formally excluded pre-transplant in all cases, but in patients with delayed graft function, the BP did not normalize under the influence of steroid treatment, but only normalized when graft function was established.

In conclusion, the findings of this study may be of great significance for future patients with intractable dialysis-associated hypotension, for whom daily activity is limited and dialysis inadequate, as transplantation could not only reverse renal failure, but intractable hypotension as well.

Acknowledgements. The authors thank Dr Curtis McMurtrey for help with the figures.

Conflict of interest statement. None declared.

References

1. Cases A, Coll E. Chronic hypotension in the dialysis patient. *J Nephrol* 2002; 15: 331–335.
2. Iwanaga K, Takamura N, Abe Y *et al*. Plasma concentrations of adrenomedullin and ghrelin in haemodialysis patients with sustained and episodic hypotension. *Endocr J* 2005; 52: 23–28.
3. Palmer BF. Why are some dialysis patients chronically hypotensive in the absence of heart disease and volume depletion? *Semin Dial* 2011; 24: 404–405.
4. Coll E, Larrousse M, de la Sierra A *et al*. Chronic hypotension in haemodialysis patients: role of functional vascular changes and vasodilator agents. *Clin Nephrol* 2008; 69: 114–120.
5. Urena P. Arterial hypotension in dialysis. *Nephrologie* 2001; 22: 105–113.
6. Daul AE, Wang XL, Michel MC *et al*. Arterial hypotension in chronic haemodialyzed patients. *Kidney Int* 1987; 32: 728–735.
7. Esforzado N, Cases A, Bono M *et al*. Vasoactive hormones in uraemic patients with chronic hypotension. *Nephrol Dial Transpl* 1997; 12: 321–324.
8. Esforzado N, Cases A, Bono M *et al*. Autonomic nervous system and adrenergic receptors in chronic hypotensive haemodialysis patients. *Nephrol Dial Transpl* 1997; 12: 939–944.
9. Cases A, Esforzado N, Lario S *et al*. Increased plasma adrenomedullin levels in haemodialysis patients with sustained hypotension. *Kidney Int* 2000; 57: 664–670.
10. Montagnac R, Clavel P, Delhotal-Landes B *et al*. Use of midodrine (Gutron) to treat permanent hypotension in a chronic haemodialysis patient. *Clin Nephrol* 2001; 56: 162–168.
11. Sun C-Y, Wu M-S. Renal transplantation reversed intractable hypotension in a diabetic patient. *Diabetes Care* 2007; 30: e65.
12. Montgomery RA, Lonze BE, King KE *et al*. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; 365: 318–326.
13. Higgins R, Lowe D, Hathaway M *et al*. HLA antibody incompatible renal transplantation: excellent medium term outcomes with negative cytotoxic crossmatch. *Transplantation* 2011; 92: 900–906.
14. Higgins R, Lowe D, Hathaway M *et al*. Double filtration plasmapheresis in antibody-incompatible kidney transplantation. *Ther Apher Dial* 2010; 14: 392–399.
15. Sinha D, Lambie M, Krishnan N *et al*. Cryofiltration as a treatment for cryoglobulinemia and HLA antibody incompatible transplantation. *Ther Apher Dial* 2012; 16: 91–96.

Received for publication: 9.2.2012; Accepted in revised form: 27.6.2012

Nephrol Dial Transplant (2012) 27: 4227–4235
doi: 10.1093/ndt/gfs409

Serum level of fibroblast growth factor 23 in maintenance renal transplant patients

Ana I. Sánchez Fructuoso[1], Maria L. Maestro[2], Isabel Pérez-Flores[1], Rosalía Valero[1], Sara Rafael[2], Silvia Veganzones[2], Natividad Calvo[1], Virginia De la Orden[2], Jose C. De la Flor[1], Francisco Valga[1], Marta Vidaurreta[2], Cristina Fernández-Pérez[3] and Alberto Barrientos[1]

[1]Nephrology Department, Hospital Clínico Universitario San Carlos, Madrid, Spain, [2]Genomic Unity, Laboratory Department, Hospital Clínico Universitario San Carlos, Madrid, Spain and [3]Medicine Preventive, Hospital Clínico Universitario San Carlos, Madrid, Spain

Correspondence and offprint requests to Ana I. Sánchez Fructuoso; E-mail: sanchezfructuoso@gmail.com

Abstract

Background. The discovery of fibroblast growth factor 23 (FGF23) provides a new conceptual framework that improves our understanding of the pathogenesis of post-transplant bone disease. Excess FGF23 is produced in the early post-transplant period; levels return to normal in the months following transplant. However, few manuscripts discuss FGF23 levels in stable long-term renal transplant recipients.

Methods. We performed a cross-sectional observational study of 279 maintenance kidney recipients with chronic kidney disease (CKD) Stages 1–4 and stable allograft function who had received their transplant at least 12 months previously. We calculated the estimated GFR (eGFR) using the MDRD4 equation.

Results. FGF23, parathyroid hormone (PTH) and phosphorus values were higher in more advanced stages, while

the serum calcitriol levels and the phosphate reabsorption rate were lower. A significant inverse correlation was found between eGFR and FGF23 ($r = -0.487$; $P < 0.001$), PTH ($r = -0.444$; $P < 0.001$), serum phosphate levels ($r = -0.315$; $P < 0.001$) and fractional excretion of magnesium ($r = -0.503$; $P < 0.001$). Multivariable analysis showed that increased time on corticosteroids ($P < 0.001$), PTH ($P < 0.001$), serum phosphate ($P = 0.003$), decreased serum calcitriol ($P = 0.049$) and estimated glomerular filtration ($P = 0.003$) rate were associated with high FGF23 levels. In contrast with pre-transplant patients and first year post-transplant patients, higher FGF23 values were not correlated with increased phosphate excretion. An elevated phosphate reabsorption rate was associated with decreased PTH ($P < 0.001$) and calciuria ($P = 0.028$) and increased serum calcitriol ($P = 0.009$), plasma bicarbonate ($P = 0.024$) and estimated glomerular filtration ($P = 0.003$).

Conclusions. Serum FGF23 concentrations remain increased in long-term kidney graft recipients, even in the early stages of CKD. It remains to be seen whether measures aimed at reducing serum levels of PTH and phosphate and/or corticosteroid doses might help to lower serum FGF23 and whether this will improve kidney recipient outcomes.

Keywords: fibroblast growth factor 23; hyperparathyroidism; hypophosphataemia; kidney transplantation; post-transplant bone disease

Introduction

Mineral and bone disorders are common in patients who have undergone kidney transplantation [1]. These conditions are caused, to a large extent, by pre-existing bone damage acquired during dialysis and chronic kidney disease (CKD). Other potential causal factors include immunosuppressive agents, hyperphosphaturia, persistent hyperparathyroidism with hypercalcaemia, acid–base balance disturbances and hypomagnesaemia. Hypercalcaemia is a severe complication that can affect graft function both acutely owing to vasoconstriction [2] and chronically [3] owing to the calcification of the tubulointerstitium [4]. Hypercalcaemia can also increase the risk of soft-tissue and vascular calcification [5], which in turn can adversely affect outcome. Hypophosphataemia is present in up to 90% of transplant recipients [6], and although it is usually seen shortly after transplantation, phosphate levels remain low for longer than in patients with CKD matched for a glomerular filtration rate (GFR) [7]. Persistent hyperparathyroidism has long been considered the cause of hypophosphataemia. However, inappropriate renal phosphate excretion can occur despite normal serum phosphate levels and low levels of parathyroid hormone (PTH) [8, 9].

The discovery of fibroblast growth factor 23 (FGF23) provides a new conceptual framework through which we can increase our understanding of the pathogenesis of post-transplant bone disease. FGF23 is mainly synthesized by osteocytes and plays a key role in the bone–kidney axis and the regulation of calcium phosphate metabolism [10–12]. It acts primarily on renal proximal tubules as a phosphaturic factor through the downregulation of sodium–phosphate co-transporters, decreased 1,25(OH)2D levels, inhibition of 25(OH)D-1α-hydroxylase and upregulation of the catabolic 25(OH)D-24-hydroxylase pathway [10]. FGF23 can also inhibit PTH synthesis [13]. The single-pass transmembrane Klotho protein is required for FGF23-mediated receptor activation *in vivo* [14, 15].

FGF23 metabolism, production and clearance are modified by CKD, and most authors have focused on this area of study [16–23]. In kidney failure, FGF23 levels increase early and rise steadily with disease progression as a physiologic adaptation to maintain normal phosphorus levels [16, 24]. Because FGF23 has a counter-regulatory effect on vitamin D action, increased FGF23 levels during CKD can induce a reduction in vitamin D activity and thus facilitate development of secondary hyperparathyroidism [16]. In patients with CKD, FGF23 can also provide prognostic information, namely, therapeutic response and cardiovascular mortality [16, 18, 24]. Information about FGF23 and bone disease in adult renal transplant patients is emerging; however, most studies focus on the immediate postoperative period [7, 9, 25–32] and validate the hypothesis that post-transplant hypophosphataemia is a syndrome of tertiary FGF23 excess [33]. The post-transplant period evaluated in these articles varies between 3 and 12 months, and the number of patients analysed is relatively low (18–50 cases). Little is known about the progress of FGF23 and bone disease 1 year after surgery, and the only available report analysed 68 pediatric patients [34]. Wolf *et al*. [35] studied FGF23 in a large cohort of stable renal transplant recipients to evaluate whether FGF23 is an independent risk factor for death and allograft loss, but these authors did not provide information about FGF23 and bone disease.

We undertook the present study to elucidate the complex associations between FGF23, PTH, 1,25(OH)2 vitamin D and phosphate in maintenance renal transplant patients. We also attempt to provide clinical evidence for the role of the newly described phosphate-centric paradigm in the pathogenesis of post-transplant bone disease.

Materials and methods

Study population

We performed a cross-sectional observational study of 279 adult maintenance kidney recipients with CKD (Stages 1–4) who had received their transplants at least 12 months previously and maintained stable allograft function (defined as no change in serum creatinine >0.2 mg/dl within the 2 months prior to the study). Diabetic patients were excluded. We included all patients who attended our outpatient clinic from September to November 2010 and who fulfilled the inclusion criteria. The study adhered to the principles of the Declaration of Helsinki and was approved by the ethics committee of our centre. All patients provided their written informed consent.

The aetiology of CKD was recorded, as were the main treatments administered (i.e. corticosteroids, calcineurin inhibitors, anti-metabolites and mineral metabolism therapy).

Patients were classified into five groups according to the international classification of CKD stages established by the Kidney Disease Outcomes Quality Initiative.

Procedures and assays

Fasting serum samples and 24-h urine samples were collected during a routine follow-up outpatient visit. Serum creatinine (Jaffe method), calcium, phosphate, magnesium and urinary creatinine, calcium, magnesium and phosphate were measured using standard assays.

The estimated GFR (eGFR) was calculated using the MDRD4 equation [36].

The ratio of the maximum rate of tubular phosphate reabsorption to GFR (TmP/GFR) was calculated using the following equation: TmP/GFR = urine phosphate − [(serum phosphate × serum creatinine)/urinary creatinine] [37]. The other parameters calculated were the 24-h fractional renal phosphate reabsorption rate, the electrolyte excretion rate (sodium, calcium and magnesium), and the urine calcium-to-creatinine ratio.

Radioimmunoassay was used to measure the concentrations of 25 hydroxyvitamin D (25D; DiaSorin, Stillwater, MN) and 1,25(OH)2 vitamin D (DiaSorin).

An intact PTH was measured using an immunoradiometric assay (Scantibodies Laboratory, Inc., Santee, CA).

A circulating C-terminal FGF23 was measured using a second-generation enzyme-linked immunosorbent assay (Immutopics, Inc., San Clemente, CA). The inter-assay variation was <3% and intra-assay variation <5% in both normal and elevated concentration ranges [38]. In a larger, adult study, the normal range was determined to be <100 RU/mL [20]. Serum samples were stored at −40°C for a maximum of 3 months.

The bone density was measured using dual energy X-ray absorptiometry (Hologic, Inc., Waltham, MA). We retrospectively reviewed bone mass in those patients for whom results were available within a maximum interval of 4 months after this cross-sectional study was performed.

Statistical analysis

Continuous variables [expressed as mean (SD)] were compared using the analysis of variance; categorical variables were compared using the chi-squared test or Fisher's exact test. Normality was assessed using the Kolgomorov–Smirnov test. Asymmetric variables were expressed as the median (IQR) and compared using the Kruskal–Wallis test. We defined hyperphosphataemia as serum phosphate ≥4.6 mg/dl and hyperparathyroidism as PTH ≥65 pg/ml according to our reference laboratory values and previously published data [20]. We calculated the percentage of patients with FGF23 ≥100 pg/ml (cut-off used elsewhere for patients with CDK) [20]. Correlations between eGFR, demographic data, treatments and mineral metabolism parameters were studied using the Spearman test. Linear regression analyses were used to explain variables associated with FGF23 and serum phosphate. After excluding colinearity, the best subset of variables was selected by backward elimination [39]. Parameters were calculated with their 95% confidence interval. In order to normalize residuals, FGF23, PTH and 1,25(OH)2 vitamin D were square root transformed. The inspection of residual plots and variance inflation factor ensured that the *a priori* assumptions for linear regression were justified. The analysis was performed using SPSS version 15.0 for Windows.

Results

Demographic characteristics and the main treatments administered are summarized in Table 1. All patients were Caucasian and had received a kidney from a deceased donor. Given the small number of patients with Stage 1 CKD, Stages 1 and 2 were considered together for purposes of the analysis.

Biochemical parameters are shown in Table 2. Impairment of renal function is accompanied by a decrease in calcitriol levels and renal phosphate excretion, and by an increase in levels of serum phosphate, FGF23 and PTH. Plasma FGF23 levels were >100 pg/ml in 49.8% of patients and lower in patients with Stage 1 and 2 CKD than in those with Stage 3 and 4 CKD. Interestingly, nine patients had FGF23 values >500 pg/mL. These patients had undergone transplantation between 12 and 282 months previously and had renal dysfunction (eGFR, 27.1 ± 5.8 ml/min/1.73 m^2), high PTH levels (median, 161 ng/ml), low 1,25(OH)2 vitamin D levels (median, 22 pg/ml) and a high serum phosphorus concentration (3.9 ± 0.7 mg/dl). The average phosphate reabsorption rate was 67.7 ± 6.3%.

We calculated the proportion of patients with normal or high FGF23 and PTH levels in each eGFR category (Table 3). The most common pattern was normal FGF/normal PTH in CKD Stages 1 and 2 and elevated FGF23/elevated PTH in Stage 4. In the intermediate Stage 3, the elevated FGF23/elevated PTH was also the most frequent pattern followed by the isolated elevated PTH, both of

Table 1. Patients demographics and concomitant treatment

	All (*n* = 279)	CKD1 (*n* = 9)	CKD2 (*n* = 65)	CKD3 (*n* = 152)	CKD4 (*n* = 53)	P
Demographics						
Age (years)	56.1 ± 12.7	57.3 ± 12.3	56.1 ± 12.3	56.6 ± 13.0	56.1 ± 12.7	0.949
Gender (men, %)	64.2	66.7	67.7	63.8	60.4	0.870
Height (cm)	164.8 ± 10.4	160.9 ± 13.4	166.0 ± 9.0	164.8 ± 11.8	164.8 ± 11.7	0.519
Weight (kg)	74.3 ± 15.2	63.4 ± 16.2	74.5 ± 14.5	75.7 ± 15.6	74.3 ± 15.2	0.172
Time on dialysis (months)	17.9 (8.8–35.5)	15.0 (6.6–61.7)	13.9 (7.6–35.3)	17.7 (9.0–41.7)	22.8 (11.7–44.0)	0.298
Post-transplant time (years)	7.1 (3.5–12.3)	9.0 (5.9–13.5)	6.8 (3.0–11.7)	6.3 (2.9–12.1)	8.4 (5.0–13.2)	0.112
Mineral metabolism therapy						
Phosphate binder (%)	3.2	0	1.5	2.6	7.5	0.237
Cholecalciferol (%)	11.5	0	15.4	11.8	7.5	0.396
Calcitriol (%)	6.5	11.1	3.1	5.9	11.3	0.295
Paricalcitol (%)	6.1	0	1.5	5.9	13.2	0.054
Bisphosphonates (%)	3.2	0	3.1	3.3	3.8	0.949
Calcium supplements (%)	18.3	0	20.0	20.4	13.2	0.319
Cinacalcet (%)	7.5	0	4.6	9.9	5.7	0.394
Immunosuppressive therapy						
Anti-metabolite (%)	95.7	77.8	100	96.1	92.5	0.01
Calcineurin inhibitor (%)	85.7	77.8	80.0	85.5	94.3	0.114
Corticosteroids (%)	48.4	33.3	26.2	45.4	86.8	<0.001
mTOR inhibitors (%)	23.7	33.3	26.2	23.0	20.8	0.809
Acute rejection (%)	35.1	22.2	33.8	35.5	37.7	0.832
Corticosteroids dose (mg/kg year)	22.8 (11.6–36.7)	10.5 (2.9–17.2)	14.2 (8.2–28.4)	22.8 (12.2–37.9)	31.7 (23.2–43.7)	<0.001

Table 2. Biochemical parameters

	All ($n = 279$)	CKD1 and 2 ($n = 74$)	CKD3 ($n = 152$)	CKD4 ($n = 53$)	P
Serum calcium (8.5–10.5 mg/dl)	9.73 ± 0.47	9.85 ± 0.56	10.0 ± 0.50	9.69 ± 0.53	<0.001
Serum phosphorus (2.5–4.5 mg/dl)	3.31 ± 0.63	3.08 ± 0.59	3.28 ± 0.57	3.72 ± 0.66	<0.001
Phos ≥4.6 (%)	1.1	0	0	5.7	<0.001
Phos <2.5 (%)	11.5	16.2	10.5	7.5	0.276
Serum magnesium (1.7–2.6 mg/dl)	1.70 ± 0.26	1.67 ± 0.24	1.70 ± 0.21	1.74 ± 0.38	0.270
Serum creatinine (0.5–1.2 mg/dl)	1.69 ± 0.69	1.05 ± 0.19	1.63 ± 0.36	2.75 ± 0.59	<0.001
Parathyroid hormone (10–65 ng/l)	79 (55–132)	58 (46–85)	75 (54–126)	155 (91–224)	<0.001
Parathyroid hormone >65 (%)	58.8	41.9	57.9	84.9	<0.001
Fibroblast growth factor (pg/ml)[a]	101 (70–153)	76 (54–96)	102 (72–145)	165 (130–318)	<0.001
Fibroblast growth factor ≥100 (%)	49.8	21.6	51.3	84.9	<0.001
25 (OH) vitamin D3 (30–100 ng/ml)	37.5 (26.5–54.0)	36.0 (26.4–53.1)	41.3 (29.0–56.0)	33.0 (23.5–51.0)	0.088
1,25(OH)2 D3 (16.0–55.5 pg/ml)	33.0 (21.0–47.5)	37.5 (26.5–56.3)	33.0 (20.5–50.0)	26.0 (18.0–39.3)	0.007
Osteocalcin (7.7–48 ng/ml)	25.0 (16.0–44.0)	19.5 (13.0–32.5)	25.0 (16.0–40.0)	43.5 (25.0–78.3)	<0.001
Alkaline phosphatase (30–120 UI/l)	89.9 ± 33.9	95.2 ± 39.7	87.9 ± 33.0	88.3 ± 27.1	0.290
Estimated glomerular filtration rate	52.4 ± 19.9	73.5 ± 12.0	44.3 ± 8.4	23.7 ± 4.2	<0.001
Phosphaturia (mg/day)	743 ± 320	772 ± 319	777 ± 339	607 ± 218	0.002
Calciuria (mg/day)	53.2 (27.0–110.4)	93.1 (36.9–171–3)	52.0 (27.0–93.6)	32.4 (17.8–55.0)	<0.001
24-h phosphate reabsorption rate (%)	73.7 ± 10.0	80.1 ± 7.3	73.6 ± 8.7	65.1 ± 10.3	<0.001
24-h fractional excretion Ca (%)	0.68 (0.37–1.15)	0.80 (0.32–1.29)	0.58 (0.38–1.08)	0.83 (0.36–1.29)	0.567
24-h fractional excretion Mg (%)	4.87 ± 2.58	3.27 ± 0.50	4.91 ± 2.34	6.99 ± 2.89	<0.001
Bone density femoral neck (*Z* score)	−0.60 (−1.30–0.20) ($n = 249$)	−0.50 (−1.30–0.20) ($n = 63$)	−0.70 (−1.30–0.20) ($n = 146$)	−0.60 (−1.65–0.10) ($n = 40$)	0.442
Bone density lumbar spine (*Z* score)	−0.60 (−1.48–0.30) ($n = 252$)	−0.60 (−1.50–0.53) ($n = 66$)	−0.70 (−1.40–0.40) ($n = 145$)	−0.40 (−1.70–0.05) ($n = 41$)	0.843

Normal ranges are shown in parenthesis according to the Central Laboratory of Hospital Clínico San Carlos (Madrid, Spain) and to those of the manufacturer.
[a]No range is applicable according to the manufacturer.

Table 3. Proportions of participants with normal or high FGF23 and PTH levels within each eGFR category

	Normal FGF23 and normal PTH	High FGF23 and normal PTH	Normal FGF23 and high PTH	High FGF23 and high PTH
CKD Stage 1 (%)	44.4	22.2	11.1	22.2
CKD Stage 2 (%)	47.7	9.2	33.8	9.2
CKD Stage 3 (%)	26.3	15.8	22.4	35.5
Patients treated with calcitriol or calcitriol analogs (%)*	8	9.4	5.3	16.1
Serum phosphate (mg/dl)**	3.2 ± 0.5	3.6 ± 0.6	3.0 ± 0.6	3.5 ± 0.7

FGF23 was considered high if ≥100 pg/ml; PTH was considered high if PTH ≥65 pg/ml. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; PTH, parathyroid hormone. No patients were treated with phosphorus supplements.
*P = 0.211; **P < 0.001.

which were far more likely than an isolated increase in FGF23.

A significant inverse correlation was found between the eGFR and FGF23 ($r = -0.487$; $P < 0.001$) (Figure 1A), PTH ($r = -0.444$; $P < 0.001$) (Figure 1B), serum phosphate levels ($r = -0.315$; $P < 0.001$) and fractional excretion of magnesium ($r = -0.503$; $P < 0.001$). A significant positive correlation was also observed between the eGFR and phosphate reabsorption rate ($r = 0.556$; $P < 0.001$) and the calcitriol levels ($r = 0.249$; $P < 0.001$).

Relationship of serum FGF23levels

In the univariable analysis (Table 4), the variables significantly associated with serum FGF23 levels were increased time post-transplant, time on corticosteroids, PTH (Figure 2A), serum phosphate levels (Figure 2B), proteinuria, decreased serum calcitriol, eGFR (Figure 1A), phosphate reabsorption rate, plasma bicarbonate, 24-h calciuria and 24-h urine magnesium. In the multivariable model, the statistically significant variables were increased time on corticosteroids, PTH, serum phosphate levels, proteinuria and decreased serum calcitriol and eGFR.

Regulation of the 24-h phosphate reabsorption rate

We compared the 24-h phosphate reabsorption rate and TmP/GFR between patients with normal and high FGF23 and PTH levels overall and according to CKD stage (Figure 3). Most patients with CKD Stage 4 had high FGF23 and high PTH levels (75%); only five patients had high FGF23 levels and normal PTH levels, and a further five had normal FGF23 levels and high PTH levels. When we analysed patients with CDK Stages 1–3, no statistical differences were found at any level between patients with normal FGF23 and normal PTH compared with patients who only had increased FGF23 ($P = 0.249$ and $P = 0.803$ for TmP/GFR at Stages 1 and 2 and Stage 3, respectively, and $P > 0.999$ and $P > 0.999$ for the phosphate reabsorption rate at Stages 1 and 2 and Stage 3, respectively). However, both figures were significantly lower in patients with increased PTH when we compared patients with normal FGF23 and high PTH with patients with high FGF23 and normal PTH levels ($P = 0.003$ and $P = 0.004$ for TmP/GFR at Stages 1 and 2 and Stage 3,

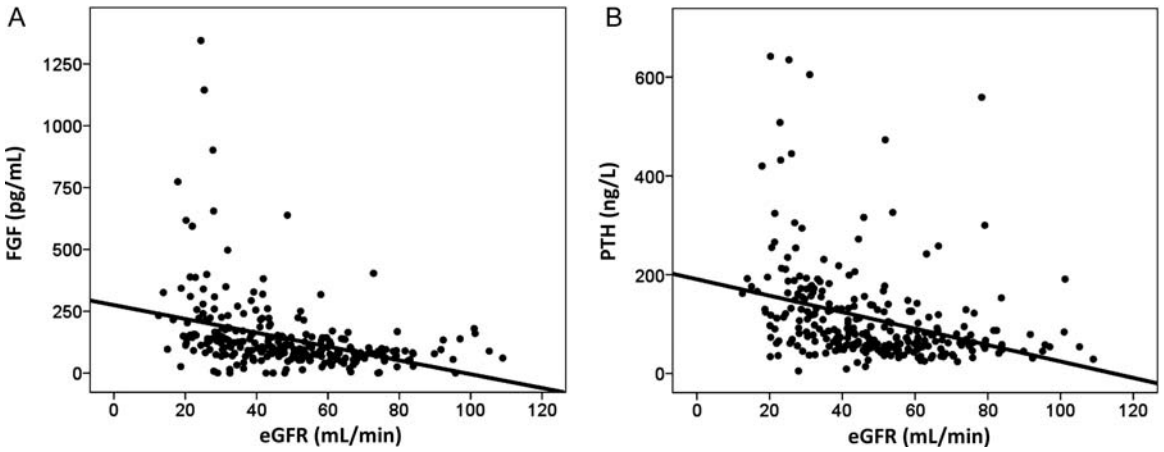

Fig. 1. (**A**) Correlation between eGFR and FGF23 (Spearman $r = -0.487$; $P < 0.001$). (**B**) Correlation between eGFR and PTH (Spearman $r = -0.444$; $P < 0.001$).

Table 4. Univariable and multivariable regression analysis for FGF23 and phosphate reabsorption rate

	Square root FGF23			Phosphate reabsorption rate		
	β	P	r^2	β	P	r^2
Univariable model						
Age	0.002	0.977	0.000	−0.021	0.712	0.000
Months post-transplant	0.004	0.021	0.019	0.026	0.650	0.001
Months on dialysis	0.056	0.350	0.003	−0.054	0.355	0.003
Square root FGF	—	—	—	−0.267	<0.001	0.071
Square root PTH	0.408	<0.001	0.166	−0.444	<0.001	0.197
Square root calcitriol	−0.206	0.001	0.043	0.187	0.002	0.035
Square root calcidiol	−0.060	0.319	0.004	−0.004	0.950	0.000
Serum phosphate	0.269	<0.001	0.073	—	—	—
Albumin-corrected calcium	0.079	0.187	0.006	−0.008	0.893	0.000
Serum magnesium	−0.051	0.396	0.003	−0.044	0.451	0.002
eGFR	−0.100	<0.001	0.158	0.276	<0.001	0.283
Plasma bicarbonate	−0.175	0.004	0.031	0.305	<0.001	0.093
Square root 24-h proteinuria	0.111	<0.001	0.116	0.158	<0.001	0.057
24-h urinary calcium	−0.177	0.003	0.031	0.100	0.096	0.010
24-h urinary magnesium	−0.131	0.029	0.017	−0069	0.249	0.005
Phosphate reabsorption rate	−0.239	<0.001	0.057	—	—	—
Tubular maximum phosphorus reabsorption/glomerular filtration rate	0.058	0.350	0.003	—	—	—
Corticosteroids dose (mg/kg year)	0.281	<0.001	0.076	−0.80	0.185	0.003
Multivariable model						
Square root PTH	0.377	<0.001	0.331	−0.555	<0.001	0.359
Square root calcitriol	−0.281	0.049		0.540	0.009	
Corticosteroid dose (mg/kg year)	0.105	0.014				
eGFR	−0.038	0.003		0.228	<0.001	
Serum phosphate	1.112	0.003				
Square root 24-h proteinuria	0.033	0.098				
Plasma bicarbonate				0.342	0.024	
24-h urinary calcium				−0.119	0.028	

FGF, fibroblast growth factor; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.

respectively, and $P = 0.003$ and $P = 0.028$ for the phosphate reabsorption rate at Stages 1 and 2 and Stage 3, respectively). The percentage of patients with CKD Stages 1, 2 and 3 treated with vitamin D analogues was similar between the four groups of patients ($P = 0.211$). No patients were treated with phosphorus supplements, although serum phosphate levels were higher in patients with high FGF23 and normal PTH levels ($P < 0.001$).We also analysed the correlation between the phosphate reabsorption rate and FGF23 levels by stratifying according to normal or high PTH. In patients with normal PTH levels, no correlation was observed between the phosphate reabsorption rate and FGF23 ($r = 0.04$, $P = 0.663$) (Figure 4A). However, if PTH was elevated, a correlation was observed between the phosphate reabsorption rate and FGF23. Moreover, when the correlation between the phosphate

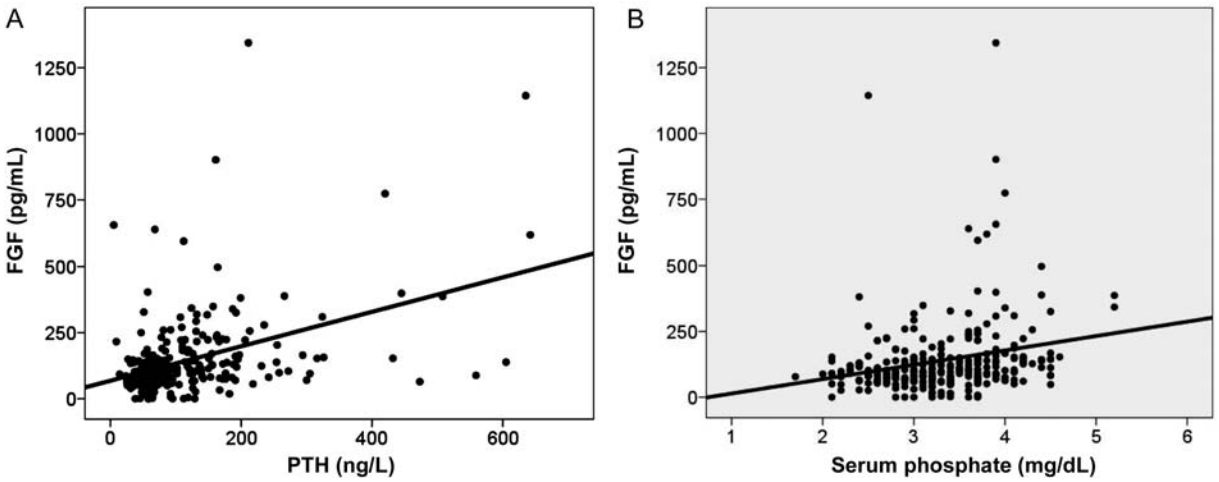

Fig. 2. (**A**) Correlation between FGF23 and PTH (Spearman $r = 0.406$; $P < 0.001$). (**B**) Correlation between FGF23 and serum phosphate levels (Spearman $r = 0.298$; $P < 0.001$).

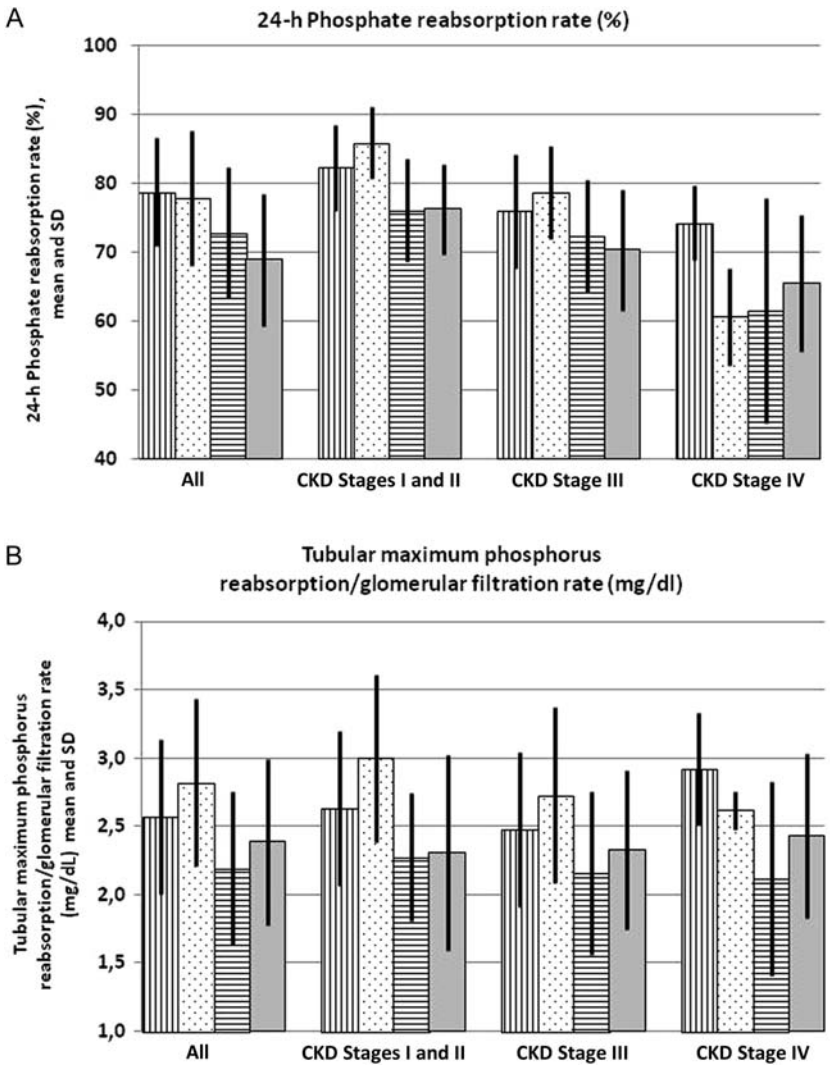

Fig. 3. The 24-h phosphate reabsorption rate and tubular maximum phosphorus reabsorption/glomerular filtration rate in patients with normal values of PTH and FGF23 (vertical lines), patients with high values of FGF23 (≥100 pg/ml) and normal values of PTH (dots), patients with normal values of FGF23 and high values of PTH (≥65 pg/ml) (horizontal lines) and patients with high values of FGF23 and high values of PTH (black). Values are expressed as the mean and standard deviation.

reabsorption rate and the PTH was analysed by dividing patients according to whether they had normal or high levels of FGF23, the correlation was significant in both ($r = 0.286$, $P = 0.001$ and $r = 0.411$, $P < 0.001$, respectively) (Figure 4B).

The results of the multivariable analysis are shown in Table 4. Increased calcitriol levels, eGFR and bicarbonate as well as decreased PTH and 24-h calciuria levels were associated with an increased 24-h phosphate reabsorption rate. FGF23 had no statistical effect on the phosphate reabsorption rate.

Discussion

Post-transplant bone disease is an important complication in a substantial proportion of patients, although its aetiology and course vary. Patients often undergo transplantation with pre-existing bone mineral disease. In addition, after transplantation, other potentially deleterious factors can appear, such as immunosuppressive therapy and impaired kidney function [40]. The levels of FGF23, a new phosphaturic hormone that has recently been implicated in the development of secondary hyperparathyroidism in patients with CKD [7, 19, 20, 26], could be increased in kidney recipients. The residual FGF23 activity may also contribute to early post-transplant hypophosphataemia [9, 25, 26, 29, 31], which could in turn play a role in the pathogenesis of post-transplant bone disease. Controversy exists over whether these losses in phosphorus persist in the long term. Studies on FGF23 in renal transplant patients evaluated in the early post-transplant period (range, 3–12 months) [9, 25–32] report a marked fall in FGF23 levels over time, with mean values at the end of follow-up very close to normal. This contrasts with our results, namely, high levels in a large number of patients, even though findings for renal function were not lower than those shown in studies analysing the immediate post-transplant period. Therefore, the production of FGF23 could increase in the medium-to-long term and under conditions of which we are unaware, since our study is not longitudinal.

As previously demonstrated in patients with CKD [16, 19–21], we found that the FGF23 values were increased in most transplant recipients with CKD (all stages) and highest in those with the most advanced stages. This inverse correlation between GFR and FGF23 levels may be partly attributable to poorer kidney function in this group. However, although the median FGF23 levels in our study were above the normal range, they were lower than those previously reported in patients with comparable eGFR who did not undergo transplant [16, 19, 20]. This finding has been corroborated by Wolf *et al*. [35]. It is possible that kidney recipients could develop abnormal FGF23 production as a result of previous chronic phosphate retention stimulating the secretion of FGF23. In addition, bone abnormalities before transplant may have led to reduced production of FGF23 in the osteocytes of recipients. In fact, the association between bone turnover and serum FGF23 can vary, as shown in several mouse models, where bone turnover was altered by a variety of exogenous and endogenous factors [41]. Finally, the factors such as immunosuppressive drugs, which can affect bone turnover, should also be taken into account. Given that our study did not include a control group of CKD patients who had not received a transplant, this finding should be further tested.

In patients with CKD, the increase in FGF23 could precede that of PTH [20, 21]. In fact, Evenepoel *et al*. [21] showed that more patients had elevated FGF23 than elevated PTH (60.8% *vs* 9.8%). However, in transplant recipients, we found that increased PTH in the setting of

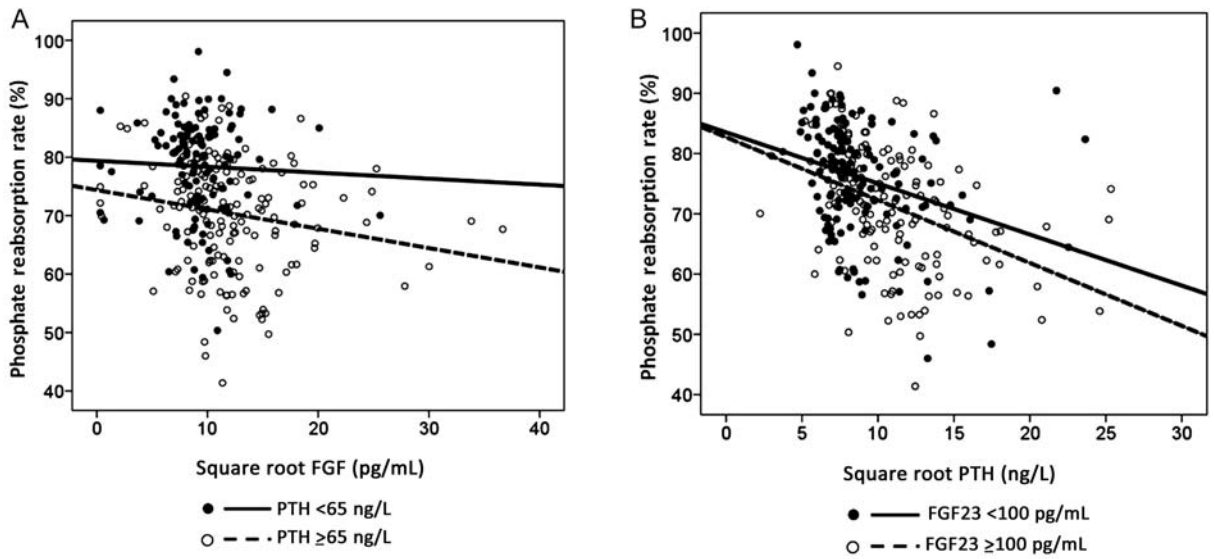

Fig. 4. (**A**) Correlation between the 24-h phosphate reabsorption rate and FGF23 levels stratified by normal PTH (black dots and continuous line) ($r = 0.04$, $P = 0.663$) or high PTH (white dots and broken line) ($r = 0.182$; $P = 0.02$). (**B**) Correlation between the 24-h phosphate reabsorption rate and PTH stratified by FGF23 <100 pg/ml (black points and continuous line) ($r = 0.286$, $P = 0.001$) or FGF3 ≥100 pg/ml (white dots and broken line) ($r = 0.411$, $P < 0.001$).

normal FGF23 levels was more frequent than an isolated increase in FGF23. Pre-transplant CKD with a stimulated PTH and a certain degree of hyperplasia in the parathyroid glands could account for the more pronounced effect of this hormone. Another possible explanation could be that bone abnormalities before transplant may have led to reduced production of FGF23. In fact, as commented on above, at similar degrees of CKD, transplant recipients seem to produce less FGF23 than patients with CKD who do not undergo transplant.

In addition to renal function, we found that other factors were associated with excess FGF23, namely, increased cumulative doses of corticosteroids, PTH and serum phosphate levels and decreased serum calcitriol. Our results are consistent with those of Bacchetta *et al*. [42], who reported that paediatric CKD patients receiving corticosteroids had higher FGF23 values than other CKD patients. This finding was confirmed in a subsequent study on paediatric kidney recipients [34], suggesting that treatment with corticosteroids can directly stimulate the bone FGF23 production and alter the relationship between FGF23 values and other biochemical variables.

The results of our multivariable analysis show that increases in serum phosphate, even within normal limits, can stimulate the production of FGF23. This finding has also been reported in patients with CKD who were not undergoing transplant [16, 20]. Wolff *et al*. [35] reported that recipients with high FGF23 levels have an increased risk of mortality and allograft loss. It has been speculated that high FGF23 levels in transplant recipients could reflect the burden of vascular calcification in the pre-transplant period and thereby predict the risk of subsequent mortality [35]. Therefore, high FGF23 levels should be prevented, because some of the factors that stimulate its production can be modified. Measures aimed at reducing doses of corticosteroids and PTH and serum phosphate levels (through the use of phosphate binders) could help to lower FGF23 levels, with a potentially positive impact on long-term survival.

Our data contrast with those from the predialysis CKD population and renal allograft recipients in the first year post-transplantation, when higher FGF23 values were correlated with increased phosphate excretion [16, 21, 25, 26, 32]. In our study, FGF23 does not seem to play an important role in renal phosphorus elimination. First, both the 24-h phosphate reabsorption rate and TmP/GFR were similar between patients with normal FGF23 and those who had high levels of FGF23 and normal PTH. Nevertheless, these values fell significantly in patients with elevated PTH. Secondly, in patients with normal PTH levels, we observed no correlation between the FGF23 and the phosphate reabsorption rate. And finally, we observed a statistically significant correlation between the PTH and the phosphate reabsorption rate, irrespective of FGF23 values (Figure 4B). Therefore, our data do not support the theory established in patients with CKD that both hormones act synergistically to induce renal phosphate excretion [21]. One of the possible explanations for this apparent lack of effect of FGF23 on the phosphate reabsorption rate could be that transplant recipients have a deficiency of or resistance to Klotho, or, that an immunosuppressive agent could interfere with Klotho.

Our study is limited by its cross-sectional design, which restricted our ability to examine longitudinal changes in FGF23, calcitriol, PTH and mineral levels. As a result, we cannot establish causal associations. However, we have attempted to limit our interpretation of the data in accordance with previously published results. In addition, we analysed a relatively large number of patients and performed an exhaustive study of bone mineral metabolism. We used the MDRD equation because this formula identifies patients with renal failure more precisely than the Cockcroft and Gault formula and is recommended in renal transplant patients [43]. However, with MDRD, the true GFR will still be overestimated by roughly 20%. Our data were also analysed using the Cockcroft and Gault formula, and the results were similar.

In conclusion, altered mineral ion homeostasis is common in the post-transplant period, even in patients who have maintained stable renal allograft function for >1 year, thus highlighting the need to apply KDIGO guidelines for bone metabolism and mineral ion homeostasis in the renal transplant population. FGF23 concentrations begin to increase early in CKD. In these patients, it seems that PTH plays a more important role in renal phosphate excretion than FGF23. It remains to be seen whether measures aimed at reducing serum levels of PTH and phosphate and/or corticosteroid doses might help to lower serum FGF23 and whether this will improve kidney recipient outcomes.

Conflict of interest statement. None declared.

References

1. Ghanekar H, Welch B, Moe O et al. Post-renal transplantation hypophosphatemia: a review and novel insights. *Curr Opin Nephrol Hypertens* 2006; 15: 97–104
2. David DS, Sakal S, Brennan L *et al*. Hypercalcemia after renal transplantation: Long-term follow-up data. *N Engl J Med* 1973; 289: 398–401
3. Ozdemir FN, Afsar B, Akgul A et al. Persistent hypercalcemia is a significant risk factor for graft dysfunction in renal transplantation recipients. *Transplant Proc* 2006; 38: 480–482
4. Gwinner W, Suppa S, Mengel M *et al*. Early calcification of renal allografts detected by protocol biopsies: causes and clinical implications. *Am J Transplant* 2005; 5: 1934–1941
5. Reynolds JL, Joannides AJ, Skepper JN *et al*. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol* 2004; 15: 2857–2867
6. Levi M. Post-transplant hypophosphatemia. *Kidney Int* 2001; 59: 2377–2387
7. Evenepoel P, Meijers B, de Jonge H *et al*. Recovery of hyperphosphatoninism and renal phosphorus wasting one year after successful renal transplantation. *Clin J Am Soc Nephrol* 2008; 3: 1829–1836
8. Green J, Debby H, Lederer Eet al. Evidence for a PTH-independent humoral mechanism in post-transplant hypophosphatemia and phosphaturia. *Kidney Int* 2001; 60: 1182–1196
9. Kawarazaki H, Shibagaki Y, Shimizu H *et al*. Persistent high level of fibroblast growth factor 23 as a cause of post-renal transplant hypophosphatemia. *Clin Exp Nephrol* 2007; 11: 255–257
10. Liu S, Quarles LD. How fibroblast growth factor 23 works. *J Am Soc Nephrol* 2007; 18: 1637–1647

11. Yamazaki Y, Tamada T, Kasai N *et al*. Anti-FGF23 neutralizing antibodies show the physiological role and structural features of FGF23. *J Bone Miner Res* 2008; 23: 1509–1518
12. Yoshiko Y, Wang H, Minamizaki T *et al*. Mineralized tissue cells are a principal source of FGF23. *Bone* 2007; 40: 1565–1573
13. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V *et al*. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest* 2007; 117: 4003–4008
14. Kurosu H, Ogawa Y, Miyoshi M *et al*. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 2006; 281: 6120–6123
15. Nakatani T, Ohnishi M, Razzaque MS. Inactivation of klotho function induces hyperphosphatemia even in presence of high serum fibroblast growth factor 23 levels in a genetically engineered hypophosphatemic (Hyp) mouse model. *FASEB J* 2009; 23: 3702–3711
16. Gutierrez OM, Mannstadt M, Isakova T *et al*. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008; 359: 584–592
17. Kazama JJ, Sato F, Omori K *et al*. Pretreatment serum FGF-23 levels predict the efficacy of calcitriol therapy in dialysis patients. *Kidney Int* 2005; 67: 1120–1125
18. Nakanishi S, Kazama JJ, Nii-Kono T *et al*. Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. *Kidney Int* 2005; 67: 1171–1178
19. Isakova T, Xie H, Yang W *et al*.; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011; 305: 2432–2439
20. Isakova T, Wahl P, Vargas GS *et al*. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 2011; 79: 1370–1378
21. Evenepoel P, Meijers B, Viaene L *et al*. Fibroblast growth factor-23 in early chronic kidney disease: additional support in favor of a phosphate-centric paradigm for the pathogenesis of secondary hyperparathyroidism. *Clin J Am Soc Nephrol* 2010; 5: 1268–1276
22. Pereira RC, Juppner H, Azucena-Serrano CE et al. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. *Bone* 2009; 45: 1161–1168
23. Filler G, Liu D, Huang SH et al. Impaired GFR is the most important determinant for FGF-23 increase in chronic kidney disease. *Clin Biochem* 2011; 44: 435–437
24. Danziger J. The bone-renal axis in early chronic kidney disease: an emerging paradigm. *Nephrol Dial Transplant* 2008; 23: 2733–2737
25. Bhan I, Shah A, Holmes J *et al*. Post-transplant hypophosphatemia: tertiary 'Hyper-Phosphatoninism'? *Kidney Int* 2006: 70: 1486–1494
26. Evenepoel P, Naesens M, Claes K et al. Tertiary 'hyperphosphatoninism' accentuates hypophosphatemia and suppresses calcitriol levels in renal transplant recipients. *Am J Transplant* 2007; 7: 1193–1200
27. Economidou D, Dovas S, Papagianni A et al. FGF-23 levels before and after renal transplantation. *J Transplant* 2009; 2009: 379–382
28. Sato T, Fukagawa M, Uchida K *et al*. 1,25-dihydroxyvitamin D synthesis after renal transplantation: the role of fibroblast growth factor 23 and cyclosporine. *Clin Transplant* 2009; 23: 368–374
29. Trombetti A, Richert L, Hadaya K *et al*. Early post-transplantation hypophosphatemia is associated with elevated FGF-23 levels. *Eur J Endocrinol* 2011; 164: 839–847
30. Kanaan N, Claes K, Devogelaer JP *et al*. Fibroblast growth factor-23 and parathyroid hormone are associated with post-transplant bone mineral density loss. *Clin J Am Soc Nephrol* 2010; 5: 1887–1892
31. Pande S, Ritter CS, Rothstein M *et al*. FGF-23 and sFRP-4 in chronic kidney disease and post-renal transplantation. *Nephron Physiol* 2006; 104: 23–32
32. Kawarazaki H, Shibagaki Y, Fukumoto S *et al*. The relative role of fibroblast growth factor 23 and parathyroid hormone in predicting future hypophosphatemia and hypercalcemia after living donor kidney transplantation: a 1-year prospective observational study. *Nephrol Dial Transplant* 2011; 26: 2691–2695
33. Seeherunvong W, Wolf M. Tertiary excess of fibroblast growth factor 23 and hypophosphatemia following kidney transplantation. *Pediatr Transplant* 2011; 15: 37–46
34. Wesseling-Perry K, Tsai EW, Ettenger RB et al. Mineral abnormalities and long-term graft function in pediatric renal transplant recipients: a role for FGF-23? *Nephrol Dial Transplant* 2011; 26: 3779–3784
35. Wolf M, Molnar MZ, Amaral AP *et al*. Elevated fibroblast growth factor 23 is a risk factor for kidney transplant loss and mortality. *J Am Soc Nephrol.* 2011; 22: 956–966
36. Manjunath G, Sarnak MJ, Levey AS. Estimating the glomerular filtration rate. Dos and don'ts for assessing kidney function. *Postgrad Med* 2001; 110: 55–62
37. Bijvoet OL. Relation of plasma phosphate concentration to renal tubular reabsorption of phosphate. *Clin Sci* 1969; 37: 23–36
38. Human intact FGF-23 ELISA Kit Enzyme-linked ImmunoSorbent Assay (ELISA) for the Determination of Human Fibroblast Growth Factor 23 Levels in Plasma or Cell Culture Media. http://www.immutopics.com/pdf/directional-inserts/60-6500.pdf
39. Kleimbaum DG, Kupper LL, Muller KE. *Applied Regression Analysis and Other Multivariable Methods*. Boston: PWS-KENT, 1988, pp. 324–325
40. Weisinger JR, Carlini RG, Rojas E et al. Bone disease after renal transplantation. *Clin J Am Soc Nephrol* 2006; 1: 1300–1313
41. Samadfam R, Richard C, Nguyen-Yamamoto L et al. Bone formation regulates circulating concentrations of fibroblast growth factor 23. *Endocrinology* 2009; 150: 4835–4845
42. Bacchetta J, Dubourg L, Harambat J *et al*. The influence of glomerular filtration rate and age on fibroblast growth factor 23 serum levels in pediatric chronic kidney disease. *J Clin Endocrinol Metab* 2010; 95: 1741–1748
43. Pöge U, Gerhardt T, Palmedo H et al. MDRD equations for estimation of GFR in renal transplant recipients. *Am J Transplant* 2005; 5: 1306–1311

Received for publication: 12.4.2012; Accepted in revised form: 31.7.2012