



Impaired GFR is the most important determinant for FGF-23 increase in chronic kidney disease

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ABSTRACT

Objectives: It is unclear whether fibroblast growth factor-23 (FGF-23) increases in response to phosphate accumulation or to decrease clearance in chronic kidney disease (CKD) as is the case with other low molecular weight proteins such as cystatin C (CysC).

Design and methods: This cross-sectional study measured serum FGF-23, CysC, and other serum markers of bone metabolism in 69 patients, aged 18 months–24 years, with various stages of CKD (eGFR = 11–214 mL/min).

Results: FGF-23 levels were significantly correlated with CysC and parathyroid hormone levels (PTH) on univariate non-linear regression analysis. In multivariate linear regression analysis, log (CysC) ($\beta = 0.660$, $p < 0.0001$), log (PTH) ($\beta = 0.038$, $p = 0.37$), and phosphate ($\beta = 0.222$, $p = 0.028$) explained 69.1% of the variance of FGF-23.

Conclusions: CysC had the largest unique contribution to FGF-23 variance in this model, supporting the hypothesis that renal clearance may be the most responsible factor for elevated FGF-23 levels in early stages of CKD.

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Introduction

Fibroblast growth factor-23 (FGF-23) was recently discovered as a phosphaturic hormone encoded by the PHEX gene that regulates phosphate and vitamin D metabolism [1]. FGF-23 levels increase as chronic kidney disease (CKD) worsens [2]. The role of FGF-23 in phosphate homeostasis in early CKD remains uncertain. Westerberg et al. examined the relationships among FGF-23, PTH, phosphorus, and estimated GFR and found that serum phosphorus is the most important predictor of FGF-23 when glomerular filtration rate (GFR) is less than 30 mL/min but not in patients with normophosphatemia and mild CKD [3]. Few studies have evaluated FGF-23 concentrations in relationship to GFR from a protein biochemistry perspective. FGF-23 is a small molecular weight protein (LMWP) of 251 amino acid residues and a molecular weight of 32 kDa [1]. Since LMWPs tend to increase in CKD as they are freely filtered across the glomerulus [4], we hypothesized that glomerular filtration rate (GFR), measured by

cystatin C (CysC), would be a major contributor to the rise of FGF-23 in CKD. Previous studies have shown an association between increased creatinine and serum FGF-23 concentrations [2,3]. We are unaware of studies on the association between CysC, a well-established LMWP marker for GFR with a molecular weight of 13 kDa [5], and FGF-23. We hypothesized that GFR would be the major predictor of FGF-23 variance in CKD patients.

Materials and methods

Specimen

After obtaining ethical approval from the University of Western Ontario Research Ethics Board (REB#16962E), we recruited 69 pediatric and young adult patients without evidence of cardiovascular disease in a cross-sectional study. All patients between the ages of 18 months and 30 years with CKD stages 1–5 at the Children's Hospital, London, Ontario, were eligible. Written and informed consents were obtained for each patient. In addition to routine blood work, we obtained serum for FGF-23 and CysC levels in each case. We also measured phosphate, calcium, ionized calcium, serum albumin and total protein, bicarbonate, vitamin D metabolites (1,25-

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dihydroxy- and 25-hydroxyvitamin D), and intact PTH (second generation assay) levels, and urinary calcium to creatinine ratio, using standard laboratory tests.

Analytical validation of FGF-23 assay

Serum FGF-23 levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA) system using two kinds of monoclonal antibodies requiring the simultaneous presence of both the N-terminal and C-terminal portions of FGF-23 (Kainos Laboratories, Inc., Tokyo, Japan) following the manufacturer's instructions. In each antibody-coated well, 50 μ L of serum sample with 50 μ L of assay diluent were added to each well. The plate was then incubated at room temperature for 2 h on a plate mixer. The plate was washed 4 times and incubated with FGF-23 conjugate mixing for 1 h at room temperature. After another 4 washes, substrate was added and allowed to develop for 30 min. The signal was read in a microplate reader at absorbance 450 nm within 10 min [6]. Inter-assay and intra-assay coefficient of variation were 5.0% and 3.0%, respectively. Cystatin C was measured using the Siemens Healthcare nephelometric assay (PETIA) on a BN ProSpec platform (Dade Behring) [4,7].

Data analysis

We used the statistical package GraphPad PRISM version 4.1 for the analysis. Continuous numerical variables were analyzed for normal distribution using the D'Agostino–Pearson omnibus normality test. Normally distributed variables were expressed as mean and standard deviation and analyzed using parametric analysis; otherwise, non-parametric tests were used. Association studies were performed using univariate one phase exponential association and multivariate regular linear regression analyses after log-transformation of non-normally distributed parameters. The relationship between CysC eGFR [7] and other parameters was also assessed using non-parametric methods and was best described as one-phase exponential decay.

Results

In this cross-sectional study, serum FGF-23, CysC, and several serum markers of bone metabolism were measured in 69 children and young adults with chronic kidney disease stages 1–5 (median cystatin C eGFR, 94.8 mL/min; range, 10–214 mL/min). The mean age of the 69 participants was 13.95 years (range, 2.5–24.0 years). Median FGF-23 level was 49 pg/mL, ranging from 21 to 971 pg/mL. FGF-23, CysC, phosphate, alkaline phosphatase, ionized calcium, parathyroid hormone, and creatinine levels were not normally distributed. Total calcium, 25-OH vitamin D, and 1,25(OH) vitamin D levels were, however, normally distributed. Table 1 lists the median (interquartile range) for each of the measurements.

To determine the co-variance between FGF-23 and other serum biochemistries, a univariate non-linear (one phase exponential)

Table 1

Median, interquartile, and range for serum markers measured in 69 pediatric and young adult patients.

	FGF-23 (pg/mL)	CysC (mg/L)	CysC eGFR (mL/min/1.73 m ²)	Creat (μ mol/L)	Ca (mmol/L)	Ionized Ca (mmol/L)	PO ₄ (mmol/L)	Alk phosph (U/L)	PTH (pmol/L)	25-OH vit D (nmol/L)	1,25(OH) vit D (pmol/L)
Number of values	64	69	69	69	70	61	70	62	61	69	59
Minimum	20.84	0.47	11	19	1.98	0.81	0.58	45	0.6	25	10
25% percentile	38.93	0.65	60	45	2.23	1.11	1.10	88	3.2	52	57
Median	49.07	0.97	95	66	2.33	1.16	1.31	157	4.8	74	75
75% percentile	73.42	1.45	150	94	2.43	1.18	1.44	226	10.5	93	99
Maximum	971.3	6.77	214	1185	2.69	1.37	3.17	478	111.5	144	164

Alk phosph indicates alkaline phosphatase; Ca, calcium; Creat, creatinine; CysC, cystatin C; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; 25-OH vit D, 25-hydroxyvitamin D; 1,25(OH) vit D, 1,25-dihydroxyvitamin D.

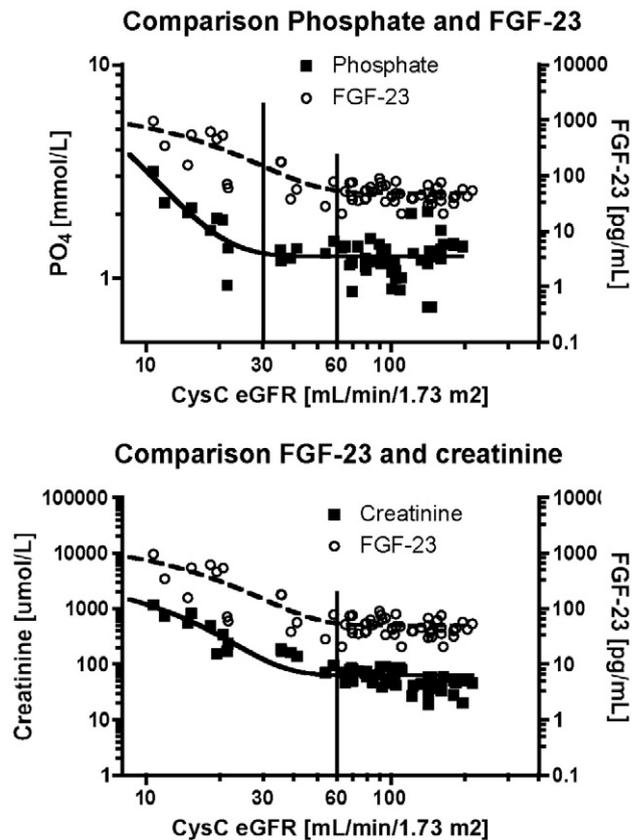


Fig. 1. The relationship between Cystatin C eGFR and FGF-23 (a marker of bone disease), phosphate (as a marker of phosphate accumulation), and creatinine (as a marker of renal function) in 65 children and young adults with chronic kidney disease. Equation for FGF-23: one-phase exponential decay $Y = \text{SPAN} \times \text{Exp}(-K \times X) + \text{PLATEAU}$, SPAN = 1821, $K = 0.09659$, PLATEAU = 48.74; half-life = 7.176. Equation for phosphate: one-phase exponential decay $Y = \text{SPAN} \times \text{Exp}(-K \times X) + \text{PLATEAU}$, SPAN = 12.46, $K = 0.1864$, PLATEAU = 1.263; half-life = 3.719. Equation for creatinine: one-phase exponential decay $Y = \text{SPAN} \times \text{Exp}(-K \times X) + \text{PLATEAU}$, SPAN = 4956, $K = 0.1472$, PLATEAU = 62.85; half-life = 4.709.

regression analysis was performed. FGF-23 levels correlated moderately with CysC levels (Spearman $r = 0.48$, $p < 0.0001$), CysC eGFR ($r = -0.47$, $p < 0.0001$), and PTH levels ($r = 0.50$, $p < 0.0001$). In addition, serum phosphate ($r = 0.25$, $p = 0.04$), 1,25(OH)₂-vitamin D ($r = -0.31$, $p = 0.02$), ionized calcium ($r = -0.32$, $p = 0.02$), and creatinine levels ($r = 0.45$, $p = 0.0002$) were weakly correlated with FGF-23 levels.

To establish the relative contribution of explanatory variables for log (FGF-23), we performed a multivariate analysis. As FGF-23, CysC, and PTH values were log-normally distributed, we performed a multivariate linear regression analysis using log (CysC), log (PTH), and phosphate as independent variables. Log (CysC) ($\beta = 0.660$, $p < 0.0001$) and log (PTH) ($\beta = 0.038$, $p = 0.37$) explained 66.7% of the

variance in FGF-23 levels. The addition of phosphate ($\beta=0.222$, $p=0.03$) increased the adjusted r^2 to 69.1% after controlling for the other independent variables. CysC had the largest unique contribution to FGF-23 in the multivariate regression model.

We also considered the relationship between CysC eGFR and FGF-23, phosphate, creatinine, and others. All parameters were most closely related to CysC eGFR as one-phase exponential decay. Fig. 1 demonstrates the relationship between GFR and FGF-23, creatinine (as a marker of renal function), and phosphate (as a marker of phosphate accumulation). Whereas FGF-23 concentrations behaved like a GFR marker and increased below a GFR of 60 mL/min/1.73 m², there was no increase in phosphate concentrations until the GFR dropped below 30 mL/min/1.73 m².

Discussion

The association between estimated GFR (eGFR) and FGF-23 levels are well established [2,3,8]. However, the interpretation of these findings differs from our interpretation of the study results. Recently, Evenepoel et al. suggested that reduced phosphate excretion was responsible for the early rise of FGF-23 levels even in patients with CKDs 1 and 2, while these patients maintained normal phosphate levels [9]. Although this is certainly a possibility, the results on oral phosphate loading in healthy volunteers make this less likely [2]. We did not measure the tubular phosphate reabsorption in all patients, but serum phosphate level does not tend to increase until CKD stage 3. Evenepoel et al. did not consider the possibility that LMWP would freely cross the glomerular filtration barrier. It is well established that CysC rises early in CKD in the so-called “creatinine-blind” range because of decreased filtration with mild CKD [4,5,7]. We hypothesized that CysC and FGF-23 would behave similarly. Indeed, FGF-23 is slightly larger than CysC in terms of molecular weight, but even the larger molecular weight protein beta-trace protein (BTP, 23–29 kDa) is an excellent marker of GFR that also does not undergo non-renal elimination [10]. FGF-23 has an identical size to BTP. In the univariate analysis, the effect of CysC and PTH on FGF-23 concentrations was similar. In the multivariate analysis, CysC, PTH, and phosphate explained 69% of the variance. However, CysC was the most important contributing factor explaining the variance of FGF-23. The figure indicating that creatinine and FGF-23 behave similarly with worsening GFR <60 mL/min/1.73 m² further supports our hypothesis, whereas phosphate levels only increase in CKD stage 4. Our data support the findings by Westerberg et al. [3]. Explaining the increases of FGF-23 levels early in CKD with protein biochemistry and decreased glomerular filtration similar to that of CysC and BTP is novel and has significant implications. FGF-23 levels need to be corrected for the degree of CKD to assess the effect of bone disease on FGF-23 concentrations. We are unaware of any such models. In other words, not all FGF-23 elevations can be explained by bone disease.

There is only one other study comparing CysC eGFR and FGF-23 concentrations: Ix et al. [8] also measured CysC eGFR and FGF-23 levels. The slightly lower correlation coefficient in their study can be explained by using a different formula for the estimation of GFR from Cystatin C, whereas we used the Filler formula [7]. The Filler

formula performed superior to other formulae in a large prospective validation study on renal transplant recipients. Ix et al. suggested that their data suggest that FGF-23 elevation is among the earliest detectable abnormalities in mineral metabolism as kidney function decline develops. However, the modest elevations in early CKD can be explained by accumulation of FGF-23 by decreased nephron endowment alone.

Our study has a few limitations. Only young patients were included. We did not measure GFR using gold-standard methods such as inulin clearance. We also require a larger patient cohort to develop and validate a model for the correction of FGF-23 levels for CysC eGFR. Nonetheless, the study clearly demonstrates that CysC is an important determinant for the variance of FGF-23 level. Our data suggest using caution when interpreting FGF-23 levels in patients with impaired GFR and the need for developing a formula to correct for impaired kidney function.

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