Fibroblast growth factor 23 and soluble klotho in children with chronic kidney disease

Mandy Wan¹, Colette Smith², Vanita Shah³, Ambrose Gullet¹, David Wells⁴, Lesley Rees¹ and Rukshana Shroff¹*

¹Renal Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK, ²Infection & Population Health, University College London, London, UK, ³Institute of Child Health, London, UK and ⁴Department of Chemical Pathology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

*Correspondence and offprint requests to: Rukshana Shroff; E-mail: rukshana.shroff@gosh.nhs.uk

Abstract

Background. Fibroblast growth factor 23 (FGF23), a bone-derived phosphaturic hormone, is elevated in chronic kidney disease (CKD). There are scarce data on the levels of its essential co-receptor klotho, and longitudinal changes in FGF23 levels are also unknown.

Methods. We examined FGF23 and soluble klotho (s-klotho) levels over 1 year in 154 children with CKD Stages 1–5 (CKD1–5), were on dialysis or who have received a transplantation.

Results. In children with CKD1–5 and who were receiving dialysis, FGF23 correlated inversely with the estimated glomerular filtration rate (eGFR) (P < 0.001), whereas a decrease in s-klotho was observed with a lower eGFR (P = 0.01). There was no correlation between FGF23 and serum phosphate (P) or parathyroid hormone (PTH) in our cohort wherein 89 and 66%, respectively, had normal levels. FGF23 increased by 6-fold over a 12-month period in children with eGFR of 15–29 mL/min/1.73 m², with an overall 5% annual increase in the CKD1–5 and dialysis cohort. High FGF23 levels were seen with high calcium (Ca) levels (P < 0.001). FGF23 levels were high when 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] were deficient (P = 0.05 and P < 0.001, respectively). s-klotho levels correlated positively with 25(OH)D (P < 0.001) and negatively with PTH (P = 0.04) and age (P = 0.03). Multivariate regression analysis demonstrated a strong relationship between FGF23 and eGFR, whereas the association between s-klotho and eGFR as observed in univariate analysis was lost following the adjustment of confounders. In transplanted patients, FGF23 correlated with eGFR (P = 0.02) and 25(OH)D (P = 0.05).

Conclusions. This study shows increasing FGF23 and reduced s-klotho levels with progressive renal failure even in a population of children with well-controlled P levels. Novel associations between FGF23 and serum Ca as well as 25(OH)D warrant further investigation.

Keywords: children; chronic kidney disease; FGF23; paediatric nephrology; s-klotho

Introduction

Mineral dysregulation with altered calcium (Ca) [1], phosphate (P) [2] and vitamin D [3,4] homeostasis is independently associated with increased cardiovascular mortality in adults with chronic kidney disease (CKD). While hyperphosphataemia is seen late in the course of renal decline [5], the risk of cardiovascular events is increased even at glomerular filtration rate (GFR) levels >60mL/min/1.73 m² [6]. Similarly, in children with CKD, early manifestations of cardiac and vascular damage in the form of left ventricular hypertrophy [7] and increased carotid intima media thickness and arterial stiffness are present in predialysis CKD stages [8].

Early in the course of CKD, fibroblast growth factor 23 (FGF23), a bone-derived protein, is elevated [9]. FGF23 increases renal P excretion by down-regulating the sodium-phosphate co-transporter in the proximal tubules, thereby, at least in early CKD, increasing P excretion [10, 11]. It also suppresses 1α-hydroxylase activity and reduces the production of 1,25-dihydroxyvitamin D [1,25(OH)₂D] [11]. Since vitamin D is thought to be a pleiotropic hormone with multiple cardioprotective, anti-inflammatory and immunomodulatory properties [12], the beneficial phosphaturic effects of FGF23 would appear to come with a ‘trade-off’ of potentially detrimental effects of suppressing 1,25(OH)₂D production [13]. Importantly, FGF23 is not simply a biomarker but is biologically active [14] and has been independently associated with left ventricular hypertrophy [15], impaired left ventricular function [16], vascular calcification [17], heart failure [18, 19], CKD progression [20] and mortality [21] in adult CKD patients.

FGF23 acts via its obligate co-receptor klotho, a membrane-bound protein that is expressed in the kidneys and parathyroid glands [22, 23]. Defects in either FGF23 or klotho cause a combination of metabolic disturbances, including hyperphosphataemia, hypercalcaemia and hyperparathyroidism [23, 24]. To date, the actions of klotho in CKD have focused on its role as a cofactor for FGF23 signalling in regulating P and vitamin D metabolism [25]. However, membrane-bound klotho is cleaved and released into the systemic circulation; little is known about the
effects of circulating soluble klotho (s-klotho), but it has been shown to act as a humoral regulator of ion transport [26] and may have direct effects on the vessel wall to mediate vascular calcification [27, 28].

There is now a growing body of literature on FGF23 and s-klotho in CKD, but several key questions remain unanswered. New studies are emerging with data on s-klotho levels in adult patients with CKD. However, these studies have yielded contradictory results [29–33]. As yet, no published data on s-klotho is available in the paediatric CKD population, and since preliminary data suggest that circulating levels of s-klotho may be influenced by age [34, 35], paediatric studies are required to examine this association. Moreover, despite a number of cross-sectional studies that show an association between FGF23 and deteriorating renal function [20, 36–44], longitudinal changes in FGF23 levels in CKD patients and their impact on mineral metabolism is poorly understood. Here, we describe FGF23 and circulating s-klotho levels in a cohort of children with CKD Stages 1–5 (CKD1–5), were on dialysis or who have received a transplantation. Longitudinal changes in FGF23 as well as the interactions between FGF23, s-klotho and the Ca-P-PTH-vitamin D axis are described.

Materials and methods

Study population

We studied FGF23 and s-klotho levels in 154 children with CKD1–5, who were on dialysis or who have received a transplantation and attended the Renal Unit at Great Ormond Street Hospital for Children. The children were recruited to participate in three separate vitamin D studies [45, 46] and the measurement of FGF23 and s-klotho was undertaken as secondary analyses. Children were divided into four groups based on their eGFR: (i) CKD1–3, (ii) CKD4–5, (iii) dialysis and (iv) transplantation. Of the children with CKD1–3 [45], 20 received daily oral ergocalciferol supplementation as per the Kidney Disease Outcomes Quality Initiative (K/DOQI) guideline [47] for a median of 12 months (range: 3–5), 23 had CKD2 and 18 had CKD3; 86% of these children were on prednisolone [46]. All studies received local research ethics committee approval. Informed written consent was obtained from all carers, and children also assented to participate, where appropriate. FGF23 and s-klotho levels were compared in 20 healthy aged-matched children.

Biochemical analysis

Blood samples were collected at baseline and every 3 months thereafter in children with CKD1–5 or who were on dialysis for a period of 12 months. Samples were centrifuged at 3000 rpm for 10 min, and the supernatant stored at −80°C until analysis. Routine serum biochemistry, FGF23 and 25(OH)D levels were analysed in all children. 1,25(OH)2D data were available in all but 30 children with CKD1–3 and 12 children from the transplant cohort. Plasma s-klotho levels were analysed in a random subset of 105 children only due to the cost of the assay.

Routine serum biochemistry was analysed at the Department of Chemical Pathology at Great Ormond Street Hospital for Children. Parathyroid hormone (PTH) levels were measured by the Immulite 2500 Intact PTH assay (Siemens Healthcare Diagnostics, Frimley, Surrey, UK). 25(OH)D [a sum of 25(OH)D2 and 25(OH)D3] was analysed by isotope-dilution liquid chromatography-tandem mass spectrometry by a single technician in the Pathology Department at the Northwick Park Hospital. Serum 1,25(OH)2D was measured by enzyme immunoassay (Immunodiagnostic Systems, Boldon, Tyne and Wear, UK) by a single technician at Great Ormond Street Hospital. The inter-assay coefficients of variation for 25(OH)D and 1,25(OH)2D were 2.7 and 4.2%, respectively. Plasma FGF23 concentrations were determined using a second-generation human FGF23 (C-Term) ELISA (Immutopics International, San Clemente, CA). The intra-assay and inter-assay coefficient of variation were 3.8 and 6.3%, respectively. Plasma s-klotho concentrations were measured by a solid-phase sandwich ELISA (Immunno-Biological Laboratories Co. Ltd, Gunma, Japan). The intra-assay and inter-assay coefficient of variation as specified by the manufacturer’s protocol were 2.7–3.5 and 2.9–11.4%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS software, version 19 (SPSS, Chicago, IL). Results are presented as the mean±SD or the median and inter-quartile range as appropriate, depending on the distribution of the variable. Descriptive analyses of mineral metabolites were performed after categorizing participants into four categories (CKD1–3, CKD4–5, dialysis and transplant cohort). Univariate comparisons of continuous variables between various groups were performed using an unpaired t-test for normally distributed data, or the non-parametric Mann–Whitney U test in the case of non-normally distributed variables. For multiple comparisons of several groups, ANOVA or the Kruskal–Wallis test were performed. Within group comparisons of continuous variables were performed using the paired t-test or Wilcoxon test, as appropriate. Cross-sectional analyses were performed considering the baseline values for each child included in the study. Spearman correlation tests were used for correlation analyses. Multivariate regression analysis was carried out, where variables with P value <0.15 on univariate analyses were entered into the multiple regression models; variables which were not normally distributed, including FGF23, s-klotho, PTH, 25(OH)D and 1,25(OH)2D were log-transformed to achieve normality. A stepwise linear multiple regression model with eGFR, Ca, log PTH, log 25(OH)D and log s-klotho as independent variables versus log FGF23 as the dependent variable were analysed in the CKD1–5 and dialysis cohort. Similarly, log s-klotho as the dependent variable versus eGFR, age, log FGF23, Ca, log PTH and log 25(OH)D as independent variables were analysed.

Longitudinal analyses were performed considering the serial measurements performed on each child. The changes in FGF23 were investigated using a linear regression model. To ensure normality, FGF23 levels were analysed on a log scale, and then back-transformed afterwards. Therefore, estimates are multiplicative. Comparisons of continuous variables between baseline and 12 months were performed using a paired t-test or the non-parametric Wilcoxon test where appropriate. Since there are very limited data on FGF3 and no data on s-klotho in healthy children, we defined FGF23 excess as ≥75 RU/mL and s-klotho deficiency as ≤765 pg/mL using the 95th and 5th percentile of FGF23 and s-klotho distributions in our control group, respectively. For all analyses, a P<0.05 was considered to be statistically significant.

Results

The baseline demographics, clinical and biochemical parameters of the 154 children are described in Table 1. There were 110 children with CKD1–5, including 28 children on dialysis (described together as the CKD1–5 and dialysis group) and 44 children with a functioning graft.

FGF23, s-klotho and eGFR

The median [inter-quartile range (IQR)] plasma FGF23 level in the CKD1–5 and dialysis group and the transplant patients was 150 (77–423) and 65 (36–103) RU/mL, respectively; both were significantly higher than in the healthy age-matched controls [13.9 (11.6–32.0) RU/mL] (P<0.001 for both comparisons). FGF23 levels were highest in dialysis patients [1120 (591–1245) RU/mL] but did not differ significantly between haemodialysis and peritoneal dialysis [1128 (956–1299) and 1060 (414–1243) RU/mL, respectively; P=0.35]. The median (IQR) s-klotho level was 1406 (968–2304) and 1698 (1110–2123) pg/mL in the CKD1–5 and dialysis group and the transplanted patients, respectively (P=0.01), as compared with 2406 (1710–3352) pg/mL in the controls (P=0.04).
FGF23 and klotho in children with CKD

Table 1. Clinical characteristics and biochemical features of the study population

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>CKD1–3 (n = 69)</th>
<th>CKD4–5 (n = 13)</th>
<th>Dialysis (n = 28)</th>
<th>Transplant (n = 44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0 ± 5.2</td>
<td>7.7 ± 5.7</td>
<td>9.9 ± 5.9</td>
<td>12.9 ± 3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender [Male; n (%)]</td>
<td>41 (59)</td>
<td>8 (62)</td>
<td>16 (57)</td>
<td>29 (66)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian/Asian/Afro-Caribbean/Others</td>
<td>53/9/7/0</td>
<td>10/1/1/0</td>
<td>14/11/3/0</td>
<td>25/16/2/1</td>
</tr>
<tr>
<td>Underlying diagnosis (n)</td>
<td>Renal dysplasia ± reflux ± obstruction</td>
<td>61</td>
<td>8</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Glomerular diseases</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Congenital nephrosis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Tubulo-interstitial disease</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Metabolic disease</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Polycystic kidney disease</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Malignancy</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Unknown aetiology</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Use of alfa-calcidol [%]</td>
<td>0</td>
<td>11 (87)</td>
<td>23 (82)</td>
<td>15 (34)</td>
<td></td>
</tr>
<tr>
<td>Alfa-calcidol dose (microgram/day)</td>
<td>—</td>
<td>0.5 (0.1–1.5)</td>
<td>0.4 (0.1–2.0)</td>
<td>0.3 (0.1–1.0)</td>
<td></td>
</tr>
<tr>
<td>Calcium-based</td>
<td>13 (19)</td>
<td>12 (92)</td>
<td>20 (71)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non-calcium-based</td>
<td>0</td>
<td>1 (8)</td>
<td>1 (4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m²)</td>
<td>71.3 ± 15.2</td>
<td>20.1 ± 6.5*</td>
<td>Dialysis</td>
<td>65.9 ± 17.2 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43, 8 ± 3.0</td>
<td>40.9 ± 5.1*</td>
<td>37.4 ± 4.1*</td>
<td>41.8 ± 3.5* &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Alumium-adjusted calcium (mmol/L)</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1*</td>
<td>2.5 ± 0.2*</td>
<td>2.3 ± 0.1 &lt;0.001</td>
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<tr>
<td>Phosphate (mmol/L)</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.2* 0.006</td>
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<tr>
<td>Calcium × phosphate (mmol²/L²)</td>
<td>3.6 ± 0.7</td>
<td>3.9 ± 0.8</td>
<td>3.4 ± 0.9</td>
<td>3.2 ± 0.5* 0.002</td>
<td></td>
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<tr>
<td>PTH (pmol/L)</td>
<td>4.0 (3.0–5.9)</td>
<td>12.7 (2.2–22.6)</td>
<td>6.5 (2.2–24.4)*</td>
<td>6.9 (3.6–10.3)* 0.011</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>213.8 ± 62.8</td>
<td>334.6 ± 195.6*</td>
<td>224.7 ± 144.5*</td>
<td>183.5 ± 83.2 0.017</td>
<td></td>
</tr>
<tr>
<td>25(OH)D (mmol/L)</td>
<td>69.0 (45.5–90.5)</td>
<td>31.5 (12.3–44.3)*</td>
<td>31.5 (19.3–44)*</td>
<td>30.2 (22–39.8)* &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>1,25(OH)₂D (pg/mL)</td>
<td>40 (21.1–51)</td>
<td>47.0 (29.3–64.3)</td>
<td>41.5 (26–69.8)</td>
<td>121.5 (97.3–146.8)* &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>FGF23 (RU/mL)</td>
<td>101 (62–258)</td>
<td>210 (142–449)*</td>
<td>1120 (591–1245)*</td>
<td>65 (36–102)* &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>s-klotho (pg/mL)</td>
<td>1736.5 (1272–2373)</td>
<td>1019 (502–1592)*</td>
<td>1148 (797–1515)*</td>
<td>1698 (1110–2123) 0.007</td>
<td></td>
</tr>
</tbody>
</table>

Children were divided into four groups based on their eGFR. eGFR was calculated using the modified Schwartz formula [68]. Unless otherwise stated, data are presented as the mean ± SD or as the median (IQR) as appropriate.

*1,25(OH)D levels were available in 39 children in the CKD1–3 group and 32 children in the transplant group.

**s-klotho levels were available in 105 children (46 in CKD1–3, 9 in CKD4–5, 21 in the dialysis group and 29 in the transplant group).

P values are for comparison across all groups obtained from ANOVA or Kruskal–Wallis. *CKD4–5, dialysis and transplant groups were compared separately with the CKD1–3 group by an unpaired t-test or the Mann–Whitney U-test, where P < 0.05.

In contrast to FGF23, s-klotho levels were significantly lower in haemodialysis compared with peritoneal dialysis patients [881 (554–1293) and 1444 (1080–3014) pg/mL, respectively; P = 0.02]. There was a strong inverse correlation between plasma FGF23 and eGFR in the total cohort (r = −0.60, P < 0.001; Figure 1a), s-klotho levels decreased with decreasing eGFR in the CKD1–5 and dialysis patients (r = 0.30, P = 0.01; Figure 1b). Decreased s-klotho was also associated with increased FGF23 levels (r = −0.31, P = 0.01; Figure 1c) in the CKD1–5 and dialysis patients.

Transplanted patients also showed an inverse correlation between FGF23 and eGFR (r = −0.36 P = 0.02; Figure 1a), but no association was found between s-klotho and eGFR (P = 0.48). The association between s-klotho and eGFR was not significant in the subset of transplanted patients with eGFR <60 mL/min/1.73 m² (P = 0.19).

FGF23 and biochemical parameters of mineral metabolism

Correlations between FGF23 and biochemical measures are presented in Table 2. In the CKD1–5 and dialysis group, FGF23 plasma levels correlated positively with albumin-adjusted serum Ca (r = 0.57, P < 0.001; Figure 2a).

There was no association between FGF23 and serum P (P = 0.25) or PTH levels (P = 0.14). Lower levels of 25-hydroxyvitamin D [25(OH)D] were associated with higher FGF23 levels (r = −0.20, P = 0.05; Figure 2b). The well-documented suppression of 1,25(OH)₂D by FGF23 was confirmed in children not receiving alfacalcidol (r = 0.46, r = −0.59, P < 0.001; Figure 2c), but was not present in patients on alfacalcidol. In children with CKD1–3, FGF23 correlated negatively with age (r = −0.32, P = 0.01); there was no association between FGF23 and age in the CKD1–5 and dialysis group. s-klotho levels correlated positively with 25(OH)D (r = 0.36, P < 0.001; Figure 3a) and negatively with PTH (r = −0.23, P = 0.04; Figure 3b). In addition, there was a negative association between s-klotho and age (r = −0.25, P = 0.03). In a multivariate regression analysis in children with CKD1–5 or on dialysis, eGFR was the only significant and independent predictor of FGF23 with each 1 mL/min/1.73 m² eGFR being associated with a −0.72 lower level of FGF23 (Table 2). Log 25(OH)D was the only significant independent predictor of s-klotho levels (Table 2).

In the transplanted group, the only significant association was between FGF23 and 25(OH)D levels (r = 0.30, P = 0.05; Figure 2b). There was no association between s-klotho and any of the biochemical variables or age.
Longitudinal analysis

In children with CKD1–5 or on dialysis, the changes in FGF23 over time were investigated using a linear regression model. The relative time effect was 1.05 per month (95% CI: 1.01, 1.10; P = 0.04), implying a 5% increase in FGF23 levels each month. When the cohort was divided into strata of decreasing eGFR, there was no significant difference in FGF23 levels between baseline and Month 12 in children with eGFR ≥30 mL/min/1.73 m² or on dialysis, whereas those with eGFR of 15–29 mL/min/1.73 m² showed a median 528% increase in FGF23 levels in 12 months (P = 0.04; Figure 4). eGFR decreased significantly over the 12-month period (P = 0.03), whereas no statistically significant differences were noted for Ca, P, PTH or vitamin D levels between these two time points. In the 20 children who received daily ergocalciferol, there was no significant change in the plasma FGF23 level after 12 months (P = 0.56), despite significant increases in both serum 25(OH)D (P < 0.001) and 1,25(OH)₂D levels with ergocalciferol supplementation. There were no significant changes in eGFR, Ca, P or PTH over the 12-month period in children on ergocalciferol.

Prevalence of biochemical abnormalities at different CKD stages

The number of children with high FGF23 increased progressively with declining eGFR (Figure 5). In the CKD1–3 cohort, 50% had high FGF23 and 13% s-klotho deficiency, whereas serum P and PTH levels were raised above normal in only 3 and 28%, respectively. 25(OH)D and 1,25(OH)₂D deficiency was widespread and seen in 68 and 51% of children with CKD1–3. In the dialysis patients, FGF23 was raised in 100% and s-klotho levels reduced in 24%, even though P and PTH levels were in the KDOQI recommended normal range in 75 and 64% of these children, respectively.

Table 2. Factors associated with FGF23 and s-klotho: univariate analysis and multivariate regression analysis using log FGF23 (n = 110) and log s-klotho (n = 76) as the dependent variable for the CKD1–5 and dialysis cohort

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Log FGF23 eGFR (mL/min/1.73m²)</td>
<td>-0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin-adjusted Ca (mmol/L)</td>
<td>0.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PO₄ (mmol/L)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Ca × PO₄ (mmol²/L²)</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Log PTH (pmol/L)</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>Log 25(OH)D (nmol/L)</td>
<td>-0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Log 1,25(OH)₂Da (pmol/L)</td>
<td>-0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Log FGF23 (RU/mL)</td>
<td>-0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.026</td>
<td>0.80</td>
</tr>
<tr>
<td>Log s-klotho eGFR (mL/min/1.73m²)</td>
<td>0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin-adjusted Ca (mmol/L)</td>
<td>-0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>PO₄ (mmol/L)</td>
<td>-0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Ca × PO₄ (mmol²/L²)</td>
<td>-0.09</td>
<td>0.44</td>
</tr>
<tr>
<td>Log PTH (pmol/L)</td>
<td>-0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Log 25(OH)D (nmol/L)</td>
<td>0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log 1,25(OH)₂Da (pmol/L)</td>
<td>0.12</td>
<td>0.49</td>
</tr>
<tr>
<td>Log FGF23 (RU/mL)</td>
<td>-0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Age</td>
<td>-0.25</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Only in children not receiving alfa-calcidol.
This study shows that even in a population of children with well-controlled serum P, high FGF23 and low s-klotho levels are seen with a progressive decline in eGFR. FGF23 levels significantly increased over a 12-month period, particularly in children with an eGFR between 15 and 29 mL/min/1.73 m². A positive correlation between serum Ca and FGF23 suggests that the calcemic effects of activated vitamin D analogues and phosphate binders need to be examined in future randomized controlled trials. Furthermore, our work reports a novel association between FGF23 and 25(OH)D, which deserves further investigation.

FGF23 is secreted by osteocytes and osteoblasts, and its circulating levels increase from the earliest stages of CKD [48]. Yet, factors that trigger FGF23 increase remain unclear. In our study FGF23 levels were increased in 50% of children with CKD1–3 when only 3% had raised P levels. Other studies have also shown a similar increase in FGF23 levels from CKD2 [36, 40, 41, 49], well before there is a critical reduction in nephron numbers, suggesting that increased FGF23 may result from an...
increased bone production rather than a decreased renal clearance [50]. It has been postulated that FGF23 increases in CKD as an appropriate response to increase P load. Indeed, circulating FGF23 levels are shown to increase in response to oral P load in healthy volunteers [51]. In our study, well-controlled serum P levels may have resulted from a protective increase in FGF23 as well as dietetic P control and appropriate use of P-binders.

While FGF23 requires its obligate co-receptor membrane-bound klotho for its actions on peripheral tissues, recent studies have shown that circulating s-klotho seems to function as a humoral regulator of ion channels and growth factors independent of FGF23 [26–28, 52]. It is therefore important to consider measurements of FGF23 in the context of s-klotho levels, but studies on circulating s-klotho levels have reported contradictory results [29–33]. We showed that as eGFR decreased s-klotho levels also decreased in parallel with an increase in FGF23. Indeed, studies have previously reported reduced expression of klotho in the urine and kidneys of patients with renal failure [27, 53]. This is further supported by a more recent study demonstrating decreased serum levels of s-klotho in adult patients with early stages of CKD [29]. Our data extend these findings to the paediatric CKD population, and collectively, these data suggest that CKD may be a state of progressive renal resistance to FGF23. It has been reported that in uraemic patients with hyperplastic parathyroid glands, the parathyroid resistance to inhibitory effects of FGF23 are mediated, in part, by down-regulation of klotho [54]. This may explain the markedly elevated levels of PTH that are commonly seen in late stages of CKD despite high FGF23 levels [49]. The reduced expression of klotho may also explain the increased prevalence of hyperparathyroidism with a progressive renal decline even though FGF23 levels are several 100-fold above normal. In addition, given the association between klotho deficiency and variable calcification [27], accelerated aging [23] and premature death [23], it may be that the association between FGF23 excess and adverse outcomes reported in previous studies are, in part, mediated by klotho deficiency. Nevertheless, a completely independent association between s-klotho and eGFR was not observed in the multiple regression analysis, and no association exists between s-klotho and eGFR in transplant recipients. It is also of interest to note that as renal function declines, the observed reduction in s-klotho is only relatively modest compared with the several 100-fold increase in FGF23. Although there are conflicting data, other studies have also failed to show a decrease in s-klotho with declining eGFR [30–33], suggesting that any changes in s-klotho cannot simply be explained by a reduction in eGFR.

Another limitation to addressing these hypotheses is that the relative importance of the transmembrane versus the soluble form of klotho is unclear and there is an assumption that lower s-klotho reflects lower tissue klotho expression. In particular, while FGF23 signalling is thought to require the transmembrane form of the protein, it remains purely speculative with regard to the predominant expression of klotho in the distal tubule when the functional effects of FGF23 are on the proximal tubule [55]. Additional research is required to examine FGF23-klotho mediated signalling before s-klotho levels in CKD patients can be accurately interpreted.

Age-dependent change in s-klotho levels has previously reported on healthy subjects [35] and in patients with X-linked hypophosphataemia [34]. This is the first report of s-klotho levels in a paediatric CKD population showing that s-klotho decreases with age. Interestingly, FGF23 also showed an inverse association with age in children with eGFR >60 mL/min/1.73 m² in our cohort, although one other study has reported increasing FGF23 with age [40]. The physiological significance of these associations are not known; however, given that the serum P level is highest at the first year of life and decreases thereafter with age, it raises the possibility that the effect of FGF23 and klotho on renal P excretion may play a role in such regulation.

A few paediatric studies exploring the association between FGF23 and various biochemical parameters have been published. Due to their cross-sectional study design and variable populations of CKD, dialysis and transplant patients, there are some conflicting results. Some studies have demonstrated a positive correlation between FGF23 and serum P [39, 41, 42] while others have shown no association between the two [40, 43]. Similarly, the associations between FGF23 and serum PTH [39–43] and FGF23 and 1,25(OH)₂D [40–43] varied. In our patients wherein 89% had normal serum P levels and 66% had PTH levels within the recommended range [56], no correlation with FGF23 was seen. Moreover, circulating s-klotho levels were inversely associated with PTH. It has been shown that klotho can directly regulate PTH synthesis; when intracellular Ca decreases within the parathyroid glands, the local expression of klotho increases, therefore inducing an increased activity of the Na–K-ATPase channel, an increased PTH synthesis and a further correction of the hypocalaemic state [57]. These data highlight the relative importance of controlling P and PTH levels from the earliest stages of CKD.
In addition to the well-known effect of FGF23 on suppressing 1,25(OH)2D, more interestingly, we observed that higher 25(OH)D levels were associated with reduced FGF23. This negative association may be explained by the stimulatory effect of FGF23 on 24-hydroxylase expression, leading to increased degradation of 25(OH)D [58]. Equally, it should be noted that the effects of low 25(OH)D are associated with hypertension, left ventricular hypertrophy and progression of CKD, all of which are also negatively affected by FGF23 excess [15, 20]. With a recent published randomized controlled trial reporting increased circulating FGF23 levels with ergocalciferol administration in healthy participants [59], this raises the intriguing question on the wider clinical implications of ergo- or cholecalciferol supplementation. Nonetheless, we acknowledge that our randomized controlled trial has failed to show an effect of ergocalciferol on FGF23 levels despite an increase in 25(OH)D levels, while others have reported opposite findings [59, 60]. Clearly, further experimental studies and clinical trials of 25(OH)D supplementation are required to reconcile these results.

On the other hand, higher 25(OH)D levels were associated with increased FGF23 in transplanted patients, where corticosteroid treatment may be a confounding factor. Previous studies in children have shown that corticosteroid therapy was associated with increased FGF23 serum levels [40, 43], but the mechanism is poorly understood. At the same time, there is evidence suggesting an association between corticosteroid usage and vitamin D levels [61–63]. As 86% of transplanted children were on prednisolone, we were unable to perform subgroup analysis to determine the effect of steroids on FGF23 and s-klotho.

We have shown a novel association between serum Ca levels and increased FGF23. Experimental data in the vitamin D receptor null mice have shown that dietary Ca supplementation significantly increased FGF23 mRNA abundance [64], implying that high Ca levels may also be a determinant of FGF23 production. Recent studies have shown that the P-binder sevelamer, but not Ca-based binders, were able to decrease FGF23 levels in predialysis CKD patients that could not be attributable to improved P or 1,25(OH)2D levels [65, 66]. A study in haemodialysis patients has shown that the use of higher dialysate Ca concentrations, as well as the administration of calcitriol and a Ca-based P-binder were associated with higher final serum FGF23 levels [67]. Future randomized studies on P-binders and vitamin D analogues would need to carefully evaluate their effects on FGF23 levels.

In the longitudinal analysis, there was a significant increase in FGF23 levels over a 12-month period in the CKD group, with the greatest increase in children with CKD4. Our result suggests that the increase in FGF23 levels is likely to be attributed to a reduction in eGFR, independent of any change in Ca, P, PTH, 25(OH)D or 1,25(OH)2D levels. While this may be partially explained by reduced clearance, increased FGF23 production in advanced CKD has also been demonstrated [14]. Since only 61% of variance in FGF23 levels was explained by the multi-regression model, other as yet unidentified factors may regulate FGF23 metabolism.

We acknowledge several limitations in our study. As with many paediatric studies small patient numbers make subgroup analysis difficult. The currently available s-klotho assays measure soluble s-klotho that may not reflect the molecule in its membrane-bound state. The relationship between circulating and membrane-bound s-klotho, as well as the interaction between circulating s-klotho and FGF23, need to be explored at different stages of CKD. Also, we were unable to measure serial s-klotho levels due to the cost of the assay. We do not have data for tubular excretion of P, a sensitive marker of FGF23 effect, or on C-reactive protein levels.

In summary, this study shows increasing FGF23 and reduced circulating s-klotho levels with a progressive renal decline even in a population of children with well-controlled P levels. The associations between FGF23 and serum Ca as well as 25(OH)D require further exploration in future randomized controlled trials of phosphate binders and vitamin D therapy.

Acknowledgements. This study was supported by the grant from the Kids Kidney Research and Kidney Research UK.

Conflict of interest statement: All the authors declared no competing interests. The results presented in this paper have not been published previously in whole or part, except in the abstract format.

References

Responsiveness of FGF-23 and mineral metabolism to altered dietary phosphate intake in chronic kidney disease (CKD): results of a randomized trial

Mhairi Sigrist1, Mila Tang1, Monica Beaulieu1,2, Gabriella Espino-Hernandez2, Lee Er2, Ognjenka Djurdjev2 and Adeera Levin1,2*

1Division of Nephrology, University of British Columbia, Vancouver, British Columbia, Canada and 2Statistics and Methodology, British Columbia Provincial Renal Agency, Vancouver, British Columbia, Canada

*Correspondence and offprint requests to: A. Levin; E-mail: alevin@providencehealth.bc.ca

Abstract

Background. High fibroblast growth factor-23 (FGF-23) levels are associated with adverse outcomes. We studied the responsiveness of FGF-23 and mineral metabolism to altered dietary phosphate intake in chronic kidney disease (CKD) and healthy control patients.

Methods. Thirty patients were enrolled: 18 normophosphatemic CKD subjects and 12 healthy controls. The study duration was 21 days with three 7-day dietary interventions; a high phosphate (HP, 2000 mg/day), low phosphate (750 mg/day) and low phosphate plus phosphate binder (aluminum hydroxide, 500 mg thrice daily with meals), with comparable macronutrient content, administered in random sequence. Baseline and weekly fasting morning measurements of FGF-23, serum phosphate (sPO4), 1,25-hydroxyvitamin D (1,25 D) and 24-h urinary calcium (uCa) and phosphate (uPO4) were collected.

Results. FGF-23 levels were higher in subjects versus controls (72 pg/mL versus 30 pg/mL) at baseline, while sPO4 remained in the normal range throughout the study. The absolute changes of uPO4 and uCa for CKD and controls vary according to diet. The absolute changes of sPO4 levels were significant in CKD patients and controls.

Conclusions. FGF-23 levels appear to be responsive to changes in diet in both CKD patients and controls.