Association of fibroblast growth factor 23, parathyroid hormone, and vitamin D with acute kidney injury

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Received 24 January 2016
Accepted 22 February 2016

Egyptian Journal of Obesity, Diabetes and Endocrinology 2016, 2:88–94

Introduction
Fibroblast growth factor 23 (FGF23) plays an important role in regulating phosphate and vitamin D homeostasis. Elevated levels of FGF23 are independently associated with mortality in patients with chronic kidney disease and End stage renal disease (ESRD). Whether FGF23 levels are elevated and associated with adverse outcomes in patients with acute kidney injury (AKI) has not been studied so far.
Objective
The aim of this study was to determine the relationship between FGF23 levels in patients with AKI and morbidity, mortality, and/or the need for renal replacement therapy.
Patients and methods
The study included two groups: group 1, which included 30 AKI patients from the general medical ward and ICUs [identified in accordance with the criteria established by Acute Kidney Injury Network (AKIN) grading of AKI]; and group 2, which included 30 healthy controls matched with the patients as regards age and sex. Plasma levels of C-terminal FGF23, 1,25-dihydroxy vitamin D [1,25$(OH)_2$D], and intact parathyroid hormone (iPTH) were measured within 24 h of AKI onset and 5 days later. The composite end point was death or need for renal replacement therapy.
Results
FGF23 levels on day 1 were significantly higher among participants with AKI than among controls (mean level: 278.20 ± 220.58 vs. 14.60 ± 9 pg/ml). There was a statistically significant negative correlation between FGF23 and vitamin D on day 1, with a *P*-value of less than 0.023, whereas there was no statistically significant negative correlation between FGF23 and vitamin D on day 5, with a *P*-value of 0.102. There was a statistically significant positive correlation between FGF23 on day 1 and both Acute Physiology and Chronic Health Evaluation and Sequential Organ Failure Assessment scores, with a *P*-value of less than 0.001. FGF23 proved to be a good predictor of mortality (sensitivity: 100%, specificity: 85%) at a cutoff value of 280 pg/ml.
Conclusion
FGF23 levels are elevated in AKI patients and are associated with increased mortality. AKI is also associated with significant reduction in the level of 1,25$(OH)_2$D and with significant elevation of PTH.

Keywords:
acute kidney injury, fibroblast growth factor 23, parathyroid hormone, vitamin D

Egypt J Obes Diabetes Endocrinol 2:88–94

2356-8062

Introduction

Acute kidney injury (AKI) is the most common reason for inpatient nephrology consultation and is associated with an inhospital mortality rate of 45–70% [1,2]. Until recently, studies on AKI focused on the epidemiology and management of AKI during the index hospitalization. However, AKI is now recognized as a disease with long-term sequelae, including increased risk for death and chronic kidney disease (CKD) progression [3,4].

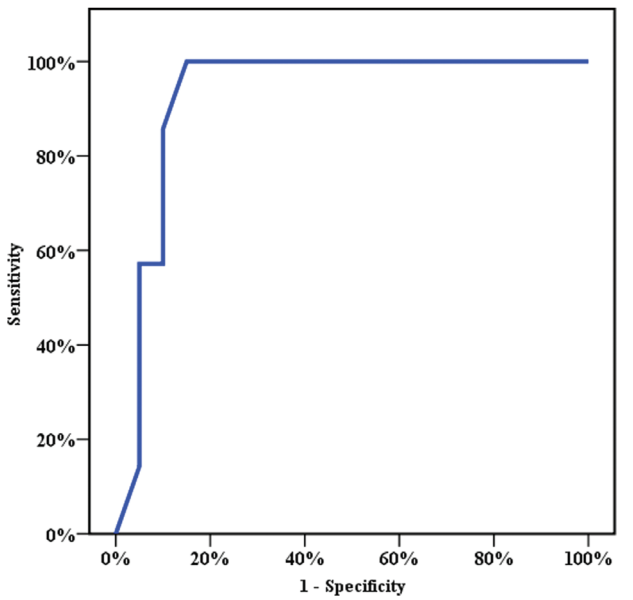
The mechanisms by which AKI is linked to adverse long-term outcomes are poorly understood. Changes commonly found in CKD patients – anemia, acid/base dysregulation, altered mineral metabolism – likely occur in AKI patients, and, as in CKD patients, may be responsible for some of these adverse long-term sequelae [5].

Interestingly, although hypocalcemia and hyperphosphatemia are commonly observed in patients with AKI, the literature on dysregulated mineral metabolism in this patient population is relatively limited.

Fibroblast growth factor 23 (FGF23) is a 26-kDa protein that is a novel, key regulator of phosphorus excretion and contributes to abnormal bone metabolism in CKD. FGF23 has been shown to be a strong, independent predictor of death in ESRD and CKD patients [6,7].

The primary physiologic actions of FGF23 involve regulation of bone and mineral metabolism through bone–kidney endocrine loops (Fig. 1). FGF23 inhibits proximal tubular phosphate reabsorption through its action on Na-dependent phosphate transporters, even though its cofactor, α-klotho, is expressed in distal and not in proximal tubular cells; the mechanisms underlying a distal-to-proximal tubular feedback loop regulating the effects of FGF23 are unclear. It is possible that the effect is mediated through soluble klotho acting as a humoral factor in inducing phosphaturia and multiple other physiologic functions [8]. FGF23 also suppresses circulating 1,25-dihydroxy vitamin D [1,25 $(OH)_2D$] levels, in part by inhibiting Cyp27b1 (1-α hydroxylase) and in part by activating Cyp24 (24-hydroxylase) [9]. The physiologic effect of FGF23 on parathyroid hormone (PTH) secretion remains contradictory. In-vivo and in-vitro studies have indicated that FGF23 suppresses PTH secretion [10], but clinically even extremely elevated FGF23 levels have not prevented the development of secondary hyperparathyroidism in CKD, and elevated FGF23 has been associated with refractory secondary hyperparathyroidism [11].

Figure 1



Receiver operating characteristic curve for fibroblast growth factor 23 as a predictor of mortality.

While physiologically elevated FGF23 represents an adaptive mechanism meant to maintain normal bone–mineral homeostasis, elevated FGF23 levels have been associated with a significant increase in adverse outcomes, such as increased mortality in patients with ESRD, with non-dialysis-dependent CKD and with kidney transplantation [12–14] and even in patients with normal kidney function [15,16]. Furthermore, elevated FGF23 levels are associated with cardiovascular (CV) events, with increased progression of CKD [17,18] with vascular calcification [19], with left ventricular hypertrophy [20,21], with arterial stiffness and endothelial dysfunction [22], and with increased levels of inflammatory markers. These associations appeared to be robust and independent of other concomitant bone–mineral abnormalities, and many have been described in patients with normal kidney function in whom the typical constellation of abnormal bone–mineral metabolism seen in CKD and ESRD was not present [23].

Aim

The aim of this study was to determine the relationship between FGF23 levels in patients with AKI and morbidity, mortality, and/or the need for the renal replacement therapy (RRT).

Patients and methods

The study included two groups: group 1, which included 30 AKI patients recruited from the general medical ward and ICUs [identified in accordance with the criteria established by Acute Kidney Injury Network (AKIN) grading of AKI] [24]; and group 2, which included 30 healthy controls matched with patients as regards age and sex.

Patients with prerenal azotemia (defined as the resolution of increased serum creatinine within 24–48 h of administration of intravenous fluid and/or discontinuation of diuretics), a history of parathyroid disease, metabolic bone disease, fat malabsorption, duodenal resection, evidence of rhabdomyolysis, and those who were currently undergoing or had recently undergone therapy with elemental vitamin D were excluded from the present study.

The patients included in this study underwent the following:

(1) Full clinical examination.
(2) Measurement of urine output every 24 h.

(3) Acute Physiology and Chronic Health Evaluation II (APACHE II) [25] score and Sequential Organ Failure Assessment (SOFA) [26].

Laboratory investigations included the following:

(1) Complete blood picture [27].
(2) Renal function tests (serum creatinine and blood urea) and cystatin C [28,29].
(3) Serum calcium and phosphorus [30,31].
(4) Serum albumin [32].
(5) C-reactive protein [33].
(6) Fasting blood sugar [34].
(7) Creatine phosphokinase, carried out on day 1 and day 5 of the onset of AKI [35].
(8) Serum aspartate aminotransferase, serum alanine aminotransferase, and lactate dehydrogenase [36].
(9) $1,25(OH)_2D$ using the enzyme-linked immunosorbent assay (ELISA) [37].
(10) Intact parathyroid hormone (iPTH) level [38].
(11) C-terminal FGF23 using the ELISA [39].

Plasma levels of C-terminal FGF23, $1,25(OH)_2D$, and iPTH were measured within 24 h of AKI onset and 5 days later, and renal function tests were carried out daily.

The composite end point was death or need for RRT.

The protocol of the study was approved by the ethical committee of Alexandria Faculty of Medicine.

The study was conducted in accordance with the ethical guidelines of 1975 Declaration of Helsinki. An informed consent was taken from the patients included in the research or from their guardians.

Statistical analysis

Data were fed into the computer and analyzed using the IBM SPSS software package (version 20.0; IBM; IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Qualitative data were expressed as number and percent. Quantitative data were expressed as range (minimum and maximum), mean, SD, and median. Significance of the obtained results was judged at the 5% level.

The following tests were used:

(1) The χ^2-test, used for categorical variables to compare between different groups.
(2) Student's *t*-test, used for normally quantitative variables to compare between the two studied groups.
(3) The paired *t*-test, used for normally quantitative variables to compare between the two periods.
(4) The Mann–Whitney test, used for abnormally quantitative variables to compare between the two studied groups.
(5) The Wilcoxon signed-ranks test, used for abnormally quantitative variables to compare between the two periods.
(6) Spearman's coefficient, used to find any correlation between two abnormally quantitative variables.

Results

Because of death or hospital discharge, only 20 patients were available for blood collections on day 5 after enrollment. In total, 10 participants reached the composite clinical end point of death and/or need for RRT (three participants required RRT and survived, four participants died without RRT, and three participants required RRT and died).

Table 1 shows age and sex distribution among the different studied groups. Group 1 included 30 patients – 16 men and 14 women. The mean age of the group was 47.87 ± 16.55 years.

Group 2 included 30 individuals – 13 men and 17 women. The mean age of the group was 43.20 ± 15.93 years.

In group 1, FGF23 level ranged from 29 to 810 pg/ml, with a mean level of 278.20 ± 220.58 pg/ml on day 1, and from 6.0 to 445.0 pg/ml, with a mean of 150.30 ± 160.45 pg/ml on day 5.

Table 1 Comparison between the two studied groups according to demographic data

	Group 1 ($n=30$) [*N* (%)]	Group 2 ($n=30$) [*N* (%)]	Test of significance	*P*
Sex				
Male	16 (53.3)	13 (43.3)	$\chi^2=0.601$	0.438
Female	14 (46.7)	17 (56.7)		
Age				
Minimum–maximum	18.0–70.0	18.0–70.0	$t=1.113$	0.270
Mean ± SD	47.87 ± 16.55	43.20 ± 15.93		
Median	50.50	40.50		

t, Student's *t*-test.

In group 2, FGF23 ranged from 7 to 30 pg/ml, with a mean level of 14.60 ± 9 pg/ml.

There was a statistically significant difference on day 1 between group 1 and group 2, with *P*-value of less than 0.001, and a statistically significant difference on day 5 between group 1 and group 2, with a *P*-value of less than 0.001.

There was no statistically significant difference between day 1 and day 5 in group 1, with a *P*-value of less than 0.059 (Table 2).

In group 1, the level of vitamin D ranged from 500 to 3300 pg/ml on day 1, with a mean of 1654.33 ± 646.88 pg/ml, and from 750.0 to 3100 pg/ml, with a mean of 1750.0 ± 522.90 pg/ml on day 5.

In group 2, the level of vitamin D ranged from 2200 to 4000 pg/ml, with a mean of 2900 ± 557.70 pg/ml.

There was a statistically significant difference on day 1 between group 1 and group 2, with a *P*-value of less than 0.001, and a statistically significant difference on group 5 between group 1 and group 2, with a *P*-value of less than 0.001.

There was no statistically significant difference between day 1 and day 5 in group 1, with a *P*-value of less than 0.307 (Table 3).

In group 1, the level of PTH ranged from 20.0 to 1853.0 pg/ml on day 1, with a mean of 312.42 ± 315.18 pg/ml, and from 20.0 to 2210.0 pg/ml on day 5, with a mean of 392.25 ± 554.90 pg/ml.

In group 2, the level of PTH ranged from 21.0 to 71.20 pg/ml, with a mean of 39.16 ± 14.06 pg/ml.

There was a statistically significant difference on day 1 between group 1 and group 2, with a *P*-value of less than 0.001, and a statistically significant difference on day 5 between group 1 and group 2, with a *P*-value of less than 0.001.

There was no statistically significant difference between day 1 and day 5 in group 1, with a *P*-value of less than 0.955 (Table 4).

Table 5 shows the correlation between FGF23 in group 1 with vitamin D level, serum PTH, APACHE score, SOFA score, cystatin C, serum creatinine, serum blood urea nitrogen (BUN), serum calcium, and serum phosphorus on day 1.

There was a statistically significant negative correlation between FGF23 and vitamin D on day 1, with a *P*-value of less than 0.023.

There was no statistically significant correlation between FGF23 and PTH levels on day 1, with a *P*-value of 0.218.

There was a statistically significant positive correlation on day 1 between FGF23 and APACHE score, with a *P*-value of less than 0.001.

Table 2 Comparison between the two studied groups according to fibroblast growth factor 23

	Group 1		Group 2 ($n=30$)	P_1	P_2	P_3
	Day 1 ($n=30$)	Day 5 ($n=20$)				
FGF23 (pg/ml)						
Minimum–maximum	29.0–810.0	6.0–445.0	7.0–30.0	<0.001*	<0.001*	0.059
Mean ± SD	278.20 ± 220.58	150.30 ± 160.43	14.60 ± 9.0			
Median	237.50	83.50	9.50			

FGF23, fibroblast growth factor 23. P_1: *P*-value for the Mann–Whitney test for comparing between group 1 (on day 1) and group 2. P_2: *P*-value for the Mann–Whitney test for comparing between group 1 (on day 5) and group 2. P_3: *P*-value for the Wilcoxon signed-ranks test for comparing between day 1 and day 5 in group 1. *Statistically significant at $P \leq 0.05$.

Table 3 Comparison between the two studied groups according to vitamin D

	Group 1		Group 2 ($n=30$)	P_1	P_2	P_3
	Day 1 ($n=30$)	Day 5 ($n=20$)				
Vitamin D (pg/ml)						
Minimum–maximum	500.0–3300.0	750.0–3100.0	2200.0–4000.0	<0.001*	<0.001*	0.307
Mean ± SD	1654.33 ± 646.88	1750.0 ± 522.90	2900.0 ± 557.70			
Median	1725.0	1850.0	2700.0			

P_1, *P*-value for Student's *t*-test for comparing between group 1 (on day 1) and group 2. P_2, *P*-value for Student's *t*-test for comparing between group 1 (on day 5) and group 2. P_3, *P*-value for the paired *t*-test for comparing between day 1 and day 5 in group 1. *Statistically significant at $P \leq 0.05$.

Table 4 Comparison between the two studied groups according to parathyroid hormone

	Group 1		Group 2 ($n=30$)	P_1	P_2
	Day 1 ($n=30$)	Day 5 ($n=20$)			
PTH (pg/ml)					
Minimum–maximum	20.0–1853.0	20.0–2210.0	21.0–71.20	<0.001*	<0.001*
Mean ± SD	312.42 ± 315.18	392.25 ± 554.90	39.16 ± 14.06		
Median	271.50	228.50	34.65		
P_3	0.955				

PTH, parathyroid hormone. P_1, *P*-value for the Mann–Whitney test for comparing between group 1 (on day 1) and group 2. P_2: *P*-value for the Mann–Whitney test for comparing between group 1 (on day 5) and group 2. P_3: *P*-value for the Wilcoxon signed-ranks test for comparing between day 1 and day 5 in group 1. *Statistically significant at $P \leq 0.05$.

Table 5 Correlation between fibroblast growth factor 23 and different parameters in group 1

	FGF23 first day	
	r_s	*P*
Vitamin D first day (pg/ml)	–0.414*	0.023
PTH on day 1 (pg/ml)	0.232	0.218
APACHE score	0.870*	<0.001
SOFA score	0.791*	<0.001
Cystatin C (mg/dl)	0.372*	0.043
Serum creatinine first day (mg/dl)	0.576*	0.001
BUN first day (mg/dl)	0.145	0.445
Calcium (mg/dl)	–0.226	0.229
Phosphorus (mg/dl)	0.310	0.096

APACHE, Acute Physiology and Chronic Health Evaluation; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; r_s, Spearman's coefficient; SOFA, Sequential Organ Failure Assessment. *Statistically significant at $P \leq 0.05$.

Table 6 Correlation between fibroblast growth factor 23 and different parameters in group 1

	FGF23 fifth day	
	r_s	*P*
Vitamin D fifth day (pg/ml)	–0.376	0.102
PTH on day 5 (pg/ml)	0.204	0.389
Cystatin C (mg/dl)	0.567*	0.009*
Serum creatinine fifth day (mg/dl)	0.703*	0.001
BUN fifth day (mg/dl)	0.227	0.336
Calcium (mg/dl)	–0.354	0.125
Phosphorus (mg/dl)	0.128	0.589

FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; r_s, Spearman's coefficient. *Statistically significant at $P \leq 0.05$.

There was a statistically significant positive correlation on day 1 between FGF23 and SOFA score, with a *P*-value of less than 0.001.

There was a statistically significant positive correlation on day 1 between FGF23 and cystatin C, with a *P*-value of 0.043.

There was a statistically significant positive correlation on day 1 between FGF23 and serum creatinine, with a *P*-value of 0.001.

There was no statistically significant correlation on day 1 between FGF23 and serum BUN, with a *P*-value of 0.445.

There was no statistically significant correlation on day 1 between FGF23 and serum calcium, with a *P*-value of 0.229.

There was no statistically significant correlation on day 1 between FGF23 and serum phosphorus, with a *P*-value of 0.096.

Table 6 shows the correlation between FGF23 in group 1 with vitamin D level, serum PTH, cystatin C, serum creatinine, serum BUN, serum calcium, and serum phosphorus on day 5.

There was no statistically significant negative correlation on day 5 between FGF23 and vitamin D, with a *P*-value of 0.102.

There was no statistically significant correlation on day 5 between FGF23 and PTH level, with a *P*-value of 0.389.

There was a statistically significant positive correlation on day 5 between FGF23 and cystatin C, with a *P*-value of 0.009.

There was a statistically significant positive correlation on day 5 between FGF23 and serum creatinine, with a *P*-value of 0.001.

There was no statistically significant correlation between on day 5 FGF23 and serum BUN, with a *P*-value of 0.336.

There was no statistically significant correlation on day 5 between FGF23 and serum calcium, with a *P*-value of 0.125.

There was no statistically significant correlation on day 5 between FGF23 and serum phosphorus, with a *P*-value of 0.589.

Figure 1 shows the evaluation of performance of FGF23 as a prognostic factor for mortality with an area under the curve (AUC) of 92.9; at a cutoff of value 280 pg/ml, it has 100% sensitivity and 85% specificity, with a high negative predictive value (100%).

Discussion

The present study confirms that FGF23 levels are elevated in AKI patients (mean level: 278.20 ± 220.58 pg/ml on day 1 and 150.30 ± 160.45 pg/ml on day 5) compared with controls (14.60 ± 9.0 pg/ml), which is in agreement with the results obtained by Leaf *et al.*[40], who conducted a case–controlled study on 30 AKI patients and 30 non-AKI controls and performed FGF23 C-terminal ELISA at enrollment (within 24 h of AKI onset) and then 5 days later. The study by Leaf *et al.*[40] showed that FGF23 C-terminal ELISA-based levels at enrollment were significantly higher in AKI patients (1471 RU/ml) compared with controls (263 RU/ml). These findings were also in agreement with those of a study conducted by Ali *et al.* [41] who found that patients who developed AKI following a cardiac surgery had elevated FGF23 C-terminal ELISA readings, both preoperatively and postoperatively, compared with the children who did not. The increase in FGF23, which occurred preoperatively, might indicate that FGF23 may act not only as a marker of AKI but also as a predictor of AKI occurrence.

Christov *et al.*[42] found that FGF23 C-terminal ELISA measurements significantly increased starting 24 h after the cardiac surgery in all patients who subsequently met the AKI criteria (≥50% increase in serum creatinine from baseline) and continued to rise by 48 h. Zhang *et al.*[43] conducted a cross-sectional study in which plasma FGF23 and iPTH levels were measured in 12 patients with AKI and eight controls. FGF23 levels were significantly higher in AKI patients than in critically ill patients without AKI, with a median FGF23 level of 1948 RU/ml in patients compared with 252 RU/ml in controls ($P=0.01$). No correlations were observed between FGF23 and severity of AKI (defined by the AKIN criteria); among patients with AKI, FGF23 levels were higher in nonsurvivors than in survivors.

In our study, there was no statistically significant correlation between FGF23 and PTH level or serum phosphorus on day 1 or day 5, with a *P*-value 0.218 and 0.96, respectively.

Leaf *et al.*[40] found that enrollment FGF23 correlated positively with phosphate ($P=0.02$) and PTH levels ($P=0.005$), whereas Zhang *et al.*[43] found that no correlation existed between phosphorous and FGF23 levels, but a significant correlation between PTH and FGF23 levels when this analysis was restricted to patients with AKI; this correlation only had borderline statistical significance.

The present study reported that participants with AKI had significantly lower levels of enrollment 1,25 $(OH)_2D$ compared with controls, with a mean of 1654.33 ± 646.88 pg/ml on day 1 and 1750.0 ± 522.90 pg/ml on day 5, whereas in controls the mean level of $1,25(OH)_2D$ was 2900 ± 557.70 pg/ml. These results were in agreement with those of a study by Leaf *et al.* [40] who demonstrated a mean level of $1,25(OH)_2D$ to be 1700 pg/ml on day 1 and 1300 on day 5 in AKI patients, whereas in controls the mean value was 2500 pg/ml.

In our study, seven patients out of 30 reached the end point of death. These patients had significantly higher FGF23 levels on day 1. Furthermore, there was a significant positive correlation between FGF23 and both APACHE and SOFA scores. This is in agreement with the results obtained by Leaf *et al.* [40], who found that enrollment FGF23 levels were significantly higher among participants who died and/or required RRT compared with those participants who survived without RRT.

Conclusion

FGF23 levels are elevated in AKI patients and that elevation appears to be independent of PTH and serum phosphorus.

AKI is associated with significant reduction in the level of $1,25(OH)_2D$ and there exist significant negative correlations between FGF23 and $1,25(OH)_2D$.

FGF23 correlates positively with both APACHE and SOFA scores (at a cutoff value of 280 pg/dl, FGF23 is a good predictor of mortality with a sensitivity of 100% and specificity of 85%).

The role of FGF23 in chronic and more so in acute illness needs to be clarified. As FGF23 seems to be such a powerful and independent predictor for outcomes in CKD, AKI and beyond, it may become a useful routine disease marker for the identification of patients with the highest risk for mortality and other complications.

Another fundamental but to date insufficiently answered question is whether FGF23 levels can be

modified by any intervention and whether this relates to improved outcomes.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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