



ORIGINAL ARTICLE

FGF23 is associated with early post-transplant hypophosphataemia and normalizes faster than iPTH in living donor renal transplant recipients: a longitudinal follow-up study

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Abstract

Background: We aimed to longitudinally analyse changes in the levels of serum fibroblast growth factor 23 (FGF23), intact parathyroid hormone (iPTH) and associated minerals in patients undergoing renal transplantation.

Methods: Sixty-three patients with end-stage renal disease (ESRD) who underwent living donor transplantation were recruited. Serum FGF23, iPTH, uric acid, inorganic phosphorous (iP), blood urea nitrogen and serum creatinine were measured pre-transplant and at 1 (M1), 3 (M3) and 12 months (M12) post-transplantation.

Results: FGF23 levels were decreased at M1, M3 and M12 by 93.81, 96.74 and 97.53%, respectively. iPTH levels were decreased by 67.95, 74.95 and 84.9%, respectively. The prevalence of hyperparathyroidism at M1, M3 and M12 post-transplantation was 63.5, 42.9 and 11.1%, respectively. FGF23 and iP levels remained above the normal range in 23 (36.5%) and 17 (27%) patients at M1, 10 (15.9%) and 5 (8%) at M3 and in none at M12 post-transplantation, respectively. A multivariate regression model revealed that, pre-transplant, iP was positively associated with iPTH ($P = 0.016$) but not with FGF 23; however, post-transplant, iP level was negatively associated with FGF23 ($P < 0.001$) but not with iPTH.

Conclusions: Post-transplant FGF23 levels settle faster than those of iPTH. However, 11% of patients continued to have hyperparathyroidism even after 12 months.

Key words: fibroblast growth factor 23, hyperparathyroidism, hypophosphataemia, intact parathyroid hormone, renal transplantation

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Introduction

Chronic kidney disease–mineral bone disorder (CKD-MBD) is common in patients with end-stage renal disease (ESRD) who undergo renal transplantation. It is associated with disturbances in the homeostasis of phosphate, calcium, calcitriol, fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) [1]. In late-stage CKD patients with abnormally high plasma FGF23 and PTH concentrations, these abnormalities exert unwarranted off-target effects, including left ventricular hypertrophy, faster CKD progression and premature mortality [2–4].

PTH is produced by the parathyroid gland, while FGF23, a 251 amino acid protein, is secreted by osteocytes and osteoblasts in bone. Both are involved in calcium phosphate homeostasis [5, 6]. Increased serum phosphate concentration induces the secretion of both PTH and FGF23. With declining renal function, circulating level of FGF23 gradually increase and enhance the excretion of phosphate per nephron, although the responsiveness to FGF23 diminishes as the number of intact nephrons declines with the progression of CKD [7–9]. This process disrupts the bone–kidney–parathyroid endocrine axis, contributing to the development of hyperphosphataemia in ESRD. Moreover, increased FGF23 levels reduce calcitriol levels, further contributing to an increase in PTH secretion [7].

After transplantation, mineral and hormone homeostasis improves with normalization of renal function. However, many abnormalities still persist in the post-transplant period. A recent study showed that excessive FGF23 causes inappropriate phosphate wasting and low calcitriol levels following renal transplantation, despite normal or mildly impaired allograft function [10]. Initially, persisting hyperparathyroidism was considered to be the cause of post-transplant hypophosphataemia [11]; however, it was subsequently observed that hypophosphataemia can persist even after elevated PTH levels have normalized, hence the role of FGF23 was discovered [12, 13]. Recent studies suggest that elevated FGF23 levels during ESRD may persist in the early post-transplant period, contributing to early post-transplant hypophosphataemia [14, 15]. FGF23 decreases renal tubular reabsorption of inorganic phosphorous (iP) and also inhibits renal 1- α -hydroxylase, which leads to decreased calcitriol synthesis in the early post-transplant period [16]. In addition, it may inhibit gastrointestinal phosphate absorption in a vitamin D–dependent manner [17]. It is still unclear whether FGF23 and PTH act alone or together in regulating phosphorous levels in the post-transplant period [18]. FGF23 has been suggested to be responsible for hypophosphataemia and, subsequently, for inappropriately low calcitriol levels after renal transplantation [14–16, 19]. The length of time required for the homeostasis of these minerals, FGF23 and iPTH (intact PTH) in the post-transplant period requires further exploration, particularly in patients receiving living donor renal transplants, where renal function normalizes faster compared with those receiving deceased donor transplantation. The majority of data in the literature concern deceased donor transplantation. We aimed to study these hormones and minerals prospectively over the post-transplantation period in a cohort of living donor renal transplant patients.

Materials and methods

Study population

In this prospective observational study, a total of 70 ESRD patients on maintenance haemodialysis (MHD) were included who underwent living donor renal transplantation at our

institute. Patients who developed delayed graft function ($n = 2$) and acute rejection ($n = 5$) were excluded. The study was approved by the institute's ethics committee. The study was conducted as per ethics standards laid down by the Declaration of Istanbul and informed written consent was obtained from each patient. In total, 63 patients [58 males, mean age 35.65 ± 11.79 years, average estimated glomerular filtration rate (eGFR) 9.58 ± 4.01 mL/min/1.73 m²] who were on MHD for a median of 8 months (range 6–24) before transplantation were included in the study. Each patient was given phosphate binders—either calcium carbonate, sevelamer carbonate or both—as required to adjust the recommended calcium, phosphorous and iPTH levels in accordance with National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines [20]. Of the 63 patients, 53 (84.12%) received calcitriol. None of the patients included in the study were treated with cinacalcit or underwent parathyroidectomy.

Assays and calculation

Fasting blood samples were collected from all patients during the pre-transplantation period prior to haemodialysis and subsequently at 1 (M1), 3 (M3) and 12 (M12) months post-transplantation. Blood samples were centrifuged at 1500 rpm for 10 min, serum aliquoted and stored at -80°C until analysis. Circulating levels of FGF23, iPTH, 25-hydroxyvitamin D [25(OH)D], calcium, iP, alkaline phosphatase, creatinine, haemoglobin, blood urea nitrogen (BUN), uric acid and albumin were measured at the above mentioned time points. Intact FGF23 levels were measured in duplicate after a single freeze-thaw cycle in batched assays by sandwich enzyme-linked immunosorbent assay (ELISA) (Millipore, San Clemente, CA, USA) [21]. The lower detection limit of the kit was 9.9 pg/mL. Serum iPTH was measured using an immunoradiometric assay (Beckman Coulter, Immunotech, Radiova, Prague, Czech Republic), which contained two antibodies directed against different epitopes of PTH molecules. The detection limit of the kit was 3.7–2500 pg/mL, with intra- and inter-assay coefficients of variation of 12.2 and 9.7%, respectively. Radioimmunoassay was used to measure the concentrations of 25 (OH)D (DiaSorin, Stillwater, MN, USA). Serum calcium, phosphate, creatinine and total alkaline phosphatase were measured simultaneously using an automatic analyzer. Hypocalcaemia was defined as corrected calcium for albumin <8.5 mg/dL and hypercalcaemia as >10.8 mg/dL. Normophosphataemia was considered if the serum iP level was 2.5–4.5 mg/dL. The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation.

Immunosuppression protocol

All patients received triple immunosuppression consisting of prednisolone (100%), calcineurin inhibitors [either tacrolimus (95%) or cyclosporine (5%)] and mycophenolate mofetil (100%). All patients received methylprednisolone 500 mg intravenously during the pre-operative period. Subsequently patients received 20 mg of prednisolone daily until 8 weeks; thereafter, the dose of prednisolone was tapered every week by 2.5 mg to reach the nadir dose of 10 mg daily by 12 weeks. The cyclosporine dose was adjusted to maintain a trough level between 300 and 400 $\mu\text{g/L}$ and then gradually tapered to 100–200 $\mu\text{g/L}$ at 12 weeks to reach the nadir level 75–150 $\mu\text{g/L}$ at 6 months. The dose of tacrolimus was adjusted to maintain a trough level between 8 and 12 ng/mL for the first 6 months and thereafter between 4 and 6 ng/mL. Of the 63 patients, 57 (74.6%) received

basiliximab and 6 (9.5%) received antithymocyte globulin induction.

Statistical analysis

Comparisons between groups were analyzed by applying the Friedman test when data were non-normally distributed. The Wilcoxon test was used to compare two groups. Normality was assessed using the Kolmogorov–Smirnov test. Associations among parameters were assessed by univariate or multivariate linear regression analysis using a backward model. Two-sided P-values <0.05 were considered to be statistically significant. Parameters were calculated with their 95% confidence interval. Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL, USA).

Results

Demographic and clinical parameters of the patients are summarized in Table 1. Of the 63 patients, 36 had chronic glomerulonephritis, 20 had interstitial nephropathy, 6 had diabetic nephropathy and 1 had polycystic kidney disease. Pre- and post-transplant values of FGF23, iPTH, 25(OH)D, BUN, serum creatinine, eGFR, calcium, iP, uric acid and alkaline phosphatase are summarized in Table 2.

Pre-transplantation FGF23 and its correlation with other parameters

Patients were categorized into three tertiles based on FGF23 levels (Table 3). Serum iPTH, iP, uric acid, BUN and creatinine levels increased significantly with increasing FGF23 tertile values. Serum calcium was significantly less in the higher tertile group.

Table 1. Demographics and clinical characteristics of patients

Characteristics	
Male/female, n/n	58/5
Age (years), mean ± SD	35.65 ± 11.79
Height (cm), mean ± SD	162.05 ± 11.27
Weight (kg), mean ± SD	55 ± 9.63
Body mass index (kg/m ²), mean ± SD	20.85 ± 2.46
Dialysis duration, months (range)	8 (6–24)
Smoker, %	7.9
Alcoholic, %	6.3
Blood-related transplant, n (%)	41 (65)
Aetiology of kidney disease, n (%)	
Chronic glomerulonephritis	36 (57.1)
Chronic interstitial nephropathy	20 (31.7)
Diabetic nephropathy	6 (9.5)
Polycystic kidney disease	1 (1.6)
Immune suppression agents, n (%)	
Steroids	63 (100)
Calcineurin inhibitor	
Tacrolimus	60 (95.2)
Cyclosporine A	3 (4.8)
Mycophenolate mofetil	63 (100)
Induction therapy	
Basiliximab	47 (74.6)
ATG	6 (9.5)
Phosphate binder, n (%)	
Calcium-based	45 (71.4)
Non-calcium-based	18 (28.6)

Haemoglobin and 25(OH)D levels were similar in all three tertiles. As expected, pre-transplant levels of iPTH, FGF23, iP, creatinine, BUN and uric acid were above the higher limit of normal. Hypercalcaemia and hyperphosphataemia were observed in 26 (41.3%) and 50 (79.4%) patients, respectively. Patients who had hypocalcaemia had significantly higher values of iPTH (439.42 ± 259.64 versus 287.92 ± 196.69 pg/mL; P = 0.016) and FGF23 (1675.05 ± 688.68 versus 1151.22 ± 882.50 pg/mL; P = 0.008) as compared with normocalcaemic patients. The percentage declines of iPTH and iP in each tertile of FGF23 at M1, M3 and M12 are presented in Table 4. It was observed that the percentage decline of iP and iPTH at the end of 1 year was greater in the higher tertile group of FGF23.

Biochemical changes in the post-transplant period

The biochemical parameters in the post-transplant period are presented in Table 2. Compared with pre-transplantation, post-transplant eGFRs improved and the values of BUN, serum creatinine, uric acid and alkaline phosphatase decreased significantly to the normal range in the first month. Moreover, there was no significant difference in 25(OH)D values post-transplant.

Changes in serum phosphorous, iPTH and FGF23 in the post-transplant period

Compared with pre-transplant, intact FGF23 levels decreased by 93.81, 96.74 and 97.53%, while iPTH levels decreased by 67.95, 74.95 and 84.9% at M1, M3 and M12 post-transplantation, respectively. FGF23 levels remained above the normal range (<50 pg/mL) in 23 (36.5%) patients at M1, in 10 (15.9%) at M3 and in none at M12 post-transplantation. Hyperparathyroidism with a cut-off value of >65 pg/mL [22] was observed in 40 (63.5%), 27 (42.9%) and 7 (11.1%) patients at M1, M3 and M12 post-transplantation, respectively (Figure 1). Forty patients who continued to have hyperparathyroidism at M1 also had higher iPTH levels pre-transplantation (397.41 ± 269.15 versus 268.76 ± 128.24 pg/mL; P = 0.013) compared with those who achieved normal iPTH levels at M1. The higher values of iPTH persisted until M3 post-transplantation (109.32 ± 74.15 versus 50.29 ± 13.64 pg/mL; P < 0.001). Patients with persisting hyperparathyroidism at M1 also had higher FGF23 (99.97 ± 86.95 versus 51.85 ± 30.71 pg/mL; P = 0.003) at M1; however, FGF23 levels gradually became normal in all patients. The percentage reductions in FGF23 and iPTH were significantly associated with each other at M1 (r = 0.35, P = 0.004), M3 (r = 0.28, P = 0.026) and M12 (r = 0.35, P = 0.004). In addition, patients with persisting hyperparathyroidism at M1 had significantly lower eGFRs and greater BUN at M1 and M3.

Phosphate levels in the post-transplant period

Hypophosphataemia was observed in 17 (27%) patients at M1, in 5 (8%) patients at M3 and in none at M12 post-transplantation. At M1, patients who developed hypophosphataemia had significantly higher levels of FGF23 (1922.53 ± 558.47 versus 1183.15 ± 772.30 pg/mL; P < 0.001) and iPTH (468.82 ± 274.10 versus 314.73 ± 209.90 pg/mL; P = 0.048) pre-transplant. The higher FGF23 (120.96 ± 102.97 versus 68.41 ± 66.04 pg/mL; P = 0.064) and iPTH (135.55 ± 111.31 versus 102.67 ± 72.77 pg/mL; P = 0.272) levels continued to persist at M1 compared with normophosphataemic patients. Calcium levels were within the normal range (8.5–10.8 mg/dL) at an early period of M1, except in one patient who developed hypercalcaemia. In all patients, levels of calcium were normalized at M3 and M12 post-transplantation. At the

Table 2. Biochemical parameters of the patients at different post-transplant periods

Variable	Pre-Tx	Month 1	Month 3	Month 12	Friedman test (P)
iPTH (pg/mL)	350.44 ± 235.16	112.3 ± 84.38	87.77 ± 65.92	52.89 ± 22.17	0.001
iPTH (pg/mL), median (range)	287 (102–1246.4)	87.8 (21–434)	61.3 (29–382.5)	49 (26.7–142)	0.001
Hyperparathyroidism (>65 pg/mL), n (%)	63 (100)	40 (63.5)	27 (42.9)	7 (11.1)	
FGF23 (pg/mL)	1367.4 ± 807.24	82.40 ± 79.78	46.92 ± 33.63	33.65 ± 15.32	0.001
FGF23 (pg/mL), median (range)	1589 (37.8–2620.3)	48.5 (10–356)	43 (10–276)	37.8 (10–112.8)	0.001
FGF23 (>50 pg/mL), n (%)	58 (92)	23 (36.5)	10 (15.9)	1 (1.6)	
25(OH)D (ng/mL)	27.29 ± 12.35	23.54 ± 9.56	27.22 ± 7.60	23.17 ± 6.29	0.002
eGFR (mL/min)	9.58 ± 4.01	81.02 ± 48.64	84.86 ± 64.90	95.71 ± 49.3	0.001
Phosphate (mg/dL)	6.42 ± 2.12	2.92 ± 0.85	3.19 ± 0.71	3.18 ± 0.51	0.001
Hypophosphataemia (<2.5 mg/dL), n (%)		17 (27)	5 (8)		
Creatinine (mg/dL)	7.49 ± 1.93	1.19 ± 0.25	1.20 ± 0.25	1.21 ± 0.26	0.001
Haemoglobin (g/dL)	9.54 ± 1.79	11.14 ± 1.61	13.38 ± 2.06	12.61 ± 2.24	0.001
BUN (mg/dL)	53.54 ± 22.51	23.26 ± 9.14	19.64 ± 7.91	18.53 ± 7.05	0.001
Uric acid (mg/dL)	6.76 ± 1.99	4.33 ± 1.32	4.21 ± 1.20	4.24 ± 1.37	0.001
Calcium (mg/dL)	8.62 ± 0.82	9.24 ± 0.50	9.45 ± 0.50	9.42 ± 0.49	0.001
Hypercalcaemia (>10.8 mg/dL), n (%)		1 (1.6)	1 (1.6)		
Hypocalcaemia (<8.5 mg/dL), n (%)	26 (41.3)				
Albumin (g/dL)	4.06 ± 0.50	4.24 ± 1.03	4.23 ± 0.88	4.3 ± 0.53	0.188
Alkaline phosphatase (U/L)	182.02 ± 97.75	99.86 ± 50.27	83.22 ± 48.34	82.87 ± 33.42	0.001

Values are presented as mean ± SD unless stated otherwise.

Table 3. Tertile of FGF23 and other biochemical parameters in the pre-transplant period

Parameters	FGF23 (pg/mL)			P-value
	Tertile 1 (≤1054.50)	Tertile 2 (1054.51–1859.19)	Tertile 3 (≥1859.20)	
iPTH (pg/mL)	220.28 ± 119.60	318.72 ± 123.74	512.33 ± 308.95	0.001
25(OH)D (ng/mL)	28.16 ± 12.63	27.51 ± 14.40	26.19 ± 10.02	NS
Phosphate (mg/dL)	5.40 ± 1.55	6.51 ± 2.26	7.35 ± 2.09	0.009
Creatinine (mg/dL)	6.56 ± 1.79	7.89 ± 1.85	8.00 ± 1.90	0.025
Haemoglobin (g/dL)	9.29 ± 1.87	9.72 ± 1.70	9.62 ± 1.84	NS
BUN (mg/dL)	44.81 ± 15.38	49.85 ± 17.63	65.94 ± 27.72	0.005
Uric acid (mg/dL)	5.91 ± 2.14	6.96 ± 1.64	7.40 ± 1.95	0.042
Calcium (mg/dL)	8.97 ± 0.88	8.71 ± 0.60	8.17 ± 0.77	0.004

Values are presented as mean ± SD.

NS; not significant.

Table 4. Percentage reduction of iPTH and iP in each tertile of FGF23

FGF23 tertile		Pre-transplant	Month 1	Month 3	Month 12
iPTH	Tertile 1 (≤1054.50 pg/mL)	220.28 ± 119.60	79.60 ± 36.40	64.08 ± 21.52	45.36 ± 9.67
	% reduction		66.86	70.90	79.40
	Tertile 2 (1054.51–1859.19 pg/mL)	318.72 ± 123.74	133.61 ± 92.55	105.45 ± 72.21	54.54 ± 24.66
	% reduction		58.07	66.91	82.88
iP	Tertile 3 (≥1859.20 pg/mL)	512.33 ± 308.95	123.69 ± 102.20	93.77 ± 82.68	58.75 ± 26.89
	% reduction		75.85	81.69	88.53
	Tertile 1 (≤1054.50 pg/mL)	5.4 ± 1.55	3.33 ± 0.75	3.64 ± 0.82	3.25 ± 0.57
	% reduction		38.33	32.59	39.81
iP	Tertile 2 (1054.51–1859.19 pg/mL)	6.51 ± 2.26	2.91 ± 0.80	3.04 ± 0.45	3.05 ± 0.36
	% reduction		55.29	53.30	53.14
	Tertile 3 (≥1859.20 pg/mL)	7.35 ± 2.09	2.52 ± 0.84	2.90 ± 0.63	3.23 ± 0.56
	% reduction		65.71	60.54	56.05

Values are presented as mean ± SD unless stated otherwise.

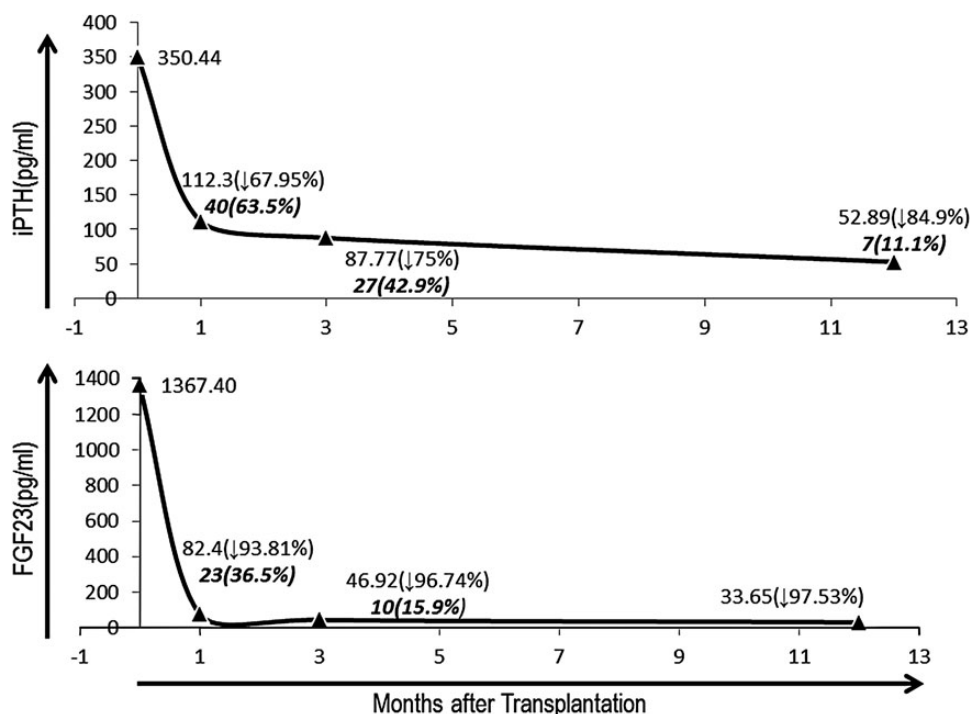


Fig. 1. Changes in FGF23 and iPTH levels at different post-transplant periods.

end of the study period, FGF23, eGFR, iP, serum creatinine, BUN, albumin and uric acid were normalized; however, hyperparathyroidism persisted in 7 (11.1%) patients.

Predictors of inorganic phosphate

To assess the relative contribution of variables involved in the determination of serum iP, univariate and multivariate linear regression models were constructed using a backward method. Pre-transplant serum iP was significantly associated with pre-transplant iPTH, FGF23, eGFR, BUN, uric acid, serum albumin and a non-vegetarian diet in a univariate linear regression model; however, upon multivariate analysis, only iPTH and uric acid levels were found to be significantly associated. Serum FGF23 was not found to be associated with serum iP on multivariate analysis in any of the regression models (Table 5). Upon univariate linear regression analysis at M1, the iP level was significantly associated with FGF23, eGFR and uric acid levels, but not with iPTH. Multivariate analysis showed that only FGF23 was an independent predictor of serum iP level at M1. The association between serum iPTH and iP at M1 was non-significant in all the models used. Of note, the regression coefficient of iP with iPTH and FGF23 was positive during the pre-transplant period and negative at M1 (Table 6).

Discussion

In the present study, it was observed that in the pre-transplant period with increasing FGF23 tertiles, iPTH and iP values increased. After renal transplantation, FGF23 levels normalized faster than those of iPTH, and FGF23 and iP levels were normalized in all at the end of 1 year; however, 11% of patients continued to have hyperparathyroidism.

Similar to our study, Domniki et al. [23] reported a similar percentage of patients with persisting hyperparathyroidism at the

Table 5. Pre-transplant factors associated with serum iP (linear regression model constructed by backward stepwise modelling procedure) in models that feature FGF23, iPTH or eGFR and iP levels

Variables	Dependent variable: pre-transplant iP			
	Regression coefficient (β)	95% CI	P-value	r^2
Univariate linear regression				
iPTH	0.004	0.002–0.006	<0.001	24.2%
FGF23	0.001	0.001–0.002	<0.001	18.8%
BUN	0.037	0.015–0.060	0.001	15.8%
Uric acid	0.467	0.216–0.707	<0.001	18.8%
Non-vegetarian			0.008	11.1%
Multivariate linear regression model (backward method)				
iPTH	0.003	0.001–0.005	0.016	41%
FGF23	0.000	0.000–0.001	0.317	
Uric acid	0.252	0.008–0.496	0.043	
Non-vegetarian			0.112	
Whole model			<0.001	

end of the first year post-transplantation. They observed that despite a significant decrease in PTH levels post-transplant, hyperparathyroidism persisted for longer in some recipients. Lobo et al. [24] showed incomplete resolution of secondary hyperparathyroidism by the end of 1 year post-transplantation in ~50% of recipients. Contrary to this, only 11% of our renal transplant recipients had persisting hyperparathyroidism, which could be due to the shorter duration of dialysis prior to transplantation. Another reason could be the inclusion of only living donor transplants in our study, where renal function and mineral metabolism normalizes relatively faster than in deceased donor transplantation. It has been postulated that persisting hyperparathyroidism for an extended duration might be due to the

Table 6. Factors associated with serum iPTH (linear regression model constructed by backward modeling procedure) in models that feature FGF23, PTH, eGFR and iPTH levels at M1 post-transplant

Variables	Dependent variable: iPTH M1			P-value	r ²
	Regression coefficient (β)	95% CI			
Univariate linear regression					
FGF23 (M1)	-0.006	-0.008 to -0.003	<0.001		28.8%
eGFR (M1)	0.004	0.000 to 0.009	0.052		6.1%
Uric acid (M1)	-0.174	-0.334 to -0.014	0.034		7.2%
Multivariate linear regression model (backward method)					
iPTH (M1)	-0.001	-0.002 to -0.003	0.700		33.1%
FGF23 (M1)	-0.005	-0.008 to -0.003	<0.001		
eGFR (M1)	0.003	0.000 to 0.007	0.082		
Uric acid (M1)	-0.051	-0.206 to 0.104	0.511		
Whole model			<0.001		

longer lifespan of parathyroid cells (~20 years, with a cell renewal rate of approximately 5% per year), which results in slow involution of the gland even after successful renal transplantation [25].

In CKD patients, the upsurge of FGF23 precedes that of PTH [22, 26], and more patients had elevated FGF23 than PTH [26]. Pre-transplant CKD with stimulated PTH levels and a certain degree of hyperplasia in the parathyroid glands could account for the more pronounced effect of this hormone in the post-transplant period. Another possible reason for the high levels of PTH compared with those of FGF23 could be persisting bone abnormalities from the pre-transplant period, which could lead to reduced production of FGF23 from the bone. It has been observed that at similar degrees of CKD, transplant recipients seem to produce less FGF23 than patients with CKD who do not undergo transplantation. Many of the available immunosuppressants [corticosteroids, calcineurin inhibitors (CNIs) and mechanistic target of rapamycin inhibitors] are known to stimulate FGF23 production [27, 28]. Despite all our patients receiving steroids and CNIs, FGF23 levels remained normal in the post-transplant period [14, 19, 23, 29]. Similar to our observations, other studies have also reported increased PTH in the setting of normal FGF23 levels in the post-transplant period [26–28].

High variability in the prevalence of hypercalcaemia (5–50%) and hypophosphataemia (30–90%) after renal transplantation has been reported in the literature [30–32]. The main reasons for these wide variations could be differing treatment strategies for hyperparathyroidism before transplantation. Some centre resort to parathyroidectomy for moderate or therapy-controlled hyperparathyroidism, while others limit parathyroidectomy to severe and uncontrolled hyperparathyroidism only. None of our patients underwent parathyroidectomy and all were treated with phosphate binders—calcium carbonate or sevelamer carbonate alone or in combination. The lower prevalence of hypercalcaemia and hypophosphataemia in our patient population could be explained by shorter dialysis vintage pre-transplantation and faster recovery from bone mineral disorder after living donor transplantation. In the present study, 27% of transplant recipients had hypophosphataemia at M1. Increased FGF23, instead of iPTH, was independently associated with hypophosphataemia. The above findings reinforce the concept of tertiary ‘hyperphosphatonism’, which accentuates hypophosphataemia in the early post-transplant period, as reported earlier [14, 16, 18, 33]. During the pre-transplant period, the response of FGF23 is diminished and levels increase as the number of intact nephrons

declines in association with reduced Klotho expression, a co-receptor for FGF23 signaling [34]. Conversely, with stable grafts, persisting high FGF23 downregulates the expression of sodium-phosphate cotransporter NPT2a and NPT2c (downstream of FGF23–Klotho signalling) and increases urinary phosphate excretion leading to hypophosphataemia [5, 35]. Evenepoel et al. [16] observed a 95% decline in FGF23 levels after M3 of successful renal transplantation; however, they also observed higher values of FGF23 levels in 61% of their patients. This could be due to deceased donor kidney transplantation in their cohort, unlike our cohort of living donor transplantations. The above findings suggest that FGF23 and iPTH may act synergistically to cause phosphaturia, as advocated in another study [16]. Moreover, hyperphosphatonism and hypophosphataemia settle to normal at M12 post-transplantation with stable graft function. Serum FGF23 levels have been previously reported to predict CKD progression and graft status [2, 36, 37].

Persisting abnormal mineral metabolism, particularly hypercalcaemia, has been shown to be associated with not only macrocalcification and microcalcification of the graft kidney in the tubular and peritubular regions, but also with chemical nephrocalcinosis characterized by functional and biochemical cellular changes because of the influence of hypercalcaemia on cell function [38]. Such changes affect the graft function. Prolonged hypercalcaemia linked with secondary hyperparathyroidism might suggest a potential causal factor in the process of vascular calcifications, which are highly prevalent in CKD patients and tend to progress even after kidney transplantation [39, 40]. Post-transplant hypercalcaemia has been suggested to play an important role in the pathogenesis of erythrocytosis and has been shown to increase the occurrence of pancreatitis in renal transplant patients [41, 42]. Persisting severe hypophosphataemia may also lead to rhabdomyolysis, haemolysis, leucocyte dysfunction, respiratory failure, impaired myocardial performance, neurological problems and osteopaenia/osteomalacia [43]. Shorter dialysis vintage and faster recovery from these mineral disorders after living donor transplantation could prevent some of these complications and gives living donor transplantation the edge over deceased donor transplantation.

PTH and FGF23 levels correlate positively with each other at the pre- and post-transplantation stages. Both FGF23 and PTH are phosphaturic hormones; one study has revealed that FGF23 is responsible for hyperparathyroidism and hypophosphataemia [44], while other observers have reported that PTH may result in increased levels of FGF23 [6, 45]. To fully understand the complex pathophysiology between PTH and FGF23, further studies are essential. Mild hypocalcaemia was found in 41% of our patients at M1, which could possibly be due to high FGF23 levels at M1. This observation is supported by an animal study by Hasegawa et al. [46], who reported that higher FGF23 levels reduce 25(OH)D production leading to hypocalcaemia. Post-transplantation, calcidiol levels decreased significantly at M1 but later remained within the normal range. No association of calcidiol was found with either FGF23 or PTH. Our findings support previous studies that have observed decreased calcidiol levels in the early post-transplant period [23, 29]. However, a limitation of our study is that we did not investigate calcitriol levels. The main strength of our study is that all recruited patients were of the same race and were treated and followed-up with similar pre- and post-transplant immunosuppression protocols to reduce bias. All patients were prospectively followed after transplantation for 1 year. The shorter dialysis vintage and better recovery from hyperparathyroidism and hyperphosphatonism in our living donor transplant recipients emphasizes the fact that ‘the earlier the

transplants, the better the outcomes' in terms of bone mineral metabolism. To avoid measurement of FGF23 terminal fragments, which accumulate in ESRD and probably do not flash endogenous FGF23 production, we analyzed only intact FGF23. The major limitations of our study are the small number of patients recruited and the fact that calcitriol levels were not monitored.

Conclusion

FGF23 levels decrease significantly after successful renal transplantation and remain within normal limits with stable graft function. The role of FGF23 in iP homeostasis is more prominent in the early period after transplantation. PTH levels decrease significantly after successful transplantation, but hyperparathyroidism can persist in some recipients for a longer duration.

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Conflicts of interest statement

None declared.

(See related article by Cianciolo and Cozzolino. FGF23 in kidney transplant: the strange case of Doctor Jekyll and Mister Hyde. *Clin Kidney J* (2016) 9: 665–668.)

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