Original Paper

The Association between Fibroblast Growth Factor-23 and Vascular Calcification Is Mitigated by Inflammation Markers

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Key Words

Fibroblast growth factor-23 · Vascular calcification · Chronic kidney disease · Inflammation markers · Uremic toxins · Chronic kidney disease

Abstract

Background: Fibroblast growth factor-23 (FGF-23) has been linked to vascular calcification, ventricular hypertrophy and mortality in chronic kidney disease (CKD), although these links may not be direct and independent. Similar grave outcomes are associated with inflammation and oxidative stress in CKD. Recently, accumulating evidence has linked components of phosphate homeostasis to inflammation and oxidative stress. The interaction between the triad of inflammation, FGF-23 and cardiovascular outcomes is underinvestigated. ***Methods:*** We studied 65 patients with stage 5 CKD on hemodialysis. Serum levels of FGF-23, high-sensitivity C-reactive protein (hsCRP), endogenous soluble receptor of advanced glycation end products (esRAGE), advanced oxidation protein products (AOPP), parathormone, lipids, calcium and phosphorous were measured. The aortic calcification index (ACI) was determined using non-contrast CT scans of the abdominal aorta. ***Results:*** FGF-23 was elevated (mean: 4,681 pg/ml, SD: 3,906) and correlated with hsCRP, esRAGE, AOPP, dialysis vintage and phosphorus in univariate analysis. In multiple regression analysis, hsCRP, AOPP and phosphorus but not esRAGE were all significantly correlated to FGF-23 ($R^2 = 0.7$, $p < 0.001$). In univariate analysis, ACI correlated with hsCRP, esRAGE, FGF-23, dialysis vintage, systolic blood pressure (BP) and serum cholesterol. In multiple regression analysis not including inflammation markers, ACI was associated with FGF-23. However, inclusion of inflammation markers in another multiple regression analyses showed that ACI correlated with hsCRP, BP, dialysis vintage and esRAGE but not with FGF-23 ($R^2 = 0.65$, $p < 0.001$). ***Conclusion:*** FGF-23 is strongly correlated to various markers of inflammation and oxidative stress in hemodialysis patients. The association between FGF-23 and vascular calcification was mitigated when corrected for inflammation markers.

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Nephron Extra 2013;3:106–112

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Introduction

Derangement of mineral metabolism has been incriminated as a harbinger of cardiovascular morbidity and mortality, not only in chronic kidney disease (CKD) but also in the general population [1, 2]. With the discovery of fibroblast growth factor-23 (FGF-23) and the plethora of studies published on this phosphaturic agent, some debatable issues have become clearer, while others have become even more confusing [3–5].

Several studies have shown an association between elevated levels of FGF-23 in CKD and left ventricular hypertrophy [6], vascular calcification [7] and mortality [8]. Antagonizing the effects of this hormone through a blockade of its actions has yielded conflicting results regarding its potential clinical benefits. The debate on the role of elevated FGF-23 in CKD as whether it is a physiologically beneficial adjustment, an ominous phenomenon or simply an innocent bystander continues [5, 6, 9]. Although the association between FGF-23 and vascular calcification has repeatedly been demonstrated [7, 10–12], it is probably not a direct cause-effect relationship [13, 14].

The role of inflammation in the pathogenesis of several complications of CKD is well documented [15]. In particular, several markers have been associated with cardiovascular morbidity and vascular calcification, including endogenous soluble receptor of advanced glycation end products (esRAGE), C-reactive protein (CRP), IL-6 and markers of oxidative stress, with some evidence of a cause-effect relationship [16–18].

Levels of FGF-23 in CKD are elevated and correlate with only a few biological markers related to components of mineral metabolism and renal dysfunction [7, 19]. Recently, observations from a few experimental and even fewer clinical studies have proposed a possible link between FGF-23 and inflammation, yet the results were not always uniform. However, the link between the triad of FGF-23, inflammation and clinical outcomes remains largely unexplored [20–23].

In this study, we investigate the relationship of FGF-23 with markers of inflammation/oxidant stress in hemodialysis patients. We also describe the impact of this relationship on the robust link between FGF-23 and vascular calcification that we previously reported in the same group of patients [7].

Methods

We included 65 patients on a dialysis schedule of 4 h/session 3 times a week, who were on regular hemodialysis with polysulfone dialyzers and a dialysate calcium concentration of 1.5 mM/l. The same patients were also included and described in more detail in 2 previous studies investigating the association between FGF-23 [7] and vascular calcification as well as esRAGE [18] and vascular calcification. Patients who had diabetes, a history of parathyroidectomy or were on oral anticoagulation were excluded. Blood samples were drawn in 3 separate tubes for further testing before dialysis and stored at –70°C. Whenever a serum sample was thawed for testing, the remaining serum was discarded afterwards. The medical history, prescriptions over the 3 preceding months and mean blood pressure (BP) recordings were registered.

Blood was tested using routine assays for calcium, phosphorus, lipid profile and albumin. The serum levels of intact parathyroid hormone were measured with an enzyme-amplified sensitivity immunoassay (Roche Diagnostics, Indianapolis, Ind., USA). Intact FGF-23 was measured with a two-site (NH2-terminal/C-terminal) ELISA (Immutopics, San Clemente, Calif., USA), esRAGE with an ELISA-based kit (Daiichi Fine Chemical Co. Ltd., Takaoka, Japan, distributed by B-Bridge International Inc.), high-sensitivity CRP (hsCRP) with ELISA (Oxis

Table 1. Characteristics of the study population

Age, years	50±11.5 (50; 19–73)
Males	28 (43)
Smokers	15 (23)
Dialysis vintage, months	31.7±39.8 (18; 0.1–204)
Systolic BP, mm Hg	150±20.2 (150; 90–190)
Diastolic BP, mm Hg	91±11.3 (90; 60–110)
Cholesterol, mg/dl	154.9±52 (146; 36–323)
Triglycerides, mg/dl	127±63.9 (102; 50–349)
Alphacalcidol*	19 (29.2)
Sevelamer, cinacalcet*	0
Erythropoietin*	35 (53.8)
$CaCO_3$*	46 (70.7)
Calcium, mg/dl	8.9±1 (8.9; 6.2–10.8)
Phosphate, mg/dl	6.8±2.7 (6.2; 3.1–16.7)
Hemoglobin, g/dl	10.1±1.3 (10.6; 8.5–12.3)
Albumin, g/dl	3.4±0.6 (3.4; 1.9–4.6)
Parathormone, pg/ml	314.7±368.2 (169.4; 13.2–1,900)
ACI, %	14.1±12 (11.7; 0–67.5)
hsCRP, μg/ml	6.8±4.7 (5.3; 0.45–21)
FGF-23, pg/ml	4,681.3±3,906.1 (4,058; 229–13,256.7)
AOPP, μM/l	133±103 (109.4; 1–448)
esRAGE, pg/ml	278±101.1 (278; 145–489)

Figures are reported as mean ± SD (median; range) or n (%).
* Number of patients using the drug over the 3 preceding months.

International Inc., Beverly Hills, Calif., USA) and advanced oxidation protein products (AOPP) was measured with spectroscopic analysis (Immundiagnostik, Bensheim, Germany).

The aortic calcification index (ACI) was determined semiquantitatively using non-contrast axial CT scans of the abdominal aorta, as previously detailed [7]. Ten consecutive slices of the abdominal aorta were subdivided radially into 12 sectors at 1-cm intervals. The sectors showing areas of calcification (≥100 HU) were counted, divided by 120 and then multiplied with 100 to express them as percentages. One of the examiners (M.M.N.) interpreted the results with an intraobserver variability of 3.7%.

After the patients had been informed about the study procedures, they gave informed consent for participation, and approval from the local research ethics committee was obtained.

The statistical package for the social sciences (SPSS; version 7.5) was used for data analysis, and the data were summarized as mean ± SD and median, range. Comparisons between the groups were made with the Student t test, and the Mann-Whitney U test was used for the analysis of nonsymmetrically distributed data. Spearman's correlation was used for bivariate analysis, and stepwise multiple regression was performed to correct for possible confounders.

Results

Characteristics of the study participants are shown in table 1.

Fibroblast Growth Factor-23
The level of FGF-23 was 4,681 ± 3,906 pg/ml. As reported previously, this level was more than 40 times higher than that of a matched control group without kidney disease [7]. In univariate analysis, FGF-23 levels correlated with ACI (R = 0.48, p < 0.0001), dialysis vintage

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Nephron Extra 2013;3:106–112

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Table 2. Stepwise multiple regression analysis of factors correlating with FGF-23, $R^2 = 0.68$ (corrected for phosphorus, hsCRP, AOPP, esRAGE and dialysis vintage)

	β	p	95% CI
hsCRP	0.6	<0.0001	387–634
AOPP	0.4	<0.0001	8–20
Phosphorus	0.3	<0.0001	273–733

Table 3. Stepwise multiple regression analysis of factors correlating with ACI, $R^2 = 0.65$ (corrected for hsCRP, systolic BP, esRAGE, AOPP, FGF-23, dialysis vintage and age)

	β	p	95% CI
hsCRP	0.47	<0.0001	0.7 to 1.7
Systolic BP	0.33	<0.0001	0.1 to 0.3
esRAGE	−0.3	0.001	−0.06 to −0.014
Vintage	0.16	0.04	0.06 to 0.1

(R = 0.6, p < 0.0001) and phosphorus (R = 0.5, p < 0.0001). In addition, all measured inflammatory markers correlated with FGF-23 (hsCRP: R = 0.6, p < 0.0001; esRAGE: R = −0.55, p < 0.0001; AOPP: R = 0.44, p < 0.0001). Stepwise multivariate analysis accounting for the correlating factors vintage, phosphorus, AOPP, esRAGE and hsCRP revealed that markers of inflammation and oxidative stress were more robustly correlated to FGF-23 than phosphorus (table 2).

Aortic Calcification Index

Calcification of the abdominal aorta was detectable in 64 out of 65 patients and showed no relation to the intake of calcium-based phosphate binders or alphacalcidol. In univariate analysis, ACI was negatively associated with esRAGE (R = −0.6, p < 0.0001) and positively correlated with hsCRP (R = 0.67, p < 0.0001), FGF-23 (R = 0.48, p < 0.0001), dialysis vintage (R = 0.44, p < 0.0001), systolic BP (R = 0.4, p = 0.001) and serum cholesterol (R = 0.27, p = 0.027).

Three models of stepwise multiple regression analysis were employed to adjust for the factors correlating with aortic calcification. The first model, also reported in a previous study, was corrected for FGF-23, age, dialysis vintage, BP, parathormone, phosphorus, triglycerides and cholesterol. In this model ($R^2 = 0.48$), the independent parameters associated with ACI were FGF-23 (β = 0.6, p < 0.001) and systolic BP [7].

The second model used in the current study, was a regression model that included the factors correlating with aortic calcification as well as inflammatory markers (FGF-23, esRAGE, hsCRP, AOPP, dialysis vintage and systolic BP) and age. After adjustment for inflammatory markers, the correlation of FGF-23 with ACI reduced the standardized regression coefficient to insignificant levels (β = 0.1, p = 0.3; table 3). In a third model, we only corrected for hsCRP and FGF-23, and again, FGF-23 showed no significant correlation with ACI after correction for hsCRP ($R^2 = 0.5$; hsCRP: β = 0.6, p < 0.0001; FGF-23: β = 0.15, p = 0.2).

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Discussion

We showed that FGF-23 was correlated to markers of inflammation and oxidative stress in end-stage renal disease patients on hemodialysis. Unlike what we demonstrated in a previous study on the same patients, the correlation of FGF-23 with aortic calcification did not seem to be independent, as it was mitigated by the correction for inflammation markers.

FGF-23 was repeatedly shown to be strikingly elevated in patients with CKD, and only few clinical markers were associated with this elevation, namely phosphorus, CKD stage and dialysis vintage [7, 19, 24]. The correlation between FGF-23 and inflammation may be a new explanation for the high levels of FGF-23 in CKD patients. Only few clinical studies have described the relation between FGF-23 and inflammatory markers. To date, Mendoza et al. [20] found the most convincing evidence of a positive association between FGF-23 and IL-6, CRP and TNF-α in predialysis CKD patients because findings from other studies were less consistent [22] or even contradictory [21]. The regression analysis (table 2) conducted in the current study showed that FGF-23 correlated well with different markers of inflammation and oxidative stress. This correlation was even more robust than the well-established correlation with phosphorus that was previously reported by us as well as other researchers [7, 11, 19, 24].

On the other hand, the association between inflammatory markers and vascular calcification is frequently reported [25, 26]. Particularly advanced glycation end products provide experimental evidence of a possible cause-effect relationship with vascular calcification [16, 25], and esRAGE may have a protective role [18].

The question that has not been approached yet is whether the interaction between inflammation and FGF-23 has an impact on the sinister associations of FGF-23 [20, 26]. Along with others, we had previously shown an independent association between FGF-23 and aortic calcification [7, 11]. The regression analysis presented here showed that adjusting for inflammatory and oxidative stress markers simply excluded FGF-23 from the independent variables correlating with aortic calcification (table 3). Indeed, the association between FGF-23 and vascular calcification may not be an independent, genuine association as suggested by recent studies in which FGF-23 failed to induce vascular calcification in vitro [13, 14].

The molecular and pathophysiological links between FGF-23 and inflammation still need to be studied in more detail. It is possible that FGF-23 is capable of stimulating and/or accentuating inflammatory cascades. FGF-23-responsive genes include several genes that encode proinflammatory cytokines [27]. FGF-23 activates ERK-1 and phospholipase C-γ isozyme pathway signalling independently of Klotho [6]. Interestingly, these pathways are also capable of signalling an increased production of IL-6, IL-8 and TNF-α [27, 28]. The second possibility is that inflammation increases FGF-23 levels. Several cytokines and inflammatory pathways, including TNF-α and NF-κB, can inhibit bone matrix PHEX and DMP-1, possibly resulting in an increased FGF-23 production from osteocytes [29–31]. A third possibility is that both inflammation and FGF-23 are aroused simultaneously by an extrinsic signal, conceivably phosphorus. Phosphorus has the capacity to increase FGF-23 production as well as stimulate inflammatory cascades and oxidative stress [24, 32, 33]. Other links between inflammation and FGF-23 could be via vitamin D or involvement of signalling pathways in the liver and adipose tissue producing cytokines and acute-phase reactants [20]. To complete the loop, all hypothesized pathways involving FGF-23, phosphorus, inflammation, PHEX, DMP-1, vitamin D, etc., are also pathways that influence bone mineralization and can affect vascular calcification directly or indirectly [29, 30]. It is possible that these multifaceted pathways interlace with each other in complex feedback interactions, affecting bone mineralization, vascular calcification and inflammation in CKD.

Nephron Extra 2013;3:106–112

DOI: 10.1159/000356118

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In this study, we reported the association between FGF-23 and 3 inflammation markers, namely (a) AOPP, a marker of oxidative stress, (b) hsCRP, a nonspecific, acute-phase marker of inflammation, and (c) esRAGE, a surrogate marker of oxidative stress and several other inflammatory cascades. These inflammatory markers are also surrogates of pathways previously shown to be associated with vascular calcification [18, 25, 26, 34]. The main drawbacks of this study are the small number of patients and the cross-sectional design; however, we tried to overcome these weaknesses by using a sensitive radiological technique for the diagnosis of vascular calcification to increase the accuracy of the collected data. The most pertinent finding of this study was that the strong relationship we previously demonstrated between FGF-23 and vascular calcification in the same patients [7] was weakened when corrected for inflammation markers. This is, to our knowledge, the first report to demonstrate an interaction between inflammation and the ominous associations of FGF-23. The nature of this interaction needs to be elucidated by experimental and longitudinal clinical studies to clarify whether there is a cause-effect relationship or not.

Disclosure Statement

The authors have no conflicts of interest to declare regarding the contents of this study.

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