RESEARCH

Perioperative changes of FGF23 in patients undergoing surgery for primary hyperparathyroidism

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Abstract

Background: Fibroblast growth factor 23 (FGF23) is a key regulator of urine phosphate excretion. The aim of the study was to investigate the perioperative (intraoperative and postoperative) changes of plasma intact and C-terminal FGF23 (iFGF23, cFGF23) concentrations in patients with primary hyperparathyroidism (pHPT) submitted to surgery.

Materials and methods: The study involved 38 adult patients with pHPT caused by adenoma. Parathyroid hormone (PTH) levels were investigated intraoperatively (just before the incision and 10 min after adenoma excision). cFGF23, iFGF23, phosphate, estimated glomerular filtration rate (eGFR), and procollagen type 1 N-terminal propetide (P1NP) were measured intraoperatively and postoperatively (next day after the surgery).

Results: PTH levels decreased intraoperatively (13.10 pmol/L vs 4.17 pmol/L, $P < 0.0001$). FGF23 levels measured intraoperatively were at the upper level of reference interval. cFGF23 decreased postoperatively compared with the values measured just before the incision (cFGF23: 89.17 RU/mL vs 22.23 RU/mL, $P < 0.0001$). iFGF23 decreased as well, but the postoperative values were low. Postoperative inorganic phosphate values increased (1.03 mmol/L vs 0.8 mmol/L, $P = 0.0025$). We proved significant negative correlation of perioperative FGF23 with inorganic phosphate (cFGF23: Spearman's r = −0.253, $P = 0.0065$; iFGF23: Spearman's r = −0.245, $P = 0.0085$). We also found that FGF23 values just before incision correlated with eGFR (cystatin C) (cFGF23: Spearman's r = −0.499, $P = 0.0014$; iFGF23: Spearman's r = −0.413, $P = 0.01$).

Conclusion: Intraoperative iFGF23 and cFGF23 did not change despite PTH decreased significantly. cFGF23 and iFGF23 significantly decreased 1 day after parathyroidectomy and are associated with increase of inorganic phosphate in pHPT patients. cFGF23 and iFGF23 just before incision correlated with eGFR (cystatin C). Similar results found in both iFGF23 and cFGF23 suggest that each could substitute the other.

Introduction

About 80–90% of primary hyperparathyroidism (pHPT) cases are caused by parathyroid adenoma. Long-term increased parathyroid hormone (PTH) secretion leads to a generalized disorder of calcium, phosphate and bone metabolism. The most relevant information on parathyroid activity is given by the determination of plasma intact PTH concentration (1). Decreased PTH concentrations after parathyroidectomy indicate successful adenoma excision.

Intraoperative determination of PTH is a frequently used indicator of successful parathyroidectomy; nevertheless, the information about changes of fibroblast growth factor 23 (FGF23) levels is scarce (2, 3). FGF23 is a phosphaturic peptide promoting phosphate excretion in urine. It is a member of the class of factors called phosphatonins which regulate the phosphate metabolism. It has a key role in maintaining normophosphatemia (4). FGF23 is highly expressed in osteocytes (5, 6, 7), some authors also mention osteoblastic expression, and it is released from the bone and, under pathophysiological conditions, from other organs, including the kidney and the liver (8, 9). FGF23 synthesis is stimulated by phosphate, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), PTH, calcium and other factors (6, 10, 11). There are limited data on the elimination of FGF23 from the body, but renal failure causes significant increase of plasma C-terminal fragments. These fragments are cleared almost twice as slow as intact FGF23. FGF23 is not connected to the membrane, and therefore, it acts like a hormone rather than a cell surface receptor. The primary target organs of FGF23 are the kidneys and the parathyroid glands (2, 11). The target organs for FGF23 are defined by the coexpression of the transmembrane protein αKlotho and FGF receptor (FGFR), and interactions between αKlotho and FGFR are necessary to mediate FGF23 signaling (12). The main source of αKlotho is the kidney, and the amount and concentration of αKlotho progressively decreases with decreasing renal function (13, 14, 15). Both target organs play role in secondary hyperparathyroidism (sHPT), which is a common complication of patients with chronic kidney disease (13, 14, 15, 16). In addition to pHPT and sHPT, diseases associated with abnormal FGF23 levels include X-linked hypophosphatemic rickets, autosomal dominant and autosomal recessive hypophosphatemic rickets, tumor-induced oncogenic osteomalacia, etc. (10, 17, 18). Primary hyperparathyroidism is associated with hypophosphatemia and hyperphosphaturia, which correlates with elevated serum PTH concentrations with higher concentrations of calcium in both serum and urine and 1,25(OH)$_2$D in the serum (16).

The aim of this study was to investigate perioperative (intraoperative and postoperative) plasma iFGF23 and cFGF23 levels, to reveal possible differences in perioperative changes and to assess the correlations with markers of mineral, renal and bone metabolism (PTH, inorganic phosphate, total calcium, eGFR creatinine, estimated glomerular filtration rate (eGFR) cystatin C and procollagen type 1 N-terminal propetide (P1NP)) in patients with pHPT subjected to parathyroidectomy.

Patients and methods

The study includes 38 patients (31 females, aged from 24 to 77 years and 7 males, aged from 38 to 75 years) with primary hyperparathyroidism subjected to parathyroidectomy. The diameter of the adenoma ranged from 5 to 30 mm. Right-sided parathyroidectomy was performed in 17 patients (7 patients with upside and 10 with downside), left-sided parathyroidectomy was performed in 13 patients (1 patient with upside, 10 patients with downside and 2 patients with both sides), 3 patients had adenoma in the mediastinum and 5 patients underwent complete thyroidectomy. Struma nodosa and thyrotoxicosis occurred in seven patients, and hypothyerosis and type 2 diabetes mellitus occurred in three patients. One patient was previously subjected to unilateral nephrectomy due to the nephroblastoma. In three patients, the second parathyroidectomy due to the adenoma was performed. Informed consent was obtained from all participants enrolled in the study. The blood samples were collected to K$_3$-EDTA tubes. Plasma concentrations of C-terminal FGF23 (cFGF23) (Immutopics Inc., San Clemente, CA, USA) (19), intact FGF23 (iFGF23) (Immutopics Inc.) (20), inorganic phosphate, creatinine, cystatin C and P1NP were measured just before the incision, immediately (10 min) after parathyroid adenoma excision and on postoperative day 1. Plasma concentrations of PTH were measured only intraoperatively (just before the incision and 10 min after adenoma excision). Plasma PTH values on postoperative day 1 were not measured. Serum calcium levels were retrieved from medical notes.

Additional calculations of eGFR cystatin C by Grubb equation and eGFR creatinine by CKD-EPI were performed. Thirty-seven patients had normal preoperative plasma creatinine levels ranging from 25 to 92 μmol/L, and one patient who underwent nephrectomy due to the nephroblastoma had elevated plasma creatinine level (112.3 μmol/L) and slightly decreased eGFR (CKD-EPI) of 86 mL/min/1.73 m^2 (reference eGFR (CKD-EPI) values ≥90 mL/min/1.73 m^2). Plasma cFGF23 and iFGF23 concentrations were measured using commercially available immunoassay ELISA kit (Immutopics Inc.). The analytical parameters of the cFGF23 kit were detection limit 1.5 RU/mL and working range 1.5–660 RU/mL (intraassay CV = 1.4–2.4%, interassay CV = 2.4–4.7%) and reference interval 21.6–91 RU/mL (21). The analytical parameters of the iFGF23 kit were detection limit 1.5 pg/mL, working range 1.5–660 pg/mL (intraassay CV = 2.0–4.1%, interassay CV = 3.5–9.1%) (20) and reference interval 11.7–48.6 pg/mL (21). Commercial ELISA determines two types of FGF23. Antibodies of iFGF23 tests detect epitopes within the amino-terminal and the carboxyl-terminal

portions. In the cFGF23 assay, antibodies detect two different epitopes in the carboxyl-terminal portion. Therefore, the cFGF23 assay measures both the intact molecule and the large carboxyl terminal fragment of human FGF23 (22) (Fig. 1). The conversion factor between RU/mL (cFGF23 assay) and pg/mL (iFGF23 assay) is expressed as: (cFGF23 (RU/mL) = 2.1 × iFGF23 (pg/mL) − 14.0) (23).

First ELISA is focused on full-length intact FGF23 (32 kDa). A murine MAB and an affinity-purified goat polyclonal antibody have been selected to detect the epitope within the amino-terminal and carboxy-terminal portion of FGF23. The MAB is biotinylated for capture and the other antibody is conjugated with the enzyme horseradish peroxidase (HRP) for detection (20). Second ELISA is focused on carboxy-terminal FGF23 fragment (aminoacids 1–24). Two affinity-purified goat polyclonal antibodies have been selected to detect the epitope within the carboxyl-terminal portion of FGF23. One antibody is biotinylated for capture and the other antibody is conjugated with the enzyme HRP for detection (19).

Additional biomarkers (cystatin C, creatinine and inorganic phosphate) were assayed by commercially used methods (immunoturbidimetry, enzymatic creatinase and end point phosphomolybdate) on Advia 1800 Siemens biochemistry analyzer. Rapid 9-min third generation Bio-intact PTH STAT assay (Cobas e601, Roche) was used to investigate plasma PTH levels. PTH reference range supplied by the manufacturer was 1.6–6.9 pmol/L.

P1NP levels were measured by electrochemiluminescence immunoassay (Cobas e601, Roche). The study was approved by the Ethical Comittee of Faculty Hospital Motol.

Statistical analysis

The normality of the data distribution was tested by D'Agostino Pearson test. Differences between subgroups were tested for statistical significance by the nonparametric Mann–Whitney *U* test. *P* value <0.05 was considered as statistically significant. Spearman's correlation was calculated to evaluate the correlation between cFGF23, iFGF23 and other biomarkers. Statistical software MedCalc version 18.02.01 (Oostende, Belgium) and GraphPad Prism version 8.02 were used for statistical evaluation.

Results

The concentrations of all biomarkers measured intraoperatively and postoperatively in patients with
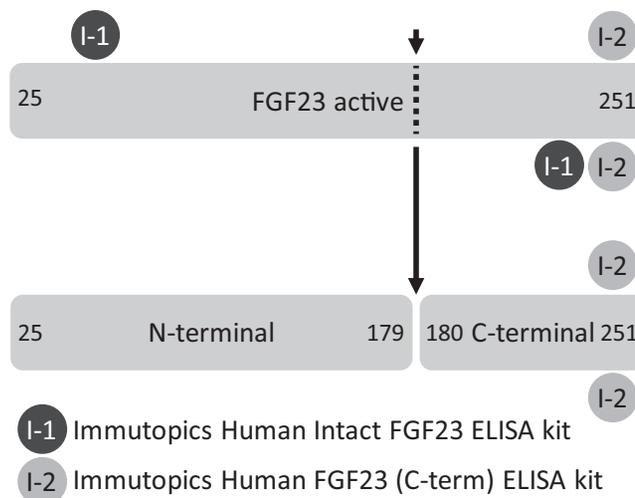

Figure 1
The principle of ELISA used to detect intact and C-teminal FGF23 (data from Donate-Correa *et al.* (22)).

pHPT are listed in Table 1. The plasma PTH levels were significantly decreased intraoperatively 10 min after surgical excision of the parathyroid adenoma (4.17 pmol/L vs 13.10 pmol/L, $P < 0.0001$), and intraoperative levels of all other biomarkers did not change. The correlations of iFGF23, cFGF23 and PTH measured just before the incision were not significant. On postoperative day 1, inorganic phosphate values increased (1.03 mmol/L vs 0.8 mmol/L, $P = 0.0025$, plasma inorganic phosphate reference values are 0.8–1.5 mmol/L) and plasma cFGF23 concentrations decreased (22.23 RU/mL vs 89.17 RU/mL, $P < 0.0001$, Fig. 2). Similarly, iFGF23 values also decreased postoperatively, but the postoperative values were lower than the detection limit of the kit. The concentrations of iFGF23 and cFGF23 are not related to age and gender and did not differ between pre- and postmenopausal women. We found significant perioperative correlation between FGF23 and inorganic phosphate (cFGF23: Spearman's $r = -0.253$, $P = 0.0065$; iFGF23: Spearman's $r = -0.245$, $P = 0.0085$) (Fig. 3), however, the intraoperative and postoperative correlations between FGF23 and inorganic phosphate were not significant. FGF23 and eGFR (cystatin C) measured just before the incision correlated significantly (cFGF23: Spearman's $r = -0.499$, $P = 0.0014$; iFGF23: Spearman's $r = -0.413$, $P = 0.01$) (Fig. 4), but the correlation between both iFGF23 and cFGF23 and eGFR (CKD-EPI) was not significant. The correlation between FGF23 and total calcium 1 day after adenoma excision (cFGF23: Spearman's $r = 0.598$, $P = 0.0008$, iFGF23: Spearman's $r = 0.41$, $P = 0.02$) was also significant. Patients were postoperatively supplemented by calcium.

Table 1 The plasma concentrations of biomarkers measured intraoperatively and postoperatively in patients with pHPT. Concentrations of biomarkers are expressed as median (95% CI for the median).

	Intraoperative		Postoperative	*P* value	
	Just before the incision (*n* = 38)	10 min after excision (*n* = 38)	Next day after excision (*n* = 38)	Before incision vs 10 min after excision	Before incision vs next day after excision
cFGF23 (RU/mL)	89 (74.58–102.6)	88 (78.42–99.54)	22 (14.41–31.91)	0.93	**<0.0001**
iFGF23 (pg/mL)	57 (45.58–67.41)	57 (78.42–99.54)	Not detectable	0.89	**<0.0001**
Inorganic phosphate (mmol/L)	0.80 (0.69–0.93)	0.80 (0.73–0.91)	1.03 (0.91–1.26)	0.76	**0.0025**
Serum calcium (mmol/L)[a]	2.71 (2.61–2.82)	–	2.22 (2.15–2.34)	–	**<0.0001**
Creatinine (μmol/L)	58.51 (54.81–62.11)	62.52 (50.16–72.38)	72.51 (62.33–75.51)	0.77	0.08
Cystatin C (mg/L)	1.01 (0.94–1.06)	0.97 (0.86–1.03)	1.06 (1.10–1.20)	0.45	0.11
P1NP (μg/L)	61.75 (54.43–78.84)	62.60 (49.47–73.27)	62.04 (54.92–67.71)	0.92	0.92
eGFR cystatin C (mL/min/1.73 m^2)	83.29 (76.79–93.97)	89.16 (80.59–109.1)	76.79 (62.35–84.69)	0.46	0.12
eGFR creatinine CKD-EPI (mL/min/1,73 m^2)	116.3 (101.4–143.6)	128.7 (103.32–136.6)	107.1 (89.8–136)	0.62	0.40
PTH (pmol/L)	13.10 (10.22–14.37)	4.17 (2.85–5.97)	–	**0.0001**	–

[a]Serum calcium values were retrieved from medical notes.
Bold indicates statistical significance.

Discussion

Intraoperative changes of PTH

The most relevant information on parathyroid activity is given by the determination of plasma PTH concentration. We proved that plasma PTH concentration was significantly decreased 10 min after parathyroidectomy compared to values before parathyroidectomy (4.17 pmol/L vs 13.10 pmol/L, *P* < 0.0001, Mann–Whitney *U* test) (Table 1). The significant decrease in PTH concentration means successful removal of the adenoma. Successful parathyroidectomy was also confirmed by adjusting the calcium concentration 1 day after the surgery.

Perioperative changes of iFGF23 and cFGF23

The intraoperative cFGF23 and iFGF23 values were at the upper level or above the upper level of reference intervals, as is shown in Fig. 2. We found similar results in the available literature, both cFGF23 and iFGF23 were elevated in pHPT patients before parathyroidectomy (2, 3). The plasma cFGF23 concentrations significantly decreased day after the surgery compared to the preoperative (just before the incision) values (Fig. 2). These findings correlated with the results of Kobayashi *et al.* (24). Kobayashi *et al.* published time course of cFGF23 in five patients showing significant decrease 6 h after parathyroidectomy. Plasma concentrations of iFGF23 showed more significant decrease, and the concentrations of iFGF23 measured 1 day after the parthyroidectomy were even lower than the

declared detection limit of the kit (1.5 pg/mL). The early perioperative changes of iFGF23 (changes until 1 day after parathyroidectomy) were not yet published. The steeper decrease in postoperative concentrations of iFGF23 is due to its shorter biological half-life (iFGF23 has shorter half-life of 5 min, while cFGF23 has a half-life of 22–94 min) (1, 21). Even iFGF23 with its short half-life of 5 min did not show any changes 10 min post-excision vs pre-incision. Of note, the concordance between intact and C-terminal FGF23 acute changes is noteworthy, suggesting that each could substitute for the other. The concordance also suggests that the decline in iFGF23 following excision of the parathyroid adenoma was relatively slow compared to the longer half-life of cFGF23.

FGF23 and PTH correlation

Correlation between PTH and FGF23 just before adenoma incision was not significant. We could not determine the postoperative correlation between PTH and FGF23 because postoperative plasma PTH values were not measured. Current review showed that measuring of postoperative plasma PTH values could be supplied by the continuously monitored albumin-adjusted serum calcium followed with clinical investigations including symptoms of hypercalcemia and presence of end organ diseases (renal stones, fragility fractures and osteoporosis) (25). We could not evaluate the continuously monitored calcium/albumin levels due to the fact that we assayed plasma samples and postoperative serum calcium levels
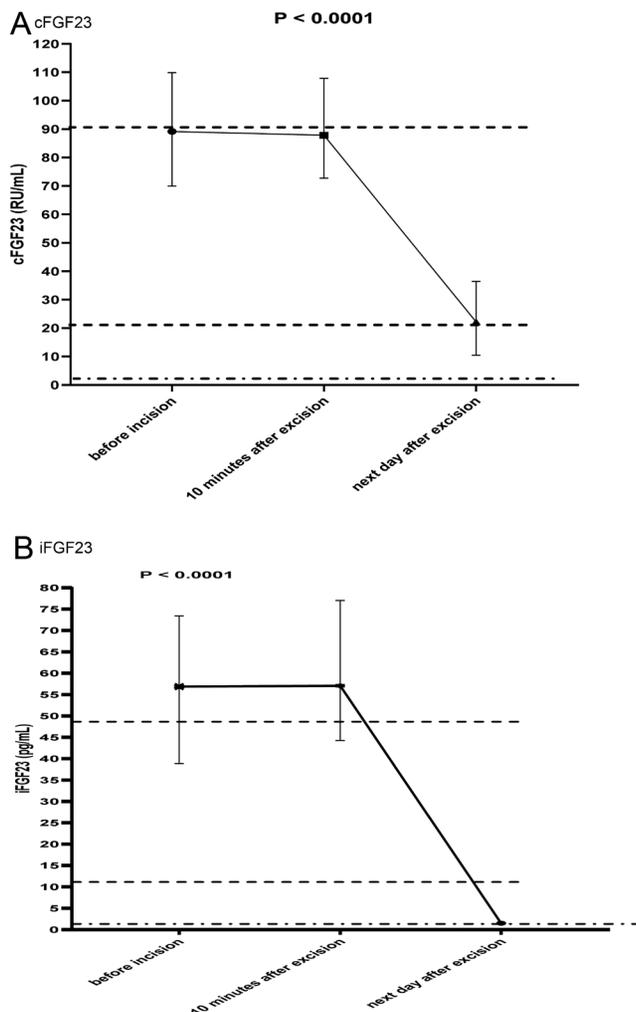
Figure 2

Results are expressed as median with interquartile range. Dotted lines show the upper and lower limit of reference interval. Semi-dotted line shows the value of detection limit set at 1.5 pg/mL for iFGF23 and 1.5 RU/mL for cFGF23, respectively.

Figure 3

Perioperative correlations of plasma iFGF23 with inorganic phosphates.

were retrieved from medical notes. The data in Fig. 2 show that the decrease iFGF23 and cFGF23 values does not occur intraoperatively, in contrast to the PTH values, which decrease immediately (10 min) after parathyroidectomy. PTH and iFGF23 have a similar half-life (PTH half-life is 3–4 min (1)). Rapid intraoperative decrease in PTH levels against the much slower decline of PTH-stimulated circulating iFGF23 despite their similar half-lives is contributed with the atrophy of non-adenomatous parts of the parathyroid gland due to the chronic dysregulated preoperative oversecretion of PTH by the adenoma in contrast to the chronically overstimulated, likely hyperplastic, revved up sites of iFGF23 production due to chronic overstimulation by chronically elevated levels of circulating PTH. Minutes after surgical excision of the hyperfunctioning PTH
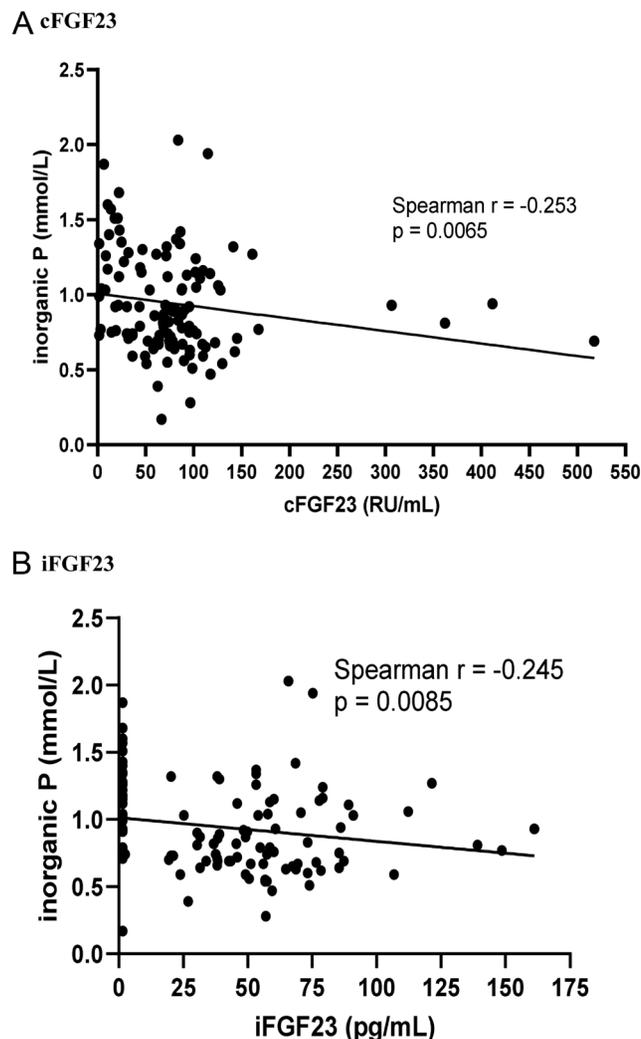
adenoma, suppressed PTH synthesis by the remaining parathyroid gland coupled with the short half-life of PTH act (synergistically) together to cause the very rapid decline in circulating PTH. In contrast, it takes longer to wind down FGF23 overproduction by hyperplastic sites of synthesis despite the sudden absence of PTH-mediated stimulation arising from excision of the PTH producing adenoma, hence the slower decline in circulating iFGF23 levels. Regulation of FGF23 is complex and multifactorial with FGF23 affecting the metabolic processes of number of important substances including hormones and factors and in turn being affected by these same moieties but not necessarily in a reciprocal manner which adds to the complexity. A case in point relevant in this study is that FGF23 directly acts on the parathyroid glands to decrease PTH synthesis and secretion (26). On the other
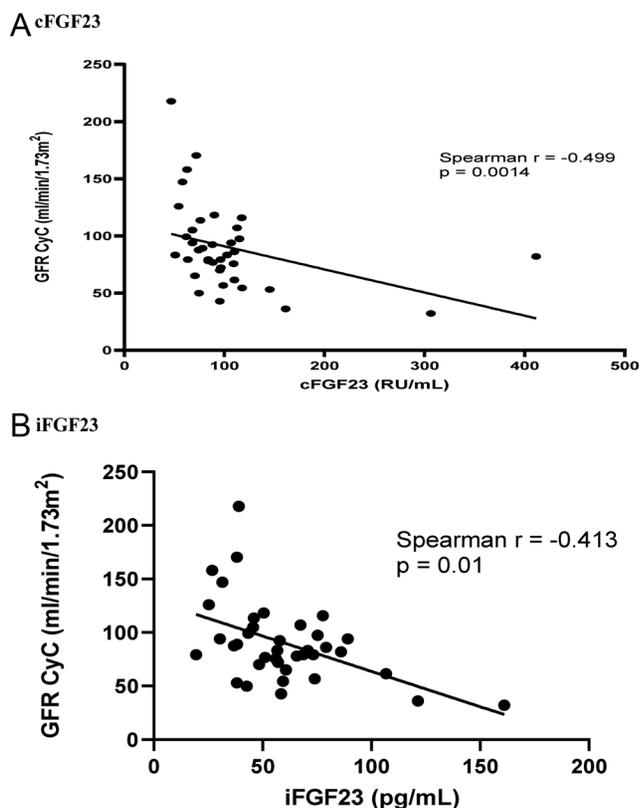
A **cFGF23**

Spearman r = -0.499
p = 0.0014

B **iFGF23**

Spearman r = -0.413
p = 0.01

Figure 4
Preoperative (just before incision) correlations of plasma FGF23 with GFR (cystatin C).

hand, FGF23 synthesis is stimulated by PTH, phosphate, calcium, $1,25(OH)_2D$ and other factors (1). Witteveen *et al.* (3) reported significant relationship between PTH and FGF23 levels in pHPT patients, and their results suggest that the increase of FGF23 levels in pHPT patients could be an adaptive mechanism to counteract the PTH-induced increase in $1,25(OH)_2D$ levels.

Correlation of cFGF23 and iFGF23 with glomerular filtration

The negative correlation of FGF23 with glomerular filtration is typical in patients with chronic kidney disease (16, 24, 27, 28). Although none of our patients enrolled in the study had significantly reduced glomerular filtration, we found significant negative correlation of cFGF23 and iFGF23 measured just before the parathyroidectomy with glomerular filtration estimated from cystatin C concentration (eGFR cystatin C cFGF23: Spearman's r = −0.499, P = 0.0014; iFGF23: Spearman's r = −0.413, P = 0.01). The correlation of both cFGF23 and iFGF23 with GFR creatinine estimated from CKD-EPI formula was not significant in our study. Similarly, Witteveen *et al.*

(3) did not find significant relationship between iFGF23 concentrations and glomerular filtration (estimated from the Modification of Diet in Renal Disease Study (MDRD) formula) in pHPT patients, and Yamashita *et al.* (29) did not show significantly increased serum iFGF23 levels in pHPT patients with normal renal function characterized by the creatinine clearance ≥70 mL/min. The discrepancies in eGFR (creatinine) and eGFR (cystatin C) are related to the association of glomerular filtration with the age as mentioned by Kang *et al.* (30). In the study, we found that FGF23 values and creatinine estimated glomerular filtration (CKD-EPI) were not associated with age, however, the association of eGFR (cystatin C) with age was significant (r = 0.50, P < 0.0001). This results indicate the higher sensitivity of cystatin C compared to creatinine in detecting renal function in patients with pHPT. Similar results were also shown by Ermetici *et al.* (31).

The role of FGF23 in maintaining calcium phosphate homeostasis

Hypercalcemia is an important diagnostic indicator of primary hyperparathyroidism. Medical records of our patients showed elevated serum calcium levels before parathyroidectomy (median = 2.71 mmol/L) with significant decrease to reference range the next day after adenoma excision (median = 2.22 mmol/L, P < 0.0001) (Table 1). Postoperative decrease of total calcium correlated with the decrease of FGF23 values (cFGF23: Spearman's r = 0.5982, P = 0.0008; iFGF23: Spearman's r = 0.41, P = 0.02). Plasma inorganic phosphate levels were at the lower level of reference intervals before parathyroidectomy. It is well known that FGF23 acts on the proximal tubule to inhibit sodium/phosphate cotransporters NaPi2a and NaPi2c and, consequently, renal phosphate reabsorption. FGF23 also inhibits the expression of renal 1α-hydroxylase and stimulates the expression of renal 24-hydroxylase leading to a decrease in serum $1,25(OH)_2D$ with a subsequent decrease in intestinal phosphate (and calcium) absorption resulting in a decrease in serum phosphate (6). On postoperative day 1, phosphate values increased (1.03 mmol/L vs 0.8 mmol/L, P = 0.0025) (see Table 1). But no difference between cFGF23 and iFGF23 with respect to postoperative phosphate was noted in this study. The elevation of perioperative plasma phosphate correlated with the decrease of plasma FGF23 (cFGF23: Spearman's r = −0.253, P = 0.0065; iFGF23: Spearman's r = −0.245, P = 0.0085). Similarly, Yamashita *et al.* found negative correlation of iFGF23 with serum phospate and $1,25(OH)_2D$ levels before the parathyroidectomy and

also observed positive correlation of iFGF23 with serum calcium levels 6 days after the parathyroidectomy. On the contrary, Bilha *et al.* (32) monitored patients with pHPT and D hypovitaminosis and showed that preoperative serum cFGF23 concentrations were within the normal range and remained unchanged 1 day postoperatively. Bilha *et al.* also mentioned that some patients followed up prospectively for up to 12 months after surgery showed constant cFGF23 levels, despite PTH and calcium normalization and vitamin D replenishment. Hassani *et al.* (33) showed positive correlation of FGF23 with $1,25(OH)_2D$ preoperatively and postoperatively (24 h and 1 week after the operation), and they did not prove significant correlations between FGF23, calcium, phosphate and PTH.

FGF23 and bone markers

A number of studies have focused on the association of FGF23 with bone metabolism (3, 27, 28, 34). We evaluated the relationship of cFGF23 and iFGF23 with P1NP as an important biomarker of bone formation and we did not find any significant correlation. Similar to our findings, Witteveen *et al.* (3) did not prove any significant relationship between FGF23 and P1NP (or between FGF23 and ALP or CTx) in patients with pHPT, nevertheless, all bone turnover markers were significantly increased in their study. Preoperative P1NP values found in our study were in the upper region of the reference range, which correspond to increased bone turnover in patients with pHPT.

The main limitation of our study is the lack of postoperative PTH values and the absence of a control group of healthy people. The advantage of the study seems to be the determination of both iFGF23 and cFGF23 in the same cohort of patients. The early perioperative changes of iFGF23 (changes less than 1 day after parathyroidectomy) were not yet published. Indeed, all the cited studies determine either iFGF23 or cFGF23 in plasma or in serum. Despite the different half-lives, the concordance between iFGF23 and cFGF23 acute changes is noteworthy in our study, suggesting each could substitute for the other. iFGF23 and cFGF23 also showed similar correlations to other markers. Similarly, the study of Shimada *et al.* demonstrated that levels of bioactive FGF23 were well correlated with the levels of immunoreactive FGF23 measured using either the iFGF23 or the cFGF23 assay and both assays would reflex the same circulating moiety (35). On the other hand, the measurements of C-terminal FGF23 are often disturbed by the clinical iron deficiency, inflammation and the use of erythrpoiesis-stimulating agents (7, 36, 37). As such, in general, the use of cFGF23 should be discouraged and

the use of iFGF23 should be prompted. Concentrations of cFGF23 and iFGF23 were increased in patients with pHPT and 1 day after parathyroidectomy they significantly decreased. Postoperative decrease of cFGF23 and iFGF23 was associated with the increase of inorganic phosphate. cFGF23 and iFGF23 did not change intraoperatively, although PTH decreased significantly. cFGF23 and iFGF23 measured just before incision correlated with eGFR (cystatin C). The investigation of cFGF23 and iFGF23 show similar results suggesting that each could substitute for the other and both cFGF23 and iFGF23 are equally relevant in maintaining laboratory evaluation of patients with pHPT.

Author contribution statement

M F – Reviewing and editing clinical data, text revision and writing, L H – Writing and data curation, R P – Manuscript reviewing and language editing and supervision, M V – Methodology and data curation, J C – Editing and reviewing, K K – Original draft preparation and conceptualization.

References

1 Rifai N, Horvath A & Wittwer C. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. St. Louis: Elsevier, 2018.

2 Nilsson IL, Norenstedt S, Granath F, Zedenius J, Pernow Y & Larsson TE. FGF23, metabolic risk factors, and blood pressure in patients with primary hyperparathyroidism undergoing parathyroid adenomectomy. *Surgery* 2016 **159** 211–217. (https://doi.org/10.1016/j.surg.2015.06.057)

3 Witteveen JE, van Lierop AH, Papapoulos SE & Hamdy NA. Increased circulating levels of FGF23: an adaptive response in primary hyperparathyroidism? *European Journal of Endocrinology* 2012 **166** 55–60. (https://doi.org/10.1530/EJE-11-0523)

4 Juppner H. Phosphate and FGF-23. *Kidney International: Supplement* 2011 **79** S24–S27. (https://doi.org/10.1038/ki.2011.27)

5 Fukumoto S & Martin TJ. Bone as an endocrine organ. *Trends in Endocrinology and Metabolism* 2009 **20** 230–236. (https://doi.org/10.1016/j.tem.2009.02.001)

6 Carpenter TO. The expanding family of hypophosphatemic syndromes. *Journal of Bone and Mineral Metabolism* 2012 **30** 1–9. (https://doi.org/10.1007/s00774-011-0340-2)

7 Agoro R, Ni P, Noonan ML & White KE. Osteocytic FGF23 and its kidney function. *Frontiers in Endocrinology* 2020 **11** 592. (https://doi.org/10.3389/fendo.2020.00592)

8 Lang F & Foller M. Enigmatic Cassandra: renal FGF23 formation in polycystic kidney disease. *Kidney International* 2014 **85** 1260–1262. (https://doi.org/10.1038/ki.2013.534)

9 Smith ER, Tan SJ, Holt SG & Hewitson TD. FGF23 is synthesised locally by renal tubules and activates injury-primed fibroblasts. *Scientific Reports* 2017 **7** 3345. (https://doi.org/10.1038/s41598-017-02709-w)

10 Athonvarangkul D & Insogna KL. New therapies for hypophosphatemia-related to FGF23 excess. *Calcified Tissue International* 2021 **108** 143–157. (https://doi.org/10.1007/s00223-020-00705-3)

11 Imel EA, Biggin A, Schindeler A & Munns CF. FGF23, hypophosphatemia, and emerging treatments. *JBMR Plus* 2019 **3** e10190. (https://doi.org/10.1002/jbm4.10190)

12 Martin A, David V & Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiological Reviews* 2012 **92** 131–155. (https://doi.org/10.1152/physrev.00002.2011)

13 Isakova T, Xie H, Barchi-Chung A, Vargas G, Sowden N, Houston J, Wahl P, Lundquist A, Epstein M, Smith K, *et al.* Fibroblast growth factor 23 in patients undergoing peritoneal dialysis. *Clinical Journal of the American Society of Nephrology* 2011 **6** 2688–2695. (https://doi.org/10.2215/CJN.04290511)

14 Quarles LD. Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. *Experimental Cell Research* 2012 **318** 1040–1048. (https://doi.org/10.1016/j.yexcr.2012.02.027)

15 Quarles LD. A systems biology preview of the relationships between mineral and metabolic complications in chronic kidney disease. *Seminars in Nephrology* 2013 **33** 130–142. (https://doi.org/10.1016/j.semnephrol.2012.12.014)

16 Takahashi H, Komaba H, Takahashi Y, Sawada K, Tatsumi R, Kanai G, Suzuki H, Kakuta T & Fukagawa M. Impact of parathyroidectomy on serum FGF23 and soluble klotho in hemodialysis patients with severe secondary hyperparathyroidism. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E652–E658. (https://doi.org/10.1210/jc.2013-4050)

17 Chong WH, Molinolo AA, Chen CC & Collins MT. Tumor-induced osteomalacia. *Endocrine-Related Cancer* 2011 **18** R53–R77. (https://doi.org/10.1530/ERC-11-0006)

18 White KE, Hum JM & Econs MJ. Hypophosphatemic rickets: revealing novel control points for phosphate homeostasis. *Current Osteoporosis Reports* 2014 **12** 252–262. (https://doi.org/10.1007/s11914-014-0223-2)

19 Immutopics. Human FGF-23 (C-term) ELISA kit 2nd generation enzyme-linked immunosorbent assay (ELISA) for the determination of human fibroblast growth factor 23 levels in plasma or cell culture media. San Clemente, CA, USA: Immutopics, 2020.

20 Immutopics. Human FGF-23 (intact) ELISA kit 2nd generation enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of human fibroblast growth factor 23 levels in plasma or cell culture media. San Clemente, CA, USA: Immutopics, 2020.

21 Smith ER, McMahon LP & Holt SG. Fibroblast growth factor 23. *Annals of Clinical Biochemistry* 2014 **51** 203–227. (https://doi.org/10.1177/0004563213510708)

22 Donate-Correa J, Muros de Fuentes M, Mora-Fernandez C & Navarro-Gonzalez JF. Pathophysiological implications of fibroblast growth factor-23 and klotho and their potential role as clinical biomarkers. *Clinical Chemistry* 2014 **60** 933–940. (https://doi.org/10.1373/clinchem.2013.206649)

23 Bozentowicz-Wikarek M, Owczarek A, Kocelak P, Olszanecka-Glinianowicz M, Wiecek A & Chudek J. C-terminal to intact fibroblast growth factor 23 ratio in relation to estimated glomerular filtration rate in elderly population. *Kidney and Blood Pressure Research* 2016 **41** 519–526. (https://doi.org/10.1159/000443452)

24 Kobayashi K, Imanishi Y, Miyauchi A, Onoda N, Kawata T, Tahara H, Goto H, Miki T, Ishimura E, Sugimoto T, *et al.* Regulation of plasma fibroblast growth factor 23 by calcium in primary hyperparathyroidism. *European Journal of Endocrinology* 2006 **154** 93–99. (https://doi.org/10.1530/eje.1.02053)

25 Aojula N, Khan S, Gittoes N & Hassan-Smith Z. Normocalcaemic primary hyperparathyroidism: what is the role of parathyroid surgery? *Therapeutic Advances in Endocrinology and Metabolism* 2021 **12** 2042018821995370. (https://doi.org/10.1177/2042018821995370)

26 Courbebaisse M & Lanske B. Biology of fibroblast growth factor 23: from physiology to pathology. *Cold Spring Harbor Perspectives in Medicine* 2018 **8** a031260. (https://doi.org/10.1101/cshperspect.a031260)

27 Komaba H & Fukagawa M. FGF23: a key player in mineral and bone disorder in CKD. *Nefrologia* 2009 **29** 392–396. (https://doi.org/10.3265/Nefrologia.2009.29.5.5400.en.full)

28 Rodriguez M, Lopez I, Munoz J, Aguilera-Tejero E & Almaden Y. FGF23 and mineral metabolism, implications in CKD-MBD. *Nefrologia* 2012 **32** 275–278. (https://doi.org/10.3265/Nefrologia.pre2012.Mar.11415)

29 Yamashita H, Yamashita T, Miyamoto M, Shigematsu T, Kazama JJ, Shimada T, Yamazaki Y, Fukumoto S, Fukagaw M & Noguchi S. Fibroblast growth factor (FGF)-23 in patients with primary hyperparathyroidism. *European Journal of Endocrinology* 2004 **151** 55–60. (https://doi.org/10.1530/eje.0.1510055)

30 Kang E, Han SS, Kim J, Park SK, Chung W, Oh YK, Chae DW, Kim YS, Ahn C & Oh KH. Discrepant glomerular filtration rate trends from creatinine and cystatin C in patients with chronic kidney disease: results from the KNOW-CKD cohort. *BMC Nephrology* 2020 **21** 280. (https://doi.org/10.1186/s12882-020-01932-4)

31 Ermetici F, Filopanti M, Verga U, Passeri E, Dito G, Malavazos AE, Mapelli C, Raggi ME, Spada A & Corbetta S. Estimated glomerular filtration rate by serum cystatin C correlates with cardiometabolic parameters in patients with primary hyperparathyroidism. *European Journal of Endocrinology* 2015 **173** 441–446. (https://doi.org/10.1530/EJE-15-0341)

32 Bilha SC, Gatu AA, Velicescu C, Bilha A, Florescu A & Branisteanu D. FGF23 and primary hyperparathyroidism: is there a link? *Endokrynologia Polska* 2020 **71** 306–312. (https://doi.org/10.5603/EP.a2020.0030)

33 Hassani S, Afkhamizadeh M, Teimouri A, Najaf Najafi M, Vazifeh Mostaan L & Mohebbi M. Evaluation of serum level of FGF23 and 1,25(OH)2D3 in primary hyperparathyroidism patients before and after parathyroidectomy. *International Journal of General Medicine* 2020 **13** 289–295. (https://doi.org/10.2147/IJGM.S253246)

34 Grabner A, Mazzaferro S, Cianciolo G, Krick S, Capelli I, Rotondi S, Ronco C, La Manna G & Faul C. Fibroblast growth factor 23: mineral metabolism and beyond. *Contributions to Nephrology* 2017 **190** 83–95. (https://doi.org/10.1159/000468952)

35 Shimada T, Urakawa I, Isakova T, Yamazaki Y, Epstein M, Wesseling-Perry K, Wolf M, Salusky IB & Jüppner H. Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 578–585. (https://doi.org/10.1210/jc.2009-1603)

36 Edmonston D & Wolf M. FGF23 at the crossroads of phosphate, iron economy and erythropoiesis. *Nature Reviews: Nephrology* 2020 **16** 7–19. (https://doi.org/10.1038/s41581-019-0189-5)

37 van Vuren AJ, Eisenga MF, van Straaten S, Glenthoj A, Gaillard CAJM, Bakker SJL, de Borst MH, van Wijk R & van Beers EJ. Interplay of erythropoietin, fibroblast growth factor 23, and erythroferrone in patients with hereditary hemolytic anemia. *Blood Advances* 2020 **4** 1678–1682. (https://doi.org/10.1182/bloodadvances.2020001595)