubular reabsorption of phosphate; TmP/GFR, maximum tubular reabsorption of phosphate per liter of glomerular filtrate.

Table 6 Correlation between serum 1,25(OH)₂D levels and other variables in patients with pHPT.

Clinical factor	<i>r</i>	P value
FGF-23	-0.227	<0.05
Serum calcium	0.162	0.118
Serum phosphate	-0.064	0.531
Serum 25OHD	0.012	0.908
Serum intact PTH	0.299	<0.05
TRP (%)	0.133	0.914
TmP/GFR	-0.002	0.984
Creatinine clearance	0.323	<0.005

FGF-23, fibroblast growth factor-23; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; pHPT, primary hyperparathyroidism; 25OHD, 25-dihydroxyvitamin D; PTH, parathyroid hormone; BUN, blood urine nitrogen; TRP, tubular reabsorption of phosphate; TmP/GFR, maximum tubular reabsorption of phosphate per liter of glomerular filtrate.

from multiple linear regression analysis because these are considered physiological sequences of PTH.

Discussion

In a previous study, we cloned FGF-23 as a causative factor of TIO and showed that FGF-23 causes hypophosphatemia, osteomalacia, and decreased

1,25(OH)₂D level *in vivo* (4). Yamashita *et al.* (20) reported that FGF-23 inhibits renal phosphate reabsorption by activation of the mitogen-activated protein kinase pathway. pHPT is representative of diseases with abnormal phosphate and vitamin D metabolism. At the beginning of our present study, we hypothesized that if serum FGF-23 plays a significant role in the homeostasis of serum phosphate, it will be suppressed in patients with pHPT, because hypersecretion of PTH causes hypophosphatemia independently of FGF-23. However, serum FGF-23 levels in pHPT patients were significantly higher than those in controls, with significant negative correlation between serum levels of FGF-23 and phosphate. Singh and Kumar (15) recently reported slightly elevated concentrations of C-terminal region of FGF-23 in 11 patients with pHPT, but it was not significant compared with 11 healthy controls. Weber *et al.* (2) observed that FGF-23 levels assayed by C-terminal ELISA increase with age in healthy persons. We did not observe this relation in control subjects or patients with pHPT, but the differences may be explained by altered metabolism and/or clearance of the C-terminal fragments.

Serum calcium and Ccr were significant determining factors of serum FGF-23 levels, according to multiple linear regression analysis findings in patients with pHPT. Serum FGF-23 level did not change after parathyroidectomy despite the normalization of serum calcium and PTH, suggesting that the serum FGF-23 level is not influenced by the serum calcium level in patients with pHPT. Finally, renal function was the only significant determining factor of serum FGF-23 in the patients with pHPT. In accordance with reports by Weber *et al.* (2) and Pande *et al.* (21), we found elevated FGF-23 levels in control patients with renal insufficiency and extremely high FGF-23 levels in end-stage renal failure patients who underwent total parathyroidectomy for secondary HPT (unpublished observation). We also reported elevated FGF-23 levels in rats with antglomerular

basement membrane nephritis, subtotal nephrectomy, or adenine-induced renal failure (22).

We did not find any difference in serum FGF-23 levels between healthy controls and pHPT patients with normal renal function, nor did we detect any significant relations between serum FGF-23 levels and Ccr, TRP%, and serum levels of iPTH, phosphate, and $1,25(\text{OH})_2\text{D}$ in pHPT patients with normal renal function. Therefore, elevated serum FGF-23 in patients with pHPT is probably due to renal insufficiency.

The physiological role of elevated serum FGF-23 in renal insufficiency and failure should be considered. PTH and the vitamin D-endocrine system play key roles in the phosphorus balance: PTH decreases renal phosphate reabsorption in the proximal tubule by inhibiting brush-border membrane Na-Pi cotransport activity, and $1,25(\text{OH})_2\text{D}$ is thought to increase both renal phosphate reabsorption and intestinal phosphate absorption (23). Therefore, with dietary phosphorus excess, PTH increases and $1,25(\text{OH})_2\text{D}$ decreases, whereas the opposite occurs with dietary phosphate depletion (23). We recently reported that clinico-pathologic features of pHPT were notably influenced by even moderate renal insufficiency (24). We observed a higher phosphate excretion rate but lower 24-h urinary phosphate excretion in patients with pHPT and renal insufficiency (Ccr of $<70\text{ ml/min}$) than in patients with normal renal function (25). These were confirmed by the present study (Table 4). The results imply that, as GFR declines, the serum FGF-23 level increases to maintain a normal serum phosphate concentration by increasing fractional phosphate excretion and inhibiting the synthesis of $1,25(\text{OH})_2\text{D}$. However, the biosynthetic failure of the diseased kidney that produces $1,25(\text{OH})_2\text{D}$ is a causative mechanism of secondary HPT, as well as hypocalcemia and hyperphosphatemia. Further studies are warranted to study the role of FGF-23 during the development of secondary HPT.

Typical features of pHPT are hypophosphatemia and $1,25(\text{OH})_2\text{D}$ elevation as well as hypercalcemia induced by excess PTH secretion. Serum FGF-23 levels correlated negatively with serum levels of both phosphate and $1,25(\text{OH})_2\text{D}$ in patients with pHPT; however, multiple-regression analysis identified serum PTH as the strongest predictive valuable for phosphate and $1,25(\text{OH})_2\text{D}$ levels. Considering that renal function was the major predictive variable for serum FGF-23, FGF-23 may not have a significant role in the regulation of phosphate and $1,25(\text{OH})_2\text{D}$, due to the greater influence of PTH on patients with pHPT, especially those with normal renal function.

Serum FGF-23 levels correlated negatively with phosphate levels and increased with the reduction in Ccr; however, FGF-23 appears not to have a significant role in the regulation of phosphate and $1,25(\text{OH})_2\text{D}$ in patients with pHPT, especially if they have normal renal function. Further studies on the role of FGF-23 in renal insufficiency and failure are warranted.

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Received 30 January 2004

Accepted 7 April 2004