

Fibroblast growth factor 23, iron and inflammation – are they related in early stages of chronic kidney disease?

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Abstract

Introduction: Fibroblast growth factor 23 (FGF-23) levels are elevated in impaired renal function. Inflammation and iron are potential regulators of FGF-23. The aim of the study was to evaluate the association between FGF-23 concentration, novel iron status biomarkers and inflammatory parameters among patients with early stages of chronic kidney disease (CKD).

Material and methods: The study population included 84 patients with CKD in the early stage. Serum hemoglobin, fibrinogen, creatinine, iron, transferrin saturation and ferritin levels were measured using standard laboratory methods. Commercially available kits were used to measure: intact FGF-23, hepcidin, soluble transferrin receptor (sTfR), interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hsCRP).

Results: In patients with CKD no differences in FGF-23 concentration according to iron status were observed. Lower iron concentration was associated with higher concentrations of hsCRP, IL-6 and fibrinogen. In univariate and multivariate analysis FGF-23 correlated with fibrinogen ($r = -0.23$, $p < 0.05$) and eGFR ($r = -0.36$, $p < 0.05$).

Conclusions: FGF-23 is affected by kidney function and fibrinogen but not iron status parameters in the early stages of CKD. Our data are paving the way for further studies on the role of FGF-23 in iron metabolism, especially in early stages of CKD.

Key words: chronic kidney disease, fibroblast growth factor 23, inflammation, iron.

Introduction

The family of fibroblast growth factors (FGFs) regulates a plethora of developmental processes; the first five subfamilies fall into this category [1]. By contrast, the FGF-19, FGF-21 and FGF-23 subfamily has recently been shown to function in an endocrine manner, dependent on the presence of Klotho proteins in their target tissues, to regulate bile acid, cholesterol, glucose, vitamin D and phosphate homeostasis [1, 2].

Fibroblast growth factor 23 (FGF-23) is a hormone produced by osteocytes and osteoblasts involved in phosphate and active vitamin D homeostasis. FGF-23 acts through two main mechanisms – it inhibits absorption of phosphate in the intestine by reducing active vitamin D synthesis and decreases the phosphate reabsorption in renal proximal tubules [3]. It is present in three major forms: biologically active intact FGF-23 (iFGF-23), the inactive C-terminal (cFGF-23) and the N-terminal fragment. Chronic kidney disease (CKD) is the principal example of secondary FGF-23 excess in response to hyperphosphatemia [4]. However, whether the increased FGF-23 levels in chronic kidney disease are beneficial or harmful is still a matter of debate [5]. CKD is also a risk factor for cardiovascular disease [6], and it has been reported that an increased level of FGF-23 is associated with progression of CKD, higher risk of cardiovascular complications (vascular dysfunction, left ventricular hypertrophy) and mortality [7, 8]. However, regulation of FGF-23 in CKD remains unclear, particularly in the early stages of chronic kidney disease, where FGF-23 concentration is elevated before the increase in serum phosphate [9]. Munoz Mendoza *et al.* reported that FGF-23 concentration is independently associated with inflammation in patients with CKD [10]. In contrast, Braithwaite *et al.* [11] did not find correlations between FGF-23 and inflammatory parameters. FGF-23 production in osteocytes also increases in response to iron deficiency; however, in healthy individuals it is accompanied by intensification of proteolytic cleavage, resulting in the increase of inactive FGF-23 forms (detectable as cFGF-23). In patients with CKD proteolytic cleavage of FGF-23 is impaired; therefore in the state of iron deficiency commonly accompanying CKD both forms of FGF-23 may be detectable (iFGF-23 and cFGF-23) [12]. Iron homeostasis is also affected by inflammation; hence FGF-23 regulation in CKD may be the result of correlations between kidney function, iron status and inflammation.

Considering the high prevalence of iron deficiency, inflammation and FGF-23 excess in patients with CKD, we investigated the associations between biologically active FGF-23 concentration, novel iron status parameters including hepcidin and inflammatory parameters in early stages of this disease. This relation might serve as an explanation of increased FGF-23 levels in early stages of CKD as well as a predictor of adverse cardiovascular outcomes.

Material and methods

A total of 84 patients with an established diagnosis of CKD and 23 healthy controls were enrolled in the study. Chronic kidney disease was defined as either an estimated glomerular filtration rate

(eGFR) ≤ 60 ml/min/1.73 m² or presence of hematuria or proteinuria for ≥ 3 months. Exclusion criteria included: acute inflammation or thrombosis; active oncological disease; acute cardiovascular complication (including uncontrolled hypertension, acute coronary syndrome, acute heart failure); any anemia and/or iron deficiency treatment; blood transfusions within 3 months preceding the study; immunosuppressive therapy. All patients were at least 18 years of age. The study protocol has been approved by the local Medical University Ethics Committee. All patients were fully informed about the study and gave their consent.

Medical history (including demographic characteristics as well as current pharmacotherapy) and blood samples were collected in all subjects at the time of enrolment in the morning after an overnight rest. Hematological measurements were made using fresh venous blood with EDTA and clotted blood. The plasma and serum were centrifuging and frozen at -70°C until further laboratory analysis. All laboratory analyses were performed in patients with CKD. In healthy volunteers several significant parameters were measured.

Serum hemoglobin, fibrinogen, creatinine, iron, total iron binding capacity (TIBC) and ferritin levels were measured using standard laboratory methods (automated system – Architect, Abbot Diagnostics, USA) in a certified local central laboratory. Transferrin saturation (TSAT) was calculated as the ratio of serum iron and TIBC and expressed as a percentage. Estimated glomerular filtration rate was calculated from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation according to the latest KDIGO guidelines [13].

Commercially available kits were used to measure: FGF-23 (Human FGF-23 Intact ELISA Kit, Immunotopics, Inc. San Clemente, USA), hepcidin-25 (EIA Kit, Peninsula Laboratories, LLC, Bachem Group, USA), soluble transferrin receptor (sTfR) (Quantikine IVD ELISA Kit, R&D Systems, Minneapolis, USA), interleukin 6 (IL-6) (Quantikine ELISA Kit, R&D Systems, Minneapolis, USA) and high sensitivity C-reactive protein (hsCRP) (CRP Elisa Kit- LDN Labor Diagnostika Nord GmbH&Co KG, Nordhorn, Germany).

Statistical analysis

Data with normal distribution were reported as mean \pm standard deviation, and non-normally distributed data were reported as median and interquartile range. Normal distribution of variables was analyzed using the Shapiro-Wilk W test. Variables with skewed distribution were log (ln)-transformed before further statistical analysis (FGF-23, hsCRP, IL-6, hepcidin, sTfR).

For comparison of differences between groups, analysis of variance (ANOVA) (with *post hoc* Tukey test for unequal groups) or Kruskal-Wallis test was

used. Analyses of the correlation of each parameter were performed using Pearson or Spearman correlation coefficients. Multiple regression analysis was used to determine independent factors affecting dependent variables. *P*-values of less than 0.05 were considered to represent statistical significance. All statistical analyses were performed using Statistica 10.0 (StatSoft) computer software.

Results

The average age of CKD patients was 69 years (Table I). The most common comorbidities were hypertension and diabetes (60% and 24% respectively).

Statistical analysis was performed regarding three tertiles of iron values (lower 16–54, middle 55–91 and upper 92–172 µg/dl) – Table II.

As expected, patients with established diagnosis of CKD had significantly higher levels of iFGF-23 and lower concentration of iron than healthy controls. Lower concentration of iron was associated with higher concentrations of inflammatory parameters such as hsCRP, IL-6, and fibrinogen.

Pearson and Spearman correlations between investigated parameters in patients with early stages of CKD are presented in Table III.

In patients with CKD iFGF-23 presented a negative correlation with fibrinogen ($r = -0.23$, $p < 0.05$) and eGFR ($r = -0.36$, $p < 0.05$). The correlation with fibrinogen was particularly marked in stage 2 ($r = -0.4$, $p < 0.05$, Figure 1).

There were no correlations between iFGF-23 and iron status parameters. Serum iron was correlated with IL-6, hsCRP and fibrinogen.

In multivariate analysis fibrinogen and GFR were found to be predictors of logFGF-23 ($\beta = -0.32$, $p = 0.003$; $\beta = -0.39$, $p < 0.001$ respectively) explaining 22% of iFGF-23 variation in patients with early stages of CKD.

Moreover, we performed regression analysis with iron as the dependent variable and log hsCRP, log hepcidin, log sTfR, log IL-6, log ferritin, and fibrinogen as possible predictor variables. In this analysis log ferritin and log hepcidin were found to be predictors of iron ($\beta = 0.22$, $p = 0.02$; $\beta = 0.28$, $p = 0.03$ respectively) explaining 43% of iron variation in our studied population.

Discussion

The results presented in this paper indicate that iFGF-23 level was not related to iron status, but was inversely associated with one of the in-

Table I. Clinical and laboratory characteristics of study participants.

Parameter	CKD (n = 84)	Control (n = 23)	<i>P</i> -value
Age [years]	69 ±12	52 ±9	< 0.001
Female/male (%)	59/25 (70/30)	15/8 (65/35)	
eGFR [ml/min/1.73 m ²]	67.7 ±16	98.1 ±16	< 0.001
G2 (eGFR 60–89 ml/min/1.73 m ²)	n = 56		
G3 (eGFR 30–59 ml/min/1.73 m ²)	n = 28		
Etiology of kidney disease (%):			
Diabetes	24		
Hypertension	60		
Other	16		
Laboratory variables:			
FGF-23 [pg/ml]	46 (32–60)	13.5 (11–17), n = 21	< 0.001
Iron [µg/dl]	76.8 ±36	95.7 ±25	0.02
TSAT (%)	28.2 ±13	29.1 ±7	0.7
WBC [$\times 10^3/\mu$ l]	7.6 ±2	5.6 ±2	< 0.001
Hemoglobin [g/dl]	13 ±2	14 ±1	0.1
Ferritin [ng/ml]	142 (90–233) n = 82	104 (61–159)	0.03
sTfR [nmol/l]	18 (14–23)	10 (8–11)	< 0.001

Results are presented as mean ± standard deviation or median (interquartile range). eGFR – estimated glomerular filtration rate, FGF-23 – fibroblast growth factor 23, sTfR – soluble transferrin receptor, TSAT – transferrin saturation, WBC – white blood count.

Table II. The comparison of subgroups according to iron tertiles

Tertiles	Lower (16–54 µg/dl) n = 28	Middle (55–91 µg/dl) n = 28	Upper (92–172 µg/dl) n = 28	P-value
iFGF-23 [pg/ml]	45.5 (30–64)	41 (34–58)	50 (33–58)	0.6
hsCRP [mg/l]	16 (5–25)	6 (2–13)	4 (1–7)	0.003
Hepcidin [ng/ml]	25.6 (3–52)	42 (28–67)	35.1 (24–51)	0.03
Ferritin [ng/ml]	98.5 (50–223)	163 (104–231)	161.7 (105–260)	0.06
sTfR [nmol/l]	22.6 (3–52)	18 (16–20)	16.7 (13–19)	0.02
IL-6 [pg/ml]	2.7 (0.6–7)	2.8 (0.4–6)	0.5 (0.2–3.4)	0.04
Fibrinogen [mg/dl]	441 ±111	421 ±95	359 ±68	0.007
eGFR [ml/min/1.73 m ²]	66 ±16	70 ±16	67 ±17	0.7
WBC [× 10 ³ /µl]	8 ±2.3	7.3 ±2.2	7.6 ±2.1	0.5
Hb [g/dl]	12.3 ±2.2	13.1 ±2	14.3 ±2	0.001
RBC [× 10 ⁶ /µl]	4.4 ±0.7	4.4 ±0.6	4.7 ±0.7	0.1

Results are presented as mean ± standard deviation or median (interquartile range). eGFR – estimated glomerular filtration rate, FGF-23 – fibroblast growth factor 23, Hb – hemoglobin, hsCRP – high-sensitivity C-reactive protein, IL-6 – interleukin 6, RBC – red blood count, sTfR – soluble transferrin receptor, WBC – white blood count.

Table III. Correlations coefficients between FGF-23, iron and inflammatory parameters in patients with CKD

	iFGF-23 [pg/ml]	hsCRP [mg/l]	Hepcidin [ng/ml]	sTfR [nmol/l]	Iron [µg/dl]	Ferritin [ng/ml]	Fibrinogen [mg/dl]	eGFR [ml/min/1.73 m ²]	IL-6 [pg/ml]
iFGF-23 [pg/ml]	x	-0.02	-0.05	0.1	0.09	-0.04	-0.23*	-0.36*	0.2
hsCRP [mg/l]		x	0.26*	0.06	-0.42*	0.29*	0.69*	-0.16	0.45*
Hepcidin [ng/ml]			x	0.36*	0.19	0.68*	0.21	-0.13	0.28*
sTfR [nmol/l]				x	-0.36*	-0.37*	0.04	-0.08	0.12
Iron [µg/dl]					x	0.27*	-0.36*	0.07	-0.28*
Ferritin [ng/ml]						x	0.13	-0.01	0.15
Fibrinogen [mg/dl]							x	-0.04	0.23*
eGFR [ml/min/1.73m ²]								x	-0.35*
IL-6 [pg/ml]									x

eGFR – estimated glomerular filtration rate, FGF-23 – fibroblast growth factor 23, hsCRP – high-sensitivity C-reactive protein, IL-6 – interleukin 6, sTfR – soluble transferrin receptor, WBC – white blood count. *Statistically significant data (p < 0.05).

flammatory parameters, i.e. fibrinogen, in patients with early stages of CKD. Serum iron was correlated with inflammatory parameters in our studied population.

Current studies concerning FGF-23 and iron status are inconsistent. In wild type mice iron defi-

ciency increases FGF-23 expression [14]. A similar observation was made in healthy volunteers [15]. In this population Imel *et al.* [15] observed the association of low serum iron levels and elevated cFGF-23 in healthy controls with iFGF-23 in the normal range, while in subjects with rickets low se-

rum iron was associated with an increase in both cFGF-23 and iFGF-23, which results in hypophosphatemia. Autosomal dominant hypophosphatemic rickets (ADHR) is caused by an FGF-23 mutation that renders it resistant to cleavage [3]. Clinical data of early stages of CKD patients showed a significant decrease in cFGF-23 concentrations after iron deficiency anemia treatment with ferric carboxymaltose [16]. In hemodialysis patients, intravenous administration of saccharated ferric oxide increased the levels of iFGF-23 [17]. In contrast, Deger *et al.* [18] reported a negative correlation between iron administration and iFGF-23 levels in a dialysis population. On the other hand, the results obtained in a recent study by Bożentowicz-Wikarek *et al.* [19], which included the largest cohort of patients so far (3780 elderly participants), revealed an association between low iron levels and increased cFGF-23 and iFGF-23 concentrations. The mechanism underlying these paradoxical findings remain unexplained. We did not measure cFGF-23 concentration; hence we cannot exclude the impact of iron status on proteolysis of iFGF-23 [8]. However, it can be postulated that iron deficiency does not increase iFGF-23 in healthy subjects, because it is cleaved by furin into smaller fragments, which are then released and can be detected with the C-terminal assay [20].

One of the most common factors influencing iron homeostasis is inflammation, which is present in CKD, especially in end-stage CKD [21]. This is usually the subclinical form of the inflammatory process, which may be the consequence of decreased uremic clearance that may result in several uremia-related immune dysfunctions, such as neutrophil apoptosis [22]. Inflammation and the iron axis have been intensively studied in the population of hemodialyzed patients [23, 24]. IL-6, hsCRP and fibrinogen significantly correlated with iron in our studied population, which is in line with previous research [23, 24].

There are contradictory results regarding inflammation and FGF-23 in previous studies. Braithwaite *et al.* [11] found no correlation between FGF-23 and inflammation defined as elevated C-reactive protein (CRP) in children with high prevalence of iron deficiency and inflammation. On the other hand, in a large population of patients with CKD, Munoz Mendoza *et al.* [10] demonstrated that increased FGF-23 concentrations are independently associated with higher levels of inflammatory parameters including CRP, interleukin 6 (IL-6), TNF- α and fibrinogen. These results are in contrast to our findings, where higher levels of iFGF-23 are associated with lower levels of fibrinogen. However, in both previously mentioned studies only cFGF-23 was investigated. Hence, proteolytic cleavage may also play an important role in the inflammatory state, not only in iron deficiency.

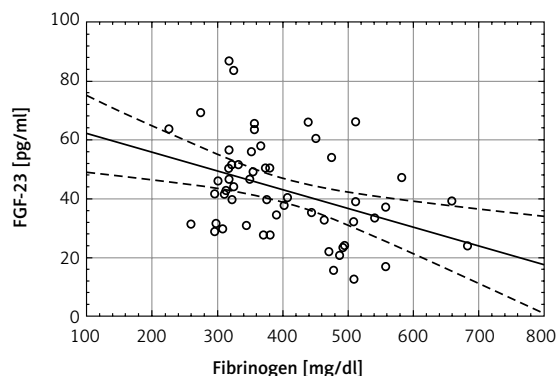


Figure 1. Correlation between iFGF-23 and fibrinogen in patients with CKD, stage 2 ($r = -0.4$, $p < 0.05$)

We would like to stress the novel conceptual approach to search for the possible associations between iron status, mineral metabolism and common comorbidities in a fairly moderate sample size of CKD patients as for a pilot study. As relations between the mineral axis and FGF-23 have been extensively studied, we focused on different aspects and looked for iron and inflammatory parameters in relation to FGF-23 in the early stages of CKD.

There is mounting evidence suggesting that micronutrient abnormalities, including those related to the bone/mineral axis, iron, gut physiology, and systemic inflammation, play a major role in accelerating CKD and cardiovascular diseases [25].

In the 21st century the discovery of FGF-23 has improved our knowledge about mineral homeostasis in both health and disease. However, we are just beginning to unravel the complex role of FGF-23, not only in bone biology, but also in iron metabolism, and our data are paving the way for further studies on the role of FGF-23 in cardiorenal syndrome or cardiorenal anemia syndrome, in particular in the early stages of CKD. In addition, given its involvement in the pathogenesis of human disease, FGF-23 holds promise as a therapeutic target, and a range of studies have confirmed its potential [1]. However, the contribution of FGF-23 to chronic kidney disease is still far from clear, but this ligand may also have pharmacological significance in this context [26].

Conflict of interest

The authors declare no conflict of interest.

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