Preliminary Communication

Fibroblast growth factor-23 and early decrements in kidney function: the Heart and Soul Study

Joachim H. Ix1,2,3, Michael G. Shlipak4,5,6, Christina L. Wassel3 and Mary A. Whooley4,5,6

1Division of Nephrology, Department of Medicine, University of California San Diego, San Diego, CA, USA, 2Nephrology Section, Veterans Affairs San Diego Healthcare System, San Diego, CA, USA, 3Division of Preventive Medicine, Department of Family and Preventive Medicine, University of California San Diego, San Diego, CA, USA, 4General Medicine Section, San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA, 5Department of Medicine, University of California San Francisco, San Francisco, CA, USA and 6Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA

Correspondence and offprint requests to: Joachim H. Ix; E-mail: joeix@ucsd.edu

Abstract

Background. Fibroblast growth factor-23 (FGF-23) is associated with mortality in dialysis patients, and concentrations are elevated in moderate chronic kidney disease (CKD). The threshold of CKD or albuminuria at which FGF-23 begins to change is unknown.

Methods. In 792 outpatients with stable cardiovascular disease (CVD) and normal kidney function to moderate CKD, we evaluate the associations of estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR) with plasma FGF-23 concentrations.

Results. Compared to participants with eGFR ≥90 ml/min/1.73m², mean FGF-23 concentrations were 7.8 RU/ml higher (4.3–11.5, P = 0.01) in those with eGFR 60–89 ml/min/1.73m² in models adjusted for age, sex, race, ACR, blood pressure, diabetes and body mass index. More advanced decrements in eGFR were associated with much higher FGF-23 concentrations. In spline analysis, the slope of change in FGF-23 concentration was evident at eGFR <90 ml/min/1.73m². Compared to participants with ACR <30 mg/g, mean FGF-23 concentrations were 18.4 RU/ml higher (9.3–29.2, P < 0.001) in those with ACR 30–299 mg/g in models adjusted for identical covariates plus eGFR and much higher in individuals with ACR ≥300 mg/g. Spline analysis demonstrated a linear relationship of ACR with FGF-23, independent of eGFR, even among persons with ACR <30 mg/g.

Conclusion. Modest decrements in eGFR or elevations in albuminuria are each independently associated with higher FGF-23 concentrations in outpatients with stable CVD.

Keywords: albuminuria; chronic; fibroblast growth factor-23; kidney disease; osteodystrophy

Introduction

In individuals with chronic kidney disease (CKD), abnormalities in mineral metabolism are important determinants of bone and vascular disease. As kidney function declines, elevated serum intact parathyroid hormone (iPTH) concentrations are frequently the earliest detectable abnormality using conventional measures of mineral metabolism. However, elevated iPTH can be detected at glomerular filtration rate (GFR) levels of approximately <60 ml/min/1.73m², and alterations in 25 (OH) vitamin D, calcium and phosphorus require even more advanced decrements in GFR [1]. Recent studies have demonstrated that higher iPTH levels and serum phosphorus levels, and lower 25 (OH) and 1,25 (OH)₂ vitamin D levels are each associated with cardiovascular disease (CVD) and all-cause mortality in the general population; even among persons with ostensibly normal kidney function [2–5]. These observations have heightened interest in understanding mineral metabolism regulation and its consequences in persons with or without CKD.

Fibroblast growth factor (FGF) 23 is a bone-derived hormone which induces phosphaturia and inhibits conversion of 25 (OH) vitamin D to its active form [6]. Higher FGF-23 levels are associated with mortality in persons with end-stage renal disease (ESRD) [7], and greater left ventricular mass in individuals with moderate to severe CKD [8]. Prior studies have demonstrated that persons with moderate CKD have higher mean FGF-23 levels than healthy controls [9]. And among individuals with CKD stage 3–5, FGF-23 levels and eGFR are inversely correlated [8]. However, the threshold of severity of CKD or albuminuria at which FGF-23 becomes elevated is unknown. We evaluate the cross-sectional associations of GFR estimated by creatinine and cystatin C and albuminuria with plasma FGF-23 levels in outpatients with stable cardiovascular disease and a spectrum of kidney function from normal to moderate CKD.
Table 1. Median (interquartile range) FGF-23 levels by kidney function categories

<table>
<thead>
<tr>
<th>Kidney function category</th>
<th>eGFRcys (ml/min/1.73 m²)</th>
<th>FGF-23 (RU/mL)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 (n = 647)</td>
<td>32.0 (22.6–49.6)</td>
<td>38.5 (24.8–53.4)</td>
<td>58.6 (35.7–109.7)</td>
<td></td>
</tr>
<tr>
<td>≥30 (n = 145)</td>
<td>40.2 (23.5–53.1)</td>
<td>48.0 (34.1–106.6)</td>
<td>93.6 (46.2–182.0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Adjusted association of kidney function and FGF-23

<table>
<thead>
<tr>
<th>Kidney function measure</th>
<th>Number (%)</th>
<th>FGF-23 (RU/mL)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFRcys (ml/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>192</td>
<td>36.0</td>
<td>31.6–40.9</td>
<td>Ref</td>
</tr>
<tr>
<td>60–89</td>
<td>417</td>
<td>43.8</td>
<td>40.3–47.5</td>
<td>0.01</td>
</tr>
<tr>
<td>&lt;60</td>
<td>183</td>
<td>76.1</td>
<td>66.8–86.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFRcys (ml/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>140</td>
<td>31.9</td>
<td>27.6–36.9</td>
<td>Ref</td>
</tr>
<tr>
<td>60–89</td>
<td>391</td>
<td>39.5</td>
<td>36.4–42.9</td>
<td>0.01</td>
</tr>
<tr>
<td>&lt;60</td>
<td>261</td>
<td>77.0</td>
<td>69.1–85.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin–creatinine ratio (mg/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 mg/g</td>
<td>647</td>
<td>44.2</td>
<td>41.4–47.1</td>
<td>Ref</td>
</tr>
<tr>
<td>30–299 mg/g</td>
<td>110</td>
<td>62.6</td>
<td>53.3–73.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥300 mg/g</td>
<td>35</td>
<td>72.4</td>
<td>54.1–97.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Materials and methods

Methods inclusion, and exclusion, criteria of the Heart and Soul study have been reported elsewhere [10]. Briefly, outpatients with prevalent coronary artery disease living in the San Francisco bay area were recruited and underwent a day-long study visit where they provided medical history, physical examination and blood and urine specimens. Participants were excluded if they were not able to walk one block, had a myocardial infarction within the past 6 months or were likely to move out of the area within 3 years. There was no exclusion for kidney dysfunction. The study protocol was approved by the Institutional Review Boards of the participating centers, and all participants provided written informed consent. Between 2000 and 2002, 1024 participants enrolled. For the present study, we excluded participants with missing serum cystatin-C (n = 37), or urine albumin-to-creatinine ratio measurements (ACR, n = 195). The remaining 792 individuals all had creatinine and FGF-23 measurements, and constituted the study sample for this analysis.

Serum creatinine was measured by the rate Jaffe method, and was used for estimated GFR (eGFRcr) by its incorporation in the abbreviated (four-variable) Modification of Diet in Renal Disease study equation [11]. Cystatin C was measured using a BNII nephelometre (Dade Behring, Inc., Deerfield, IL). The intra-assay coefficient of variation (CV) was <2.9% and interassay CV was <3.2% [12]. Cystatin C was used to calculate estimated GFR (eGFRcys) using the formula eGFRcys = 76.7 × cystatin C^{-1.19}. This equation has been validated with comparison to iothalamate measured GFR in a pooled cohort of kidney disease studies [13]. Urine albumin and creatinine were measured using nephelometry and the rate Jaffe method, respectively, and ACR was calculated (milligram per gram) [10]. Plasma FGF-23 concentrations were measured using a C-terminal human ELISA (Immunotopics, San Clemente, CA) [14]. Measurements were made in duplicate and averaged. The intra-assay CV was <5.1%; the inter-assay CV was 10.0% at a concentration of 36.4 RU/mL and 12.7% at a level 379 RU/mL.

Graphical methods demonstrated that FGF-23 levels were right-skewed, and FGF-23 levels were therefore natural log-transformed. Spearman correlation coefficients were calculated for the unadjusted associations of eGFRcys, eGFRcys, and ACR with FGF-23. Categories of kidney function were defined according to standard clinical cut-points. For eGFRcys and eGFRcys, participants were categorized into three groups (eGFR ≥90, 60–89 and <60). Similarly, subjects were categorized into three groups by ACR (<30, 30–299, and ≥300 mg/g) [15,16]. The highest eGFR category and lowest ACR category served as reference groups in analysis. Linear regression was used to evaluate the association of each kidney function measure with natural log-FGF-23 levels. Subsequently, geometric means were calculated to provide the adjusted mean FGF-23 level for each category of kidney function on the natural scale. All models were adjusted for age, sex, race (black, white, other), systolic blood pressure, diastolic blood pressure, diabetes, and body mass index. Models evaluating eGFRcys and ACR were further adjusted for ACR. The models evaluating ACR were further adjusted for eGFRcys. To explore a threshold of kidney function required to observe differences in plasma FGF-23 levels, we developed cubic B-spline functions using general additive models, adjusted for the same covariates. The extreme 2.5% of kidney function measurements were excluded from spline functions to avoid implausible extrapolations from the extremes of the data distribution. Analyses were conducted using STATA version 11 (Stata Corp., College Station, Texas) and SPlus version 6.1 (Insightful Corp., Seattle, Washington).

Results

The mean age of the 792 study participants was 67 ± 11 years, 18% (n = 143) were female and 16% (n = 123) were African-American. Twenty-eight percent (n = 220) had diabetes mellitus, and 72% (n = 570) had hypertension. Mean eGFRcr was 76 ± 23 ml/min/1.73 m², 54% (n = 417) had eGFRcr <90 ml/min/1.73 m², 22% (n = 171) had eGFRcr <60 ml/min/1.73 m² and 2% (n = 12) had eGFRcr <30 ml/min/1.73 m². The latter two categories were combined in subsequent analysis due to low numbers of individuals with eGFR <30 ml/min/1.73 m². Mean eGFRcys was 70 ± 23 ml/min/1.73 m². The median ACR was 10 mg/g (interquartile range 6–20), 14% (n = 110) had microalbuminuria (ACR 30–299 mg/g) and 4% (n = 35) had macroalbuminuria (ACR ≥300mg/g).

The distribution of FGF-23 levels was right-skewed with a median of 43 RU/mL (interquartile range 29–73

Fig. 1. Cubic spline function demonstrating that the adjusted association of (A) eGFRcys, (B) eGFRcys, and (C) ACR with plasma FGF-23 levels. Solid lines represent the adjusted point estimates, and dotted lines represent the 95% confidence intervals. The y-axis demonstrates the beta coefficient, representing the change in natural log transformed FGF-23 levels per unit change in kidney function. *The spline functions for eGFRcys and eGFRcys were adjusted for age, sex, race, ACR, systolic blood pressure, diastolic blood pressure, diabetes and body mass index. Spline function for ACR was adjusted for age, sex, race, eGFRcys, systolic blood pressure, diastolic blood pressure, diabetes and body mass index.
Urine Albumin to Creatinine Ratio (mg/g)

Difference Ln FGF-23 (RU/ml)

0.5 1.0 1.5 2.0 2.5 3.0

120 100 80 60 40
eGFR_Cr (ml/min/1.73m²)

Difference in Ln FGF-23 (RU/ml)

0.5 1.0 1.5 2.0 2.5 3.0

100 80 60 40
eGFR_Cys (ml/min/1.73m²)

Difference Ln FGF-23 (RU/ml)

0.5 1.0 1.5 2.0 2.5 3.0

Urine Albumin to Creatinine Ratio (mg/g)
The unadjusted Spearman correlations of eGFR_Cr, eGFR_Cys, and ACR with FGF-23 were of moderate strength \((r = -0.32, -0.37\) and 0.24, respectively). All \(P\)-values <0.001. Table 1 shows the median FGF-23 level by categories cross-classified by eGFR_Cys and ACR. Individuals with either higher ACR or lower eGFR_Cys had higher median FGF-23 levels in unadjusted analysis. Results were similar with adjustment for age, sex, race, systolic and diastolic blood pressure, diabetes and body mass index (Table 2). Participants with eGFR_Cr between 60 and 89 and eGFR <60 had significantly higher FGF-23 levels compared to participants with eGFR_Cr \(\geq 90\)ml/min/1.73m². Results were similar when kidney function was classified using eGFR_Cys. Compared to participants with ACR <30 mg/g, those with ACR 30–299 mg/g and with ACR >300mg/g also had higher FGF-23 levels in models adjusted for similar covariates and eGFR_Cys. Results were similar when eGFR_Cr replaced eGFR_Cys in this model, and with additional adjustment for calcium and phosphorus levels (data not shown).

Spline functions were used to identify the threshold of decrement in kidney function at which the slope of FGF-23 levels became steeper. This occurred at eGFR levels approximately <90 ml/min/1.73m² by both eGFR_Cr and eGFR_Cys, independent of age, sex, race, ACR, blood pressure, diabetes and body mass index (Figure 1A and B). Interaction terms confirmed this finding, demonstrating that slope of the association of eGFR with FGF-23 differed significantly among individuals with eGFR_Cys >90 versus those with eGFR_Cys 60–89. Results were similar with eGFR_Cr (interaction \(P\) for both comparisons <0.001). In contrast, the slope of the relationship of ACR with FGF-23 was positive and fairly linear throughout the distribution of ACR values (Figure 1C). Significant differences were observed even among individuals with <30 mg/g in this analysis.

**Discussion**

We demonstrate that modest decrements in eGFR or modest albuminuria are sufficient to detect higher FGF-23 levels in a cohort of out-patients with stable cardiovascular disease and a range of kidney function from normal to moderate CKD. Decrement in GFR and elevations in albuminuria were each associated with elevated FGF-23 independent of one another. These data suggest that FGF-23 is among the earliest detectable abnormalities in mineral metabolism as kidney function decline develops.

Recently studies have demonstrated that FGF-23 levels are strongly associated with mortality in incident dialysis patients, and with greater left ventricular mass in persons with CKD [7,8]. Yet even modest decrements in GFR are strongly associated with CVD events [17,18]. On the basis of results demonstrated here, future studies should evaluate the association of FGF-23 with mortality and CVD events in the general population. Moreover, as FGF-23 induces phosphaturia and inhibits conversion of 25 (OH) vitamin D to its active form, it is possible that FGF-23 levels may be associated with lower bone mineral density and fractures. Studies are needed to evaluate whether FGF-23 may contribute to osteoporosis in the general population, and/or to metabolic bone disease in persons with mild to moderate CKD.

Strengths of this study include its relatively large sample size, availability of creatinine, cystatin C, and albumin–creatinine ratio concurrently, and inclusion of individuals with normal to moderate decrements in kidney function. Limitations include a preponderance of older men, all with prevalent CVD, and few subjects with advanced CKD. Results may differ in other settings.

We conclude that modest decrements in eGFR and elevations in albuminuria are each associated with higher FGF-23 levels independent of one another.

**Acknowledgements.** The authors thank Ms. Clydene Nee for review and assistance with the manuscript. This study was supported by an American Heart Association Fellow to Faculty Transition Award (JH). The Heart and Soul Study was supported by the Department of Veterans Epidemiology Merit Review Program; the Department of Veterans Affairs Health Services Research and Development service; the National Heart Lung and Blood Institute (R01 HL079235); the American Federation for Aging Research (Paul Bessone Scholars Program); the Robert Wood Johnson Foundation (Generalist Physician Faculty Scholars Program); and the Ischemia Research and Education Foundation.

**Conflict of interest statement.** None declared.

**References**

12. Ix JH, Shlipak MG, Chertow GM et al. Association of cystatin C with mortality, cardiovascular events, and incident heart failure


Received for publication: 1.10.09; Accepted in revised form: 24.11.09