Associations between Peritoneal Daily Phosphate Removal and Regulators of Phosphate Metabolism Including Fibroblast Growth Factor 23 in Peritoneal Dialysis Patients

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Abstract

Aim: Increased FGF23 blood levels were found intact and biologically active in peritoneal dialysis (PD) patients. We aimed to evaluate in PD patients the associations between regulators of phosphate (Pi) metabolism including FGF23 and peritoneal daily Pi removal (PDPiR).

Methods: Sixty-four adult patients (male 56%, mean age 48 ± 13 years, median time on PD 3.4 months) were evaluated in a cross-sectional study (ST1). Daily Pi intake was assessed by food frequency questionnaire. Intact FGF23 blood levels were evaluated by ELISA (Immutopics, Inc., San Clemente, CA). The first 33 patients who completed 1 year after the first evaluation were re-evaluated. (longitudinal study, ST2).

Results: In ST1, both circulating FGF23 and Pi serum levels were associated with PDPiR (β=0.533, p=0.002 and β=0.342, p=0.045, respectively) independent of iPTH, dietary Pi intake, renal daily Pi removal, dialysate/plasma creatinine concentration ratio (D/P Cr), residual renal function (RRF), ultrafiltration volume, dwell volume, number of exchanges and dialysis vintage. In ST2, the increase in PDPiR was only correlated with the increase in FGF23 (β=0.480, p=0.012) independently of changes in serum Pi, RRF, renal daily Pi removal and D/P Cr.

Conclusions: Differences in circulating FGF23 and Pi serum levels contribute both independently to heterogeneity of PDPiR in PD patients

Keywords: Fibroblast growth factor 23; Peritoneal dialysis; Peritoneal daily phosphate removal; Serum phosphate

Introduction

Elevated serum phosphate (Pi) concentrations contribute to the development of arterial calcification, myocardial hypertrophy and endothelial dysfunction [1-3] and numerous observational studies have shown a significant and independent association between elevated serum Pi levels and cardiovascular mortality in patients with chronic kidney disease (CKD) [4-7]. Recently, fibroblast growth factor-23 (FGF23) was described as an endocrine hormone produced by osteocytes that directly regulates Pi homeostasis [8-10]. The expression of FGF23 is up regulated by increased serum Pi levels and directly reduces tubular Pi reabsorption by decreasing the expression of sodium-dependent Pi transporters in renal proximal tubules [11-14]. In pre-dialysis CKD patients, FGF23 blood levels are elevated earlier than either Pi or parathormone (iPTH) thus suggesting that FGF23 might be a sensitive early biomarker of disordered Pi metabolism that helps maintain normal serum Pi levels despite reduced renal function [15,16]. Although the exact CKD stage when FGF23 levels first become significantly elevated differs across studies, higher FGF23 on a continuous scale was consistently associated with higher serum Pi levels, higher fractional excretion of Pi, lower estimated glomerular filtration rate (eGFR) and lower levels of 1,25-dihydroxyvitamin D [17-20]. Circulating FGF23 in PD patients was found to be mostly intact and biologically active [21]. However, the specific effects of FGF23 on Pi balance are less clear in this population. Recent studies consistently provided evidence for a strong positive association between serum Pi and circulating FGF23 in both incident and prevalent PD patients

[22-24]. In addition, Isakova et al. reported in adult PD patients that among those with residual renal function (RRF) there is a continuous relationship between higher FGF23 and lower RRF and that longer dialysis vintage also independently associates with higher FGF23 [24]. It remains unclear, however, whether elevated FGF23 levels are associated with peritoneal Pi elimination. In studies that examined associations between FGF23 serum levels and peritoneal Pi clearance there was no significant relationship in patients with or without RRF [22,24]. However, we must bear in mind that peritoneal Pi clearance is calculated by the ratio of peritoneal daily Pi removal (PDPiR) and serum Pi levels and the latter are well recognized to be associated independently with FGF23. Moreover, although it has been suggested that PDPiR is indicative merely of Pi intake in the steady state [25] the association between PDPiR and regulators of Pi homeostasis such as FGF23, Pi serum levels, iPTH and dietary Pi intake was not

examined so far. In the present study we aimed to evaluate in stable adult PD patients the associations between PDPiR and regulators of Pi metabolism including FGF23. We also examined in a longitudinal study the time-dependent changes in RRF and their association with FGF23 serum levels and PDPiR.

Materials and Methods

Study population

Patients aged 18 years or older who had been undergoing chronic PD for the treatment of ESRD for at least 45 days at São João Hospital Centre were invited to participate in the study. Patients with a recent history (less than 3 months) of infection or acute cardiovascular episode were excluded. The medical records for each subject such as underlying kidney disease, duration of PD, cardiovascular disease history and other illnesses were abstracted. The study protocol was developed in adherence to the Declaration of Helsinki and was approved by the Ethics Committee for Health and Institutional Review Board of São João Hospital Centre. All patients provided written informed consent prior to the inclusion in the study.

Procedures

Sixty-four patients were included in a cross-sectional study. Patients were asked to attend to our Unit in two consecutive days. On day 1 they were instructed to bring 24-hour dialysate and urine collections and fasting plasma samples were obtained; on day 2 a combined peritoneal equilibration test (PET) was performed [26]. In particular, a combination of bicarbonate/lactate (25/15 mmol/L) was used as buffer, with a nominal pH of 7.4 and a sodium concentration of 132 mmol/L. Dietary Pi intake was assessed in a subset of 51 patients using a semiquantitative food

frequency questionnaire (FFQ), validated for the Portuguese adult population [27]. The FFQ is an 86-item questionnaire that assesses usual dietary intake over the previous 12 months, including food groups and beverages. Food intake was calculated by weighting one of the nine possibilities of frequency of consumption (from never or less than once per month, to six or more times a day), by the weight of the standard portion size of the food-item in grams. In addition, the same subset of 51 patients completed a food record to estimate average daily dietary intake of Pi, on the 2-day period before blood and dialysate collections. The conversion of food intake into nutrients was conducted based on the software Food Processor Plus (ESHA Research, Salem, Oregon), with nutritional information from food composition tables of United States Department of Agriculture, adapted to foods typically Portuguese [28]. Similar clinical and laboratory assessments were performed in a subset of 33 patients evaluated longitudinally, 12 months after the first evaluation. Patients enrolled in the longitudinal study were the first to complete a year after the first evaluation.

Assays

Routine laboratory testing of blood, dialysate, and urinary samples was performed by local laboratory using standard procedures. Dialysate and urinary Pi concentrations were measured using standard autoanalysers. All patients used commercially available PD solutions. The dialysate creatinine concentration was corrected for interference by glucose according to laboratory standards (correction factor=0.0001887). In the 64 patients included in cross-sectional study serum FGF23 levels were measured using Human Intact FGF23 ELISA kits (Immutopics, San Clemente, CA, USA), in duplicate. Blood was collected in BD Vacutainer tubes (Becton Dickinson, Madrid, Spain) containing aprotinin to prevent proteolysis and K3EDTA as anticoagulant. The reported assay parameters were sensitivity 1.0 pg/mL, intra-assay coefficient of variation 4.4% at 14.6 pg/mL and 2.6% at 148 pg/mL, and inter-assay coefficient of variation, 6.1% at 15.6 pg/mL and 6.5% at 166 pg/mL.

The upper limit of detection for this assay is 200 pg/ml; samples greater than 200 pg/ml required serial dilutions to obtain a measurement. In the subset of 33 patients included in the longitudinal study we used a second generation Human Intact FGF23 ELISA kit (Immutopics, San Clemente, CA, USA), in duplicate, for both the first and second evaluation. The reported assay parameters were sensitivity 1.5 pg/mL, intra-assay coefficient of variation 4.1% at 43 pg/mL and 2.0% at 426 pg/mL, and interassay coefficient of variation 9.1% at 46 pg/mL and 3.5% at 441 pg/mL. The upper limit of detection for this assay is 660 pg/ml; samples greater than 660 pg/ml required serial dilutions to obtain a measurement.

Calculations

We calculated parameters of dialysis adequacy, including weekly total, renal and peritoneal Kt/V and creatinine clearance, using standard methods. We analyzed renal creatinine clearance (in ml/min per 1.73 m^2) as the measure of RRF. Presence of RRF was defined as renal creatinine clearance greater than zero. We quantified the PDPiR or urinary excretion of Pi (mg/d) as follows: dialysate [or urine] Pi (mg/dl) x 24-hour dialysate [or urine] volume (ml/d) x 0.01. Total daily Pi removal equalled the sum of PDPiR and renal Pi removal. Peritoneal and renal Pi clearances (L/d per 1.73m^2) were calculated as follows: dialysate [or urine] Pi (mg/dl)/plasma Pi (mg/dl) x 24-hour dialysate [or urine] volume (ml/d) x 0.001 (L/ml) x (1.73 m^2 BSA). Total Pi clearance equaled the sum of peritoneal and renal Pi clearance. Dialysate/plasma creatinine concentration (D/PCreat) ratios were also calculated and used to classify patients as low, low average, high average or high transporters according to the criteria defined by Twardowski et al. [29]. Dietary protein intake was estimated from the protein equivalent of total nitrogen appearance (PNA) using the Randerson equation: PNA (g/24 h)=10.76 (0.69 urea nitrogen appearance + 1.46). Urea nitrogen appearance was determined by measuring the urea in the patient's urine and dialysate. PNA was normalized to actual edema-free body weight. (nPNA).

Statistical analysis

Results were expressed as frequencies and percentages for categorical variables. For continuous variables we present mean ± SD for those with Normal distribution, and median and interquartile range otherwise. All variables were tested for Normal distribution using the Kolmogorov–Smirnov test. Continuous variables non Normally distributed were logistically transformed to reduce the kurtosis: y=(1 + e (a-x)⁄b) , with x being the original variable, y being the transformed variable, a value chosen close to the median and b a value chosen close to half of the interquartile range. For the values of FGF23 obtained in the cross-sectional study we used a=300 and b=100. For the values of FGF23 obtained in the longitudinal study we used a=2500 and b=2500. After applying these transformations, we verified that the new variables followed a Normal distribution Correlations between two continuous variables were expressed as Pearson's correlation coefficients. Multivariate analysis using multiple linear regression models was performed to analyze the determinants of tFGF23, in which were included as independent variables serum Pi, iPTH, serum urea, serum creatinine, RRF, daily Pi intake, age and dialysis vintage. In order to analyze the determinants of PDPiR, the following variables

were included in another model:serum Pi, iPTH, RRF, D/PCreat, renal daily Pi removal, FGF23, dietary Pi intake, ultrafiltration volume, dwell volume, number of exchanges and dialysis vintage. The absence of collinearity among explanatory factors was checked in all models based on tolerance values greater than 0.10 and variable inflation factor less than 5.0. We compared laboratory and clinical characteristics of the subset of 33 patients in the longitudinal study using t test or Wilcoxon, as appropriate. Statistical analysis was performed using SPSS, version 17.0, for Windows software (SPSS, Chicago, IL). A $p < 0.05$ was considered statistically significant.

Results

Cross-sectional study

Demographic, clinical, laboratory and dialysis adequacy characteristics of the cross-sectional study population are shown in (Table 1). The study population consisted of 64 patients with mean (± SD) age of 48 ± 13 years; 56% were men; 100% were Caucasians. The median dialysis vintage was of 3.4 months [1.6 to 7.7 months]; 100% were treated on continuous ambulatory PD (CAPD). Hypertension and diabetes were present in 84.4% and 27% of patients, respectively. Residual renal function was present in 95.3% of participants. When patients were classified by membrane transport category, 22 (34.4%) were fast transporters, 33 (51.6%) were fast-average transporters, and 9 (14.1%) were slow-average or slow transporters. The mean daily nPNA was 1.53 ± 0.4 g/kg. Dietary Pi intake assessed by FFQ was closely related

with dietary Pi intake assessed by 2-day food record (r=0.723, p<0.001) as well as with daily nPNA (r=0.697, p<0.001).

In unadjusted analysis, tFGF23 serum levels positively correlated with serum Pi (r=0.486, p<0.001), serum urea (r=0.272, p=0.037), serum creatinine (r=0.415, p=0.001), iPTH (r=0.270, p=0.035), dialysis vintage (r=0.333, p=0.009), PDPiR (r=0.563, p<0.001) and dietary Pi intake (r=0.303, p=0.034) and negatively correlated with RRF (r=-0.254, p=0.048) and age (r=-0.294, p=0.022) (Figure 1). No significant relationship was observed between tFGF23 and peritoneal creatinine clearance (r=0.070, p=0.591). Also, tFGF23 did not correlate with both renal and peritoneal Pi clearance (r=-0.138, p=0.290 and r=0.131, p=0.315, respectively). Multivariate analysis showed that serum Pi and dialysis vintage were closely related with tFGF23 (β=0.553, p=0.006 and β=0.337, p=0.009 respectively) independently of age, iPTH, RRF, daily Pi intake, serum urea and serum creatinine (Table 2) In unadjusted analysis, PDPiR also positively associated with serum Pi (r=0.604, p<0.001), peritoneal creatinine clearance (r=0.238, p=0.048) and negatively correlated with RRF (r=-0.455, p<0.001). No significant relationship was observed between PDPiR and peritoneal urea Kt/V (r=0.096, p=0.455), age (r=0.008, p=0.951), dialysis vintage (r=-0.083, p=0.513), daily dwell volume (r=0.128, p=0.312), ultrafiltration volume (r=0.047, p=0.713), D/PCreat (r=0.144, p=0.258) and number of exchanges (r=0.163, p=0.198). In addition PDPiR was not associated with daily nPNA (r=-0.028, p=0.845) and daily Pi intake (r=0.142, p=0.320). On the other hand daily Pi intake was positively correlated

Demographic	
Age (years)[a]	48 ± 13
Sex (male)	(56%)
Clinical data	
Peritoneal dialysis vintage (months)[b]	3.4 (1.6 to 7.7)
Diabetes (%)	(27%)
Diabetes vintage (years)[a] n=17	16.6 ± 10.5
Hypertension (%)	(84.4%)
Hypertension vintage (years)[a] n=56	11.9 ± 8.7
CAPD	64 (100%)
Systolic blood pressure (mmHg)[a]	133 ± 24
Diastolic blood pressure (mmHg)[a]	79 ± 13
24-hour urine volume (ml)[b]	1725 (962 to 2187)
24-hour dialysate volume (ml)[b]	6572 (587 to 7560)
Ultrafiltration volume (ml)[b]	425 (0 to 800)
Number of exchanges[b]	3 (2 to 4)
Dwell volume (l/day)[b]	6.0 (6.0-8.0)
Residual renal function (ml/min per 1.73 m²)[a]	3.9 ± 2.3
Etiology of ESRD	
Glomerulonephritis	4(6.3%)
Diabetes	14(21.8%)
Polycystic kidney disease	6(9.4%)
Unknown	19(29.7%)
Others	21 (32.8%)

Laboratory Values	
Albumin (g/dl)[a]	36.8 ± 4.0
Calcium (mEq/L)[a]	4.46 ± 0.37
Phosphate (mg/dl)[a]	4.63 ± 1.18
PTH (pg/ml)[a]	438.0 ± 260.3
Urea (mg/dl)[a]	134.3 ± 38.5
Creatinin e (mg/dl)[a]	7.1 ± 2.5
C reactive protein (C-RP) (mg/L)[b]	2.9 (1.4 to 7.7)
FGF23 (pg/ml)[b]	288.7 (216.1 to 345.6)
Indices of dialysis adequacy	
Peritoneal weekly urea (Kt/V)[a]	1.2 ± 0.4
Renal weekly urea (Kt/V)[a]	1.1 ± 0.7
Total weekly urea (Kt/V)[a]	2.3 ± 0.7
Peritoneal creatinin e clearance (L/week per 1.73 m²)[a]	20.7 ± 6.7
Renal creatinine clearance (L/week per 1.73 m²)[a]	39.4 ± 22.5
Total creatinine clearance (L/week per 1.73 m²)[a]	60.1 ± 21.1
Phosphate clearance	
Peritoneal ph osphate clearan ce (L/week per 1.7 3 m²)[a]	36.8 ± 9.9
Renal phosphate clearance (L/week per 1.73 m²)[a]	45.7 ± 35.2
Total phosphate clearance (L/week per 1.73 m²)[a]	82.5 ± 33.9
Peritoneal daily phosphate removal (mg/d)[a]	240.8 ± 85.7.9
Renal daily phosphate removal (mg/d)[a]	283.1 ± 169.3
Total daily phosphate removal (mg/d)[a]	523.9 ±177.8
Peritoneal transport status	
Dialysate/Plasma Creatinine	22/64(34.4%)
Fast transporter	22/64(34.4%)
Fast-average transporter	33/64(51.6%)
Slow-average transporter	8/64(12.5%)
Slow transporter	1/64(1.6%)
Diet and medications	
Dietary phosphate (mg/day)[a] n=52	1178 ± 327.1
nPNA (g/kg/day)[a]	1.53 ± 0.4
Phosphate binders use (%)	48.3
Active vitamin D use (%)	53.1

CAPD, Continuous ambulatory peritoneal dialysis; APD, Automated peritoneal dialysis; Values are expressed as frequencies and percentages for categorical variables. Patients are categorized, concerning creatinine transport status as slow (D/P Cr=0.49), slow-average (0.50=D/P Cr=0.64), fast-average (0.65=D/P Cr=0.8) and fast (D/P Cr=0.8) transporters, according to the criteria defin ed by Twardowsky et al.; nPNA, normalized protein equivalent of total nitrogen appearance.
[a]Values are expressed as mean ± SD, [b]Values are medians with interquartile range in parentheses.

Table 1: Demographic, clinical, laboratory and dialysis adequacy characteristics of the cross-sectional study population (n=64).

with total daily Pi removal (r=0.394, P=0.004). To explore further the predictors of PDPiR we included in multivariable regression model tFGF23, Pi serum levels, iPTH, dietary Pi intake, renal daily Pi removal, D/PCreat, RRF, ultrafiltration volume, dwell volume, number of exchanges and dialysis vintage as independent variables and PDPiR as dependent variable. As shown in Table 3, both tFGF23 and Pi serum levels closely predicted PDPiR (β=0.533, p=0.002 and β=0.342, p=0.045 respectively), independently of other confounders (Table 3).

Longitudinal study

The first 33 patients who completed a year after the first evaluation (54% male; 100% on CAPD) were reassessed to study longitudinal changes in RRF and PDPiR and their association with changes in regulators of Pi metabolism including FGF23. Clinical, laboratory and dialysis adequacy characteristics of the longitudinal study population on the first and second evaluation periods are shown in Table 4. As can be
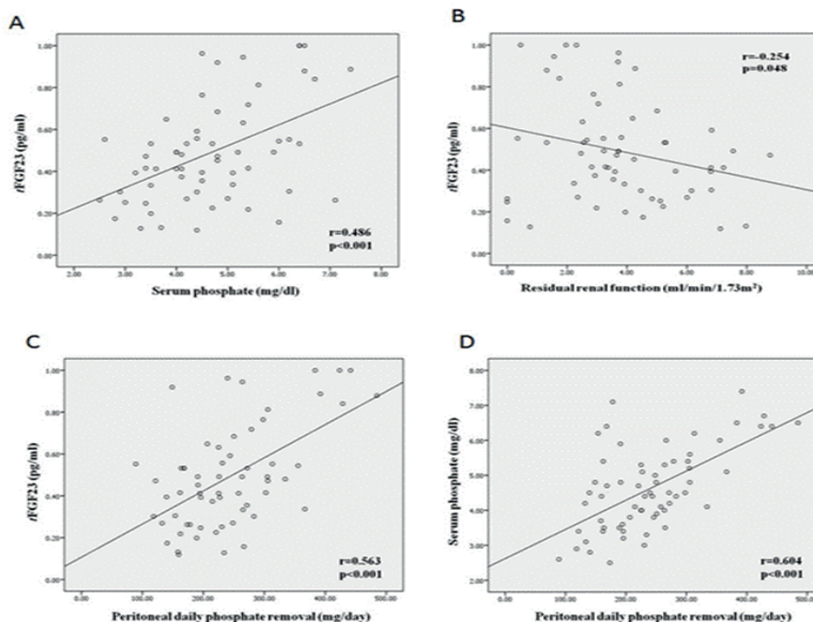
Figure 1

Figure 1: Scatter plots depict the unadjusted associations of tFGF23 with serum Pi (A), tFGF23 with RRF (B), tFGF23 with peritoneal daily Pi removal (C) and between serum Pi levels with peritoneal daily Pi removal (D) in the cross-sectional study (n=64).

Variables	B (SE)	β	p
Serum phosphate (mg/dl)	0.113 (0.39)	0.553	0.00 6*
iPTH (pg/ml)	-0.0008 (0.0002)	-0. 089	0. 615
RRF (ml/min/1.73$^{2)}$)	0.004 (0.022)	0.034	0. 848
Dietaryphosphate intake (mg/day)	-0.0007 (0.0001)	0.108	0. 466
Dialysis Vintage (months)	0.088 (0.032)	0.377	0.00 9*
Age (years)	-0.003 (0.002)	-0. 018	0. 207
Serum urea (mg/dl)	-0.002 (0.001)	-0. 357	0. 074
Serum creatinine (mg/dl)	0.026 (0. 021)	0.286	0. 277

Coefficient of determination (R^2)=0.429; Coefficient of determination adjusted (R^2 adjusted)=0.312; B, Regression coefficient; SE, Standard deviation; β, standardized regression coefficient, *p<0.05

Table 2: Predictive variables of FGF23 in the cross-sectional study population according to multiple linear regression analysis (n=64).

observed, during the 12 months follow-up period, a significant decrease was observed in RRF. This was accompanied by significant decreases in daily urine volume, renal weekly urea Kt/V, renal creatinine clearance, total creatinine clearance, renal Pi clearance, renal daily Pi removal and total daily Pi removal (Table 4). On the other hand, significant increases were observed over time in 24h dialysate volume, number of exchanges, serum creatinine, peritoneal weekly urea Kt/V, PDPiR and circulating FGF23. No significant changes were observed between the 1st and the 2nd evaluation periods in serum Pi levels, daily Pi intake, nPNA, total weekly urea Kt/V and total Pi clearance. Serum FGF23 levels positively correlated with serum Pi levels on both the 1st and the 2nd evaluation

Variables	B (SE)	B	p
Serum phosphate (mg/dl)	23.2 (11.2)	0.342	0.045*
iPTH (pg/ml)	-0.036 (0.053)	-0.170	0.499
RRF (ml/min/1.73$^{2)}$)	-8.2 (6.7)	-0.205	0.231
D/P C$_r$	-35.8 (1 13.9)	-0.047	0.754
Renal daily phosphate removal (mg/day)	-0.01 (0.09)	-0.028	0.881
t FGF23 (pg/ml)	179.9 (53.7)	0.533	0.002*
Dietary phosphate intake (mg/day)	-0.001 (0.034)	-0.006	0.968
Dwell volume (l/day)	4.46 (11.79)	0.086	0.708
Number of exchanges	-8.61 (31.8)	-0.065	0.788
Ultrafiltration volume (ml/day)	-0.008 (0.015)	-0.09	0.585
Dialysis Vintage (months)	-20.83 (11.93)	-0.267	0.089

Coefficient of determination (R^2)=0.537; Coefficient of determination adjusted (R^2 adjusted)=0.399; B, Regression coefficient; SE, Standard deviation; β, standardized regression coefficient, *p<0.05

Table 3: Predictive variables of peritoneal daily Pi removal in cross-sectional study population according to multiple linear regression analysis (n=64).

Variable	1st evaluation	2nd evaluation	p
24-hour urine volume (ml)[b]	1850 (975 to 2325)	1050 (387 to 1578)	<0.001*
24-hour dialysate volume (ml)[b]	6340 (5727 to 7092)	9217 (6692 to 12585)	<0.001*
Ultrafiltration volume (ml)[b]	300 (-175 to 800)	600 (100 to 1300)	0.205
Number of exchanges[b]	3 (2 to 4)	4 (3 to 5)	0.005*
Dwell volume (ml)[b]	6000 (6000 to 7000)	8000 (6000 to 10000)	0.056
Residual renal function (ml/min per 1,73 m²)[a]	4.17 ± 1.9	2.26 ± 1.8	<0.001*
Laboratory Values			
Albumin (g/dl)[a]	35.7 ± 3.8	36.9 ± 3.8	0.023*
Calcium (mEq/L)[a]	4.4 ± 0.3	4.3 ± 0.5	0.382
Phosphate (mg/dl)[a]	4.7 ± 1.0	4.7 ± 1.0	0.839
PTH (ppg/ml)[a]	433.5 ± 244.7	472 ± 270.9	0.586
Urea (mg/dl)[a]	133.1 ± 36.1	134.2 ± 34.4	0.755
Creatinine (mg/dl)[a]	7.0 ± 2.5	8.5 ± 2.3	0.002*
C reactive protein (C-RP, mg/L)[b]	1.9 (1.4 to 4.8)	2.4 (1.1 to 5.7)	0.439
Vitamin D (ng/ml)[b]	7.5 (5.0 to 11.7)	11 (6.0 to 21.7)	0.017*
FGF23 (pg/ml)[b]	1555.8 (786.2 to 3956.2)	3728.8 (960.8 to 8931.2)	0.040*[†]
Indices of dialysis adequacy			
Peritoneal weekly urea (Kt/V)[a]	1.1 ± 0.4	1.4 ± 0.5	0.001*
Renal weekly urea (Kt/V)[a]	1.2 ± 0.5	0.7 ± 0.5	<0.001*
Total weekly urea (Kt/V)[a]	2.3 ± 0.6	2.1 ± 0.5	0.097
Peritoneal creatinine clearance (L/week per 1,73 m²)[a]	20.7 ± 5.5	22.7 ± 7.6	0.055
Renal creatinine clearance (L/week per 1,73 m²)[a]	42.0 ± 20.03	23.4 ± 18.5	<0.001*
Total creatinine clearance (L/week per 1,73 m²)[a]	62.7 ± 19.9	46.9 ± 15.87	<0.001*
Phosphate clearance			
Peritoneal phosphate clearance (L/week per 1,73 m²)[a]	35.8 ± 8.4	38.5 ± 11.3	0.166
Renal phosphate clearance (L/week per 1,73 m²)	43.7 ± 23.2	29.9 ± 31.9	0.030*
Total phosphate clearance (L/week per 1,73 m²)[a]	79.6 ± 22.9	68.4 ± 32.2	0.112
Peritoneal daily phosphate removal (mg/d)[a]	230.5 ± 74.5	271.5 ± 121.3	0.037*
Renal daily phosphate removal (mg/d)[a]	278.3 ± 141.7	185.1 ± 144.3	<0.001*
Total daily phosphate removal (mg/d)[a]	508.6 ± 161.6	456.5 ± 182.9	0.028*
Peritoneal transport status			
Dialysate/Plasma Creatinine			
Fast transporter	14/33 (42.4%)	8/29 (27.6%)	
Fast-average transporter	12/33 (36.4%)	13/29 (44.8%)	

Slow-average transporter	6/33 (18.2%)	7/29 (24.1%)	
Slow transporter	1/33 (3%)	1/29 (3.4%)	
Diet and medications			
Dietary phosphate intake (mg/day)[a] n=24	1205.8± 381.9	1146.1±254.4	0.426
nPNA (mg/kg/day)[a]	1.65 ± 0.43	1.57 ± 3.7	0.395
Phosphate binders use (%)	46.4	66.7	
Active vitamin D use (%)	57.5	51.5	

[a]Values are expressed as mean ± SD, [b]Values are medians with interquartile range in parentheses, [†] p value was obtained using the transformed variable.

Table 4: Changes in clinical, laboratory and dialysis adequacy parameters in the longitudinal study population (n=33).

periods (r=0.486, p=0.004; r=0.512, p=0.003, respectively). In addition, serum FGF23 levels positively correlated with PDPiR on both the 1st and the 2nd evaluation periods (r=0.533, p=0.001; r=0.575, p=0.001, respectively). Moreover, serum Pi levels positively correlated with PDPiR on both the 1st and the 2nd evaluation periods (r=0.583, p=0.001; r=0.822, p=0.001, respectively). In unadjusted analysis, the changes in PDPiR over time were positively correlated with the changes in both FGF23 and Pi serum levels (Figure 2). However, in multivariate analysis the changes in PDPiR over time were only correlated with the changes in FGF23 (Table 4). This relationship was independent of changes in serum Pi, RRF, renal daily Pi removal and D/Pcreat (Table 5).

Discussion

In the present study we could confirm that FGF23 serum levels are markedly elevated in patients on PD and associate with Pi serum levels, longer dialysis vintage and lower RRF [22,24]. The finding of the present study is that FGF23 and Pi serum levels are both important determinants of PDPiR. We were also able to study longitudinal changes in RRF in a subset of PD patients and found that the increase in PDPiR over time was independently associated with the increase in FGF23. Taken together, our data provide evidence favoring the view that in addition to differences in serum Pi, differences in FGF23 closely predict changes in PDPiR and further suggest that these associations occur through distinct mechanisms. Studies carried out in pre-dialysis CKD patients all provided evidence that reduced renal function and Pi serum levels are major determinants of FGF23 [19,30]. Although data on FGF23 in PD patients are scarce, it has been reported in both pediatric and adult PD patients that absence of RRF is associated with higher FGF23 [24,31]. In addition, studies in adult PD patients showed that higher Pi serum levels, lesser RRF and longer dialysis vintage are associated with higher levels of FGF23 [22-24]. In the present study, we report similar findings in adult PD patients with RRF and extend this observation by showing that there is a continuous relationship between higher FGF23 and PDPiR and that Pi serum levels also associates with PDPiR, independently of other confounders. It appears, therefore, that Pi serum levels and FGF23 are two major factors that are associated with heterogeneity of PDPiR in PD patients. In the longitudinal study we found that the decreases in RRF and in renal Pi removal over time were accompanied by significant increases in PDPiR as well as in FGF23. Because PDPiR was found to be closely associated with Pi serum levels, we could speculate that more severe hyperphosphatemia maintained the concentration gradient for Pi removal through the peritoneal membrane on the second evaluation period. Yet, the Pi serum levels were identical in the two evaluation periods in the setting of similar dietary Pi intake and greater use of Pi binders. Moreover, in

multivariate analysis, we found that the changes in PDPiR over time were closely predicted by FGF23, independent of serum Pi and other confounders. Taken together, these findings further reinforce the view that FGF23 and Pi serum levels may contribute to heterogeneity of peritoneal Pi removal in PD patients, through distinct mechanisms.

In the present study, we could confirm previous observations showing that FGF23 is not associated with peritoneal clearance of Pi [22,24]. This is not an unexpected finding if one takes into consideration that peritoneal Pi clearance is calculated dividing the PDPiR by Pi serum levels. Actually, Pi serum levels are well recognized to be independently associated with FGF23 and the results of the present study further show that differences in PDPiR are also independently associated with FGF23. Although the association between PDPiR and regulators of Pi homeostasis was not previously examined, it has been suggested that PDPiR is indicative merely of Pi intake in the steady state [25]. In the present study, no significant relationship was observed
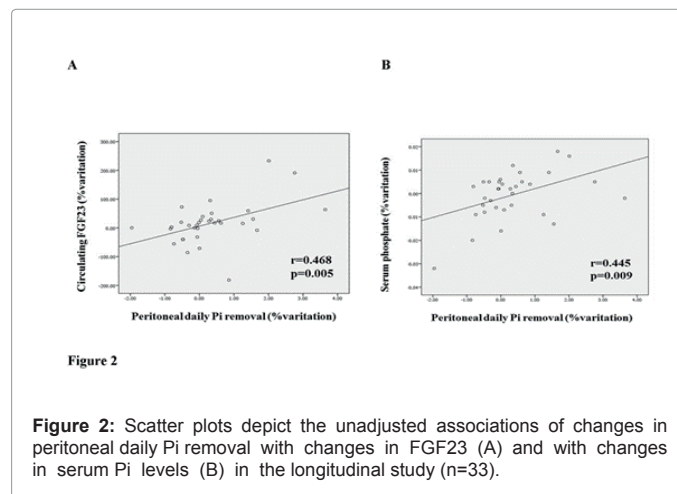

Figure 2: Scatter plots depict the unadjusted associations of changes in peritoneal daily Pi removal with changes in FGF23 (A) and with changes in serum Pi levels (B) in the longitudinal study (n=33).

Variables	B (SE)	β	p
Circulating FGF23 (% variation)	0.008 (0.003)	0.480	0.012*
Serum Pi (% variation)	27.2 (17.7)	0.251	0.137
RRF (% variation) -2.34 (6.26)	-0.061	0.711	
D/P Cr (% variation)	-44.1 (104.9)	-0.066	0.677
Renal daily phosphate removal (% variation)	0.023 (0.162)	0.024	0.889

Coefficient of determination (R^2)=0.413; Coefficient of determination adjusted (R^2 adjusted)=0.300; B, Regression coefficient; SE, Standard deviation; β, standardized regression coefficient, *p<0.05.

Table 5: Predictive changes of peritoneal daily Pi removal in the longitudinal study population according multiple linear regression analysis (n=33).

between dietary Pi intake and PDPiR. This is not an unexpected finding giving that ~95% of patients presented RRF. Accordingly, dietary Pi intake was closely associated with total daily Pi removal (renal + peritoneal). Our assessment of dietary intake of Pi was based on both a long-term (one year) and a short-term (2 days) food record carried out in the same subset of patients. In addition, the finding that dietary Pi intake was similar with both methods allowed us to appropriately assess the components of Pi balance antecedent to our evaluation. In our study, a strong correlation was observed between PDPiR and peritoneal creatinine clearance but not with peritoneal Kt/V urea. These findings agree well with the previous suggestions that peritoneal creatinine clearance can be used as surrogate marker for peritoneal Pi clearance [7,25] and further highlight that one cannot be based on the clearance of urea to evaluate peritoneal transport, particularly as it relates to Pi transport. In PD, diffusive and convective transport of Pi across the peritoneal membrane contributes to PDPiR [32]. In addition, the transport of Pi between the plasma and dialysate compartments in PD was suggested to be influenced by an electric potential difference [32,33]. However the mechanisms that regulate these components of peritoneal Pi transport have not been clearly elucidated. Because vascular tissues are well recognized to be endowed with FGF23 receptors [34] our present results raise interest in evaluating the presence of peritoneal FGF23 receptors and their potential role in peritoneal Pi transport. Strengths of our study include adjustment by multivariate linear regression analysis of the known variables that could influence PDPiR. Moreover we excluded all the measurements of dialysis dose and Pi removal carried out in the first 45 days of PD, to remove the possible bias of incident patients with inadequate dialysis. We were also able to study longitudinal changes in RRF and their association with regulators of Pi metabolism including FGF23 over a 12-month period in a subset of the studied population. One limitation is that this is an observational study. Also, we did not take into account the fact that Pi serum levels have known circadian pattern and demonstrate significantly higher overall within-subject variation than FGF23 [35]. In conclusion, our study highlights the strong positive association between FGF23 and PDPiR in stable PD patients. This relationship is independent of Pi serum levels, iPTH, dietary Pi intake, renal daily Pi removal, D/PCreat, RRF, ultrafiltration volume, dwell volume, number of exchanges and dialysis vintage. Studies examining the involvement of FGF23 on peritoneal Pi transport are thus warranted.

Acknowledgments

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