Regulation of fibroblast growth factor-23 in chronic kidney disease

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Abstract

Background. Fibroblast growth factor-23 (FGF23) is a circulating factor that regulates the renal reabsorption of inorganic phosphate (Pi) and is increased in chronic kidney disease (CKD). The aim of the current investigation was to study the regulation of FGF23 in CKD subjects with various degree of renal function. As such, we analysed the relationship between FGF23, Pi, calcium, parathyroid hormone (PTH), 25(OH) vitamin D₃(25(OH)D₃), 1,25(OH)₂ vitamin D₃(1,25(OH)₂D₃) and estimated glomerular filtration rate (eGFR).

Methods. Intact FGF23 and other biochemical variables were analysed in 72 consecutive adult out-patients with various stages of CKD (eGFR ranging from 4–96 ml/min.) Association studies were performed using linear univariate and multivariate analysis.

Results. FGF23 was significantly elevated at CKD stage 4 (266 ± 315 pg/ml, P < 0.001) and 5 (702 ± 489 pg/ml, P < 0.001) compared with CKD 1–2 (46 ± 43 pg/ml). In CKD 4–5 an independent association between log FGF23 and Pi (P < 0.001), 25(OH)D₃ (P < 0.05) as well as eGFR (P < 0.01) was observed. In contrast, in CKD 1–3 log PTH (P < 0.05) was the only independent predictor of log FGF23 in multivariate analysis. In CKD 1–5, Pi (P < 0.00001) and log PTH (P < 0.01) were explanatory variables for log FGF23 in multivariate analysis.

Conclusions: We conclude that serum FGF23 increases in CKD 4–5, in parallel with the emerging hyperphosphatemia. Serum Pi is the most important predictor of FGF23 when GFR is less than 30 ml/min. In contrast, our data suggest that Pi may not be an important determinant of FGF23 in normophosphatemic CKD subjects. Finally, the association between FGF23 and PTH in CKD may suggest a co-regulation that remains to be further elucidated.

Keywords: chronic kidney disease; fibroblast growth factor-23(FGF23); hyperphosphatemia; parathyroid hormone; phosphate

Introduction

Fibroblast growth factor-23 (FGF23) is a novel circulating phosphaturic factor that plays a critical role in renal inorganic phosphate (Pi) reabsorption [1,2]. Importantly, two genetic disorders of disturbed Pi homeostasis, autosomal dominant hypophosphatemic rickets (ADHR, OMIM#193100) [2] and hyperphosphatemic familial tumoral calcinosis (HFTC, OMIM#211900) [3,4] are caused by activating and inactivating mutations in the human FGF23 gene. Unlike most other members of the FGF family, FGF23 contains a signal peptide and is secreted into the circulation. Bone is the major physiological site of FGF23 expression [5].

FGF23 decreases renal Pi reabsorption, and subsequently serum Pi levels, by reducing the apical expression of the sodium-dependent Pi co-transporter IIa (NPT2a) in the brush-border membrane of the renal proximal tubules [1]. Consequently, hypophosphatemia is a hallmark of a variety of disorders associated with increased FGF23 levels, such as tumour-induced osteomalacia [6] and X-linked hypophosphatemic rickets [5].

The role of FGF23 in chronic kidney disease (CKD) and secondary hyperparathyroidism has been subject to investigation by several independent research groups. We and others have previously demonstrated that serum FGF23 increases as renal function declines [7–12]. The aetiology of increased FGF23 levels in renal failure is likely a compensatory mechanism to the hyperphosphatemia, although retention of FGF23 in the circulation [10,11] and calcitriol treatment may also contribute [12,13]. Recent data suggest that FGF23 directly signals in the parathyroid glands [14] and Nakanishi et al. [7] demonstrated that elevated FGF23 levels are an important predictor of future secondary hyperparathyroidism in dialysis patients.
Although over-expression of FGF23 in vivo unequivocally causes phosphaturia and hypophosphataemia, it is still not entirely clear to what extent FGF23 regulates Pi levels in normal physiology. In this regard, several groups have reported a small increase of FGF23 levels after a dietary Pi load [11,15–17]. This effect does not occur rapidly, thus it is unlikely that acute changes or adaptations in serum Pi regulate FGF23 expression.

In this cross-sectional study, we wanted to elucidate the regulation of intact, biologically active, FGF23 in CKD patients with a wide range of renal function. Additionally, we wanted to establish at which degree of renal function elevated FGF23 levels are manifest.

**Subjects and methods**

**Subjects**

We recruited 72 consecutive individuals with CKD and varying degrees of residual renal function at the out-patient clinic at the Department of Nephrology, Uppsala University Hospital. Blood samples were drawn and stored at −70°C until analysis. Written consent was obtained from all patients and samples were drawn and stored according to ethical guidelines (ethical approval number 2006:094). The aetiology of CKD was 15% diabetes mellitus, 30% glomerulonephritis, 6% polycystic kidney disease, 24% other renal disease and 25% unknown renal disease. 25% (n=18) were treated with low dose of per oral active vitamin D, 25% (n=18) used calcium carbonate as Pi binder and 7% (n=5) were on sevelamer treatment. Mean age ± SD was 59.6 ± 18 years; gender distribution was 53% males/47% females; mean BMI (kg/m²) ± SD was 26.1 ± 4.9; mean systolic blood pressure (mmHg) ± SD was 131 ± 19 and mean diastolic blood pressure (mmHg) ± SD was 78 ± 10.

**Biochemical analysis**

Routine serum biochemistries for Pi, calcium, albumin and cystatin C were assessed by standard methods at the Department of Clinical Chemistry at Uppsala University Hospital. All calcium levels presented within this paper were corrected for albumin concentration as follows: 
\[
\text{[calcium (corrected)]} = \text{[calcium]} - \left[0.018(\text{albumin})\right] - 42.4
\]
Estimated glomerular filtration rate (eGFR) was indirectly calculated by using the following estimate:
\[
\text{eGFR} = \frac{[79.901(\text{cystatin C})^{1.4389}]}{\text{ml/min/1.73 m}^2}
\]
Parathyroid hormone (PTH) levels were analysed for biointact PTH (1–84) measured on an automated immunoassay system (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Measurement of 25(OH)D3 was performed on the Nichols Advantage automated assay system (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) and 1,25(OH)2D3 using an ELISA kit from Immundiagnostik Inc., (Bensheim, Germany). Serum intact FGF23 concentrations were measured using an ELISA according to the manufacturer’s protocol (Kainos Laboratories International; Tokyo, Japan) [18]. This second-generation, two-site, monoclonal antibody ELISA has previously been shown to recognize the biologically active, intact FGF23 [18].

**Statistical analysis**

All statistical analyses were performed using STATISTICA software (StatSoft Inc., Tulsa, USA). Univariate correlation analyses were used where the Pearson correlation coefficient, \(r\), measured the degree of linear relationship between two normally distributed continuous variables. Multiple linear regression analysis was used to investigate the relationship among the target and several independent predictor variables simultaneously and to define the relative contributions of the independent variables to the variation of the target variable. For all analyses, standardized \(\beta\)-values are presented and a \(P\)-value <0.05 was considered as statistically significant. A comparison of biochemical changes between various stages of CKD was performed using ANOVA followed by Tukey’s HSD post-hoc test.

**Results**

A biochemical summary of the CKD cohort is presented in Table 1. Serum FGF23 levels in the entire cohort were 235 ± 367 pg/ml, which is higher than the previously determined mean values (28.9 ± 1.1 pg/ml, ranging from 8.2 to 54.3 pg/ml) among 104 healthy control individuals [18]. To further analyse serum biochemistries, we stratified the cohort into four parts, depending on degree of renal function: CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage 1–2 (eGFR>60 ml/min), CKD stage

<table>
<thead>
<tr>
<th>Table 1. Summary of serum biochemistries of the entire CKD cohort as well as stratified into CKD stages 1–5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>FGF23 (pg/ml)</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
</tr>
<tr>
<td>25(OH) vitamin D (ng/ml)</td>
</tr>
<tr>
<td>1,25(OH)2 vitamin D (pg/ml)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
</tr>
</tbody>
</table>

Significantly changed variables compared with CKD 1–2 are in bold.

<sup>*</sup><sup>P</sup> < 0.05; <sup>**P</sup> < 0.01.
3 (eGFR 30–60 ml/min), CKD stage 4 (eGFR 15–30 ml/min) and CKD stage 5 (eGFR <15 ml/min).

Since FGF23 and PTH did not follow a Gaussian distribution, the log values of FGF23 and PTH were used for subsequent association analysis.

Mean FGF23 levels were 46 ± 43 pg/ml in CKD 1–2 and did not change in CKD 3 (56 ± 31 pg/ml) as determined by ANOVA and Tukey’s HSD post-hoc test. FGF23 was significantly increased in CKD 4 (266 ± 315 pg/ml, P < 0.001) and CKD 5 (702 ± 489 pg/ml, P < 0.001) (Table 1) compared with CKD 1–2. Since significant changes in serum FGF23 occurred between CKD 3 and 4, we further subdivided CKD 3 subjects into CKD 3a (eGFR 45–60 ml/min) and CKD 3b (eGFR 30–45 ml/min). No difference in FGF23 levels were observed between the two groups (CKD 3a; 54 ± 31 pg/ml and CKD 3b; 60 ± 33 pg/ml).

Thus, our results indicate that FGF23 significantly rises when eGFR is below 30 ml/min. Similarly, serum Pi was in the normal range for subjects with CKD 1–3, although increased in CKD 4 (P < 0.01) and CKD 5 (P < 0.001) (Table 1). PTH levels were normal in CKD 1–3 but also increased in CKD 4 (P < 0.001) and CKD 5 (P < 0.001) (Table 1). No difference in serum calcium, 25(OH)D3 or 1,25(OH)2D3 between the different groups was observed (Table 1).

We then performed linear univariate analysis with log FGF23 as dependent variable. Importantly, Pi (r = 0.69, P < 0.001), log PTH (r = 0.56, P < 0.001), 1,25(OH)2D3 (r = −0.24, P < 0.05) and eGFR (r = −0.68, P < 0.001) all correlated to log FGF23 levels when all subjects were included (Table 2). In contrast, in a multivariate analysis, only Pi (β = 0.46, P < 0.00001) and log PTH (β = 0.56, P < 0.01) remained as independent predictors of log FGF23 levels (Table 2).

Based on the finding that FGF23 were significantly changed at eGFR below 30 ml/min, we split the cohort into two parts, namely CKD 1–3 and CKD 4–5. There was no significant correlation between log FGF23 and eGFR in CKD 1–3 in a univariate model (Figure 1B). In contrast, a strong association was found between log FGF23 and eGFR in CKD 4–5 (r = −0.62, P < 0.001) (Figure 1A). No correlation was found

Table 2. Linear univariate and multivariate analysis of biochemical variables in the CKD cohort with log FGF23 as dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-value</th>
<th>P-value</th>
<th>β-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>0.69</td>
<td>&lt;0.001</td>
<td>0.46</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.00</td>
<td>N.S.</td>
<td>0.16</td>
<td>0.075 N.S.</td>
</tr>
<tr>
<td>Log PTH</td>
<td>0.36</td>
<td>&lt;0.001</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>25(OH)D3</td>
<td>0.0009</td>
<td>0.99 N.S.</td>
<td>0.13</td>
<td>0.12 N.S.</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>−0.24</td>
<td>&lt;0.05</td>
<td>−0.054</td>
<td>0.52 N.S.</td>
</tr>
<tr>
<td>eGFR</td>
<td>−0.68</td>
<td>&lt;0.001</td>
<td>−0.17</td>
<td>0.16 N.S.</td>
</tr>
</tbody>
</table>

N.S. means not significant, significant P-values are in bold.

Fig. 1. Correlation between log FGF23 and eGFR; (A) CKD stage 4–5 (r = −0.62, P < 0.001); (B) CKD 1–3 (r = −0.24, P = 0.13).
between log FGF23 and Pi in CKD 1–3 (Figure 2B), however, there was a linear correlation between FGF23 and Pi in CKD 4–5 ($r = 0.75$, $P < 0.001$) (Figure 2A). Importantly, we also found a significant correlation between log FGF23 and log PTH in CKD 1–3 (Figure 3B) ($r = 0.44$, $P < 0.01$), but no such correlation was found in CKD 4–5 (Figure 3A). Finally, there was no association between log

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**Fig. 2.** Correlation between log FGF23 and Pi; (A) CKD stage 4–5 ($r = 0.75$, $P < 0.001$); (B) CKD stage 1–3 ($r = 0.03$, $P = 0.83$).

**Fig. 3.** Correlation between log FGF23 and log PTH; (A) CKD stage 4–5 ($r = 0.17$, $P = 0.35$); (B) CKD stage 1–3 ($r = 0.44$, $P < 0.01$).
FGF23, calcium, 25(OH)D₃ or 1,25(OH)₂D₃ in neither of the two groups (data not shown).

In a multivariate analysis with log FGF23 as dependent variable, log PTH was the only significant predictor of log FGF23 levels in CKD 1–3 (β = 0.39, P < 0.05) (Table 3). In contrast, in CKD 4–5, Pi (β = 0.60, P < 0.001), eGFR (β = −0.37, P < 0.01) and 25(OH)D₃ were also independent predictors of log FGF23 (Table 4).

Finally, we sought to further elucidate the association between FGF23 and PTH at earlier stages of CKD. Notably, median values of log PTH were proportionally higher in CKD 4 compared with CKD 3, than that of median FGF23 levels (Figure 4). Moreover, only 18 subjects revealed PTH within the normal reference range in our cohort, whereas 30 individuals displayed FGF23 levels <50 pg/ml (data not shown). These findings may imply that a gradual increase in PTH occurs before a reciprocal rise in FGF23 in CKD.

**Discussion**

In the current study, we sought to determine the regulation of serum FGF23 in relation to other biochemical parameters in CKD subjects with various degrees of renal function. In agreement with previous reports, we demonstrate that FGF23 are increased at later stages of CKD [9–12]. Importantly, we did not detect a substantial rise in FGF23 until CKD 4, which coincided with significant changes in serum Pi and PTH. In parallel, multivariate analysis revealed that eGFR was negatively associated to log FGF23 in CKD 4–5, but not in CKD 1–3, supporting that GFR is an independent determinant of serum FGF23 at later stages of CKD. Further studies are needed to determine whether minor changes in GFR at earlier stages of CKD are associated to variations in serum FGF23. We conclude that intact FGF23 significantly rises at GFR below 30 ml/min., in the presence of other biochemical abnormalities including hyperphosphataemia.

The association between FGF23 and Pi in normal physiology has been subject to extensive investigation. Dietary Pi load appears to increase serum FGF23, although the effect is small [11,15–17]. Other studies support the notion that supraphysiological concentrations of Pi may be required to induce FGF23 expression [19]. In the current study, we did not find any correlation between log FGF23 and Pi in CKD 1–3, whereas Pi was the most significant predictor of log FGF23 in CKD 4–5. Our data favours the idea that a rise in FGF23 is detected at a critical

**Table 3.** Linear univariate and multivariate analysis of biochemical variables in CKD 1–3 with log FGF23 as dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-value</th>
<th>P-value</th>
<th>β-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>−0.03</td>
<td>0.84 N.S.</td>
<td>0.034</td>
<td>0.83 N.S.</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.28</td>
<td>0.079 N.S.</td>
<td>0.22</td>
<td>0.18 N.S.</td>
</tr>
<tr>
<td>Log PTH</td>
<td>0.44</td>
<td>&lt;0.01</td>
<td>0.40</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>25(OH) vitamin D</td>
<td>0.25</td>
<td>0.12 N.S.</td>
<td>0.24</td>
<td>0.13 N.S.</td>
</tr>
<tr>
<td>1,25(OH)₂ vitamin D₁</td>
<td>0.31</td>
<td>0.052 N.S.</td>
<td>−0.28</td>
<td>0.11 N.S.</td>
</tr>
<tr>
<td>eGFR</td>
<td>−0.24</td>
<td>0.13 N.S.</td>
<td>0.073</td>
<td>0.69</td>
</tr>
</tbody>
</table>

N.S. means not significant. Significant P-values are in bold.

**Table 4.** Linear univariate and multivariate analysis of biochemical variables in CKD 4–5 with log FGF23 as dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-value</th>
<th>P-value</th>
<th>β-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>0.75</td>
<td>&lt;0.001</td>
<td>0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.05</td>
<td>0.77 N.S.</td>
<td>0.29</td>
<td>0.050 N.S.</td>
</tr>
<tr>
<td>Log PTH</td>
<td>0.17</td>
<td>0.35 N.S.</td>
<td>0.23</td>
<td>0.12 N.S.</td>
</tr>
<tr>
<td>25(OH) vitamin D</td>
<td>0.060</td>
<td>0.75 N.S.</td>
<td>0.26</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1,25(OH)₂ vitamin D₁</td>
<td>0.041</td>
<td>0.83 N.S.</td>
<td>0.035</td>
<td>0.77 N.S.</td>
</tr>
<tr>
<td>eGFR</td>
<td>−0.62</td>
<td>&lt;0.001</td>
<td>−0.37</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

N.S. means not significant, significant P-values are in bold.

**Fig. 4.** Medians and quartiles for log PTH and log FGF23 categorized for various CKD stages; (A) log PTH; (B) log FGF23.
FGF23 regulation in CKD

point where manifest hyperphosphataemia occurs and that hyperphosphataemia is one major stimulus to elevated FGF23 levels in CKD.

Interestingly, log PTH was positively and independently associated to log FGF23 in CKD 1–3. This may imply a possible co-regulation of FGF23 and PTH in normophosphataemic CKD subjects, although further studies are needed to clarify such a regulation.

FGF23 and PTH are both phosphaturic factors, decreasing renal Pi reabsorption in the kidney proximal tubules. In CKD, FGF23 and PTH gradually increase with declining renal function, which mainly occurred in CKD 4–5 in our study. Importantly, we observed small increments in PTH in many patients before a reciprocal rise in FGF23 was detected. The physiological relevance of this is unclear but may be explained by several facts. A mild hyperphosphataemia may be a stronger stimulus for PTH than for FGF23. The phosphaturic potency of PTH in vivo may also be stronger than that of FGF23. Furthermore, an early rise in PTH may be more beneficial in CKD because it promotes renal Pi secretion and protects systemic calcium levels by increasing circulating serum calcitriol.

In summary, we conclude that serum FGF23 is increased in CKD 4–5, mainly due to manifest hyperphosphataemia and decreased renal clearance of FGF23. Our data suggest that FGF23 is not a suitable marker for early Pi retention, but rather a sign that renal Pi excretion no longer can be sufficiently increased by a further rise in PTH. A positive association between FGF23 and PTH in CKD 1–3 supports a possible co-regulation of FGF23 and PTH that needs to be further explored.

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