The Association of Fibroblast Growth Factor-23 with Mineral Factors (Ca, P, and Mg), Parathyroid Hormone, and 25-Hydroxyvitamin D in Hemodialysis Patients: A Multicenter Study

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Abstract

Background: Fibroblast growth factor-23 (FGF23) is a phosphaturic factor that is released from bone. A variety of bone diseases can occur in renal diseases.

Objectives: This study aimed to investigate the association of FGF23 with mineral factors, PTH, and 25-hydroxyvitamin D among hemodialysis patients.

Methods: This cross-sectional multicenter study was performed on 135 patients aged 18 years or over with end-stage renal disease treated with hemodialysis maintenance. FGF-23, phosphorus, Ca, Mg, PTH, 25-hydroxyvitamin D, Uric Acid, Na, and K were measured in each patient’s fasting blood sample. We used univariate and multivariate linear regressions.

Results: The mean age of patients was 56.45 ± 13.64 years. The mean and median FGF23 concentration in patients were 855.07 ± 43.33 and 762.6 (IQR = 456.6 - 1430.3) pg/mL, respectively. Different variables did not show any significant difference between the two sexes. After adjustment for age, sex, dialysis time, uric acid, Na, K, and kt/V, FGF23 had a linear association with 25-hydroxyvitamin D and every 10-unit (pg/mL) increase in FGF23 was significantly associated with a 0.03 mg/mL increase in 25-hydroxyvitamin D (P = 0.04). In addition, other variables showed no significant association with FGF23.

Conclusions: According to the results, FGF23 had a linear association with vitamin D and an increase in FGF23 was significantly associated with an increase in vitamin D. In addition, there was no significant association between mineral factors and PTH, and FGF23.

Keywords: Fibroblast Growth Factor-23, Mineral Factors, Parathyroid Hormone, 25-hydroxyvitamin D, Hemodialysis Patients

1. Background

Fibroblast growth factor 23 (FGF23) is a hormone released from bone to plasma to increase phosphate excretion from the kidney. Besides, it is a predictor for the outcome in both acute and chronic illnesses [1-3]. Increasing FGF23 levels have been shown in different stages of kidney diseases [1]. Moreover, in adult patients with CKD, increased plasma FGF23 is associated with adverse outcomes. Bone disorders may occur very soon in CKD and there is a strong association between FGF23 and the risk of mortality mainly from cardiovascular disease, left ventricular hypertrophy, kidney disease progression, and vascular dysfunction in CKD [4-8].

Parathyroid hormone (PTH) is secreted to correct low levels of serum calcium. PTH increases serum Ca level through bone and kidney [9]. PTH is commonly elevated in patients with CKD. The kidney is also the major site for producing 1,25 (OH) 2 D and 24,25 (OH) 2 D from 25 (OH) D [10, 11]. High PTH often is found in people with vitamin D insufficiency. In contrast, FGF23 levels were elevated in those who were vitamin D replete [12]. In addition, FGF23 is an important factor in regulating phosphorus (P), Ca, and vitamin D metabolism and it can lead to vascular calcifications [13]. FGF23 that regulates vitamin D and P homeostasis independent of the PTH shows potential molecular mechanisms of bone and mineral disorders in CKD [14].

The progressive derangement of mineral homeostasis is accompanied by CKD. Besides, there is an imbalance be-
between blood and tissue concentrations of Ca and P (15). Calcium and vitamin D can suppress PTH expression and parathyroid gland hyperplasia. It is believed that FGF23 and PTH mutually regulate each other in a negative feedback loop where PTH stimulates FGF23 production and FGF23, in turn, suppresses PTH synthesis (9). Therefore, FGF23, vitamin D, and PTH accompany each other and interact in the regulation of metabolism of Ca, P, and bone (13). Although variant derangements in calcium, phosphate, and vitamin D metabolism and complex bone diseases can occur in CKD, the association between different levels of these factors and the outcomes in hemodialysis patients is not completely understood (12).

Therefore, this study aimed to investigate the association of FGF23 with mineral factors (Ca, P, and Mg), PTH, and 25-hydroxyvitamin D among hemodialysis patients.

2. Methods

2.1. Data Collection

This cross-sectional multicenter study was performed on 135 patients aged 18 years or over with end-stage renal disease treated with hemodialysis maintenance from the hemodialysis center of Imam Khomeini Hospital in Tehran province and the hemodialysis center of Razi Hospital in Rasht city, Iran. Some necessary data regarding the patient’s age, sex, and dialysis time were extracted from the records of the patients using a data collection form.

2.2. Mineral Factors (Ca, P, and Mg), Parathyroid Hormone (PTH), and 25-Hydroxyvitamin D Measurement in Patients

Phosphorus (P), calcium (Ca), magnesium (Mg), parathyroid hormone (PTH), and 25-hydroxyvitamin D were measured in each patient’s blood sample before the beginning of dialysis using audit kits from Audit Company made in Ireland for Ca, P, and Mg; PTH was measured using chemiluminescent assay in IMMULITIE2000 with Siemens kits from Siemens Company made in China; and 25-hydroxyvitamin D was determined using the ELFA (enzyme linked fluorescent assay) technique with Vidas kits from Vidas Company made in China.

2.3. Biochemical Markers Measurement in Patients

Uric acid, sodium (Na), and potassium (K) were measured using blood tests while fasting. In addition, kt/V was calculated in all patients.

2.4. Measurement of Fibroblast Growth Factor 23

FGF23 was measured by ELISA kits from BIOTECH company made in China before the beginning of dialysis sessions in pg/mL unit.

2.5. Statistical Analysis

For a description of continuous data, first, the normality assumption was assessed using the Kolmogorov-Smirnov test. If data had a normal distribution, means and standard deviations were presented for them; otherwise, the medians and interquartile ranges were presented. For comparison of continuous variables among males and females, we used a t-test for normal variables and Mann-Whitney test for non-normal distributed variables.

We used univariate and multivariate linear regressions to investigate the association between FGF23 and mineral factors (Ca, P, and Mg), parathyroid hormone, and 25-hydroxyvitamin D. To better report and interpret the results, the FGF23 was divided by 10. Hence, each unit change in FGF23 equals 10 pg/mL. Mineral factors (Ca, P, and Mg), PTH, and 25-hydroxyvitamin D were modeled in separate models against FGF23, dialysis time, age, sex, uric acid, Na, K, and kt/V.

For P and Mg, because the residuals of the models were normal, we did not use any outcome transformation. For PTH, because the residuals of the models were not normal, we did use log transformations. In addition, for Ca and 25-hydroxyvitamin D, because the residuals of the models did not show normal distribution after various transformations, to get the correct P value and confidence interval, we used 300 bootstrap sampling. Data were analyzed by the Stata software (version 12). For all statistical tests, $P < 0.05$ was considered statistically significant.

3. Results

Using the Kolmogorov-Smirnov test, P, Mg, Na, K, and uric acid had normal distributions; hence, their means and standard deviations are presented. Other study variables had non-normal distribution and medians and interquartile ranges are presented for them.

Totally, 135 patients with end-stage renal disease were studied. The mean age of the patients was 56.45 ± 13.64 years (54.28 ± 14.24 in males and 59.72 ± 12.11 in females). Sixty percent (81) of the patients were male (sex ratio: 1.5 male/female). The median dialysis time in patients was 7 (IQR = 5 - 10) months. In addition, the mean and median FGF23 in patients were 855.07 ± 43.33 and 762.6 (IQR = 456.6 - 1430.3) pg/mL, respectively. The distribution of P, Mg, Na, K, and uric acid among males and females was calculated by t-test and the distribution of dialysis time, FGF23, Ca, Vit. D, PTH, and kt/V among males and females was calculated by the Mann-Whitney test.

The mean age, dialysis time, kt/V, FGF23, Ca, P, PTH, 25-hydroxyvitamin D, and biochemical markers did not show any significant difference between the two sexes. The description of the study variables is presented in Table 1.
There was a statistically significant association between FGF23 and dialysis time adjusted for sex and age. For every month increase in the duration of dialysis time, the concentration of FGF23 increased by 58.66 pg/dL (P = 0.001).

3.1. Association of Mineral Factors (Ca, P, and Mg), Parathyroid Hormone, and 25-Hydroxyvitamin D Pressure with FGF23

As Table 2 shows in univariate regression, there was no significant association between mineral factors (Ca, P, and Mg), PTH, and 25-hydroxyvitamin D, and FGF23 (P > 0.05).

After adjustment for age, sex, dialysis time, uric acid, Na, K, and kt/V, FGF23 had a linear association with 25-hydroxyvitamin D and every 10-unit (pg/mL) increase in FGF23 was significantly associated with a 0.03 mg/mL increase in 25-hydroxyvitamin D (P = 0.04). In addition, other variables did not show any significant association with FGF23 (Table 2).

4. Discussion

The aim of this study was to investigate the association between FGF23 and mineral factors (Ca, P, and Mg), parathyroid hormone, and 25-hydroxyvitamin D among patients with end-stage renal disease treated with hemodialysis maintenance.

Homeostasis of calcium and phosphate is maintained by active vitamin D in accompanying with FGF23 and PTH (16). In the present study, after adjustment for different variables, FGF23 had a linear association with vitamin D and every 10-unit (pg/mL) increase in FGF23 was significantly associated with a 0.03 mg/mL increase in 25-hydroxyvitamin D. Similarly, in a multicenter hemodialysis cohort in Taiwan, Chao et al. evaluated the relationship between 25(OH) D and 1,25(OH)2 D, vitamin D-binding protein, and FGF23. In multiple regression analyses, the serum FGF23 level had a strong association with total, free, and bioavailable 25 (OH) D and total, free, and bioavailable 1,25 (OH)2 D levels (17). In Mizuiri et al. study on 32 Japanese maintenance hemodialysis patients, the determinants of the serum FGF23 level in patients were age, serum Ca, P, PTH levels, the active vitamin D dose, and the GNRI (18). In addition, in Hsu et al. study, patients with higher FGF23 had higher 25 (OH) vitamin D, serum phosphorus, and age (19).

Previous studies have shown that FGF23 is adjusted by Ca, P, and PTH. FGF23 can suppress 1,25-dihydroxyvitamin D production and PTH secretion (16). On the other hand, the serum P level had a positive correlation with elevated FGF23 levels in patients with ESRD and the effects of PTH were variable (20). In addition, studies have shown that FGF23 could affect bone formation and mineralization, independently of its effect on phosphate regulation (21, 22).

Nevertheless, in our study, there was no significant association between mineral factors (Ca, P, and Mg) and PTH, and FGF23. Contrary to our results, Sridharan et al. showed a strong association between different levels of FGF23 and the bone formation marker PINP. They also showed FGF23 was up-regulated following intermittent PTH levels (23). In contrast, in a study, the exogenous PTH administration reduced circulating FGF23 concentrations (24).

The reason why we did not find a relationship between calcium and phosphorus, and parathyroid hormone can be that despite a better understanding of FGF23 biology in systemic regulation of P turnover (25), factors inducing its skeletal expression have not been yet fully documented. 1,25-dihydroxyvitamin D, P, Ca, leptin, iron, secreted klotho, acidosis, and PTH are the factors currently known to induce FGF23 production (9). Perhaps, another reason we could not relate these factors is that our patients were not identical regarding the flux of dialysis filters. According to the results of Kendrick study, in hemodialysis patients, FGF23 levels are even more than 1000 times higher than in the normal population (26). Early increase in the level of FGF23 in CKD is an adaptive mechanism to prevent phosphate overload (27). In another study, over a period of 12 months, high-flux hemodialysis was associated with stable FGF23 levels whereas the low-flux hemodialysis group showed an increase in FGF23 (28).

In Wetmore et al. study, increased baseline log FGF23 levels had a significant correlation with putative alterations in gland mass as estimated by significantly shallower slopes of the iCa/PTH suppression curves. Besides, they reported that FGF23 levels decrease during dialysis, but the decrease does not appear to be associated with the changes in PTH or decrements of P during the hemodialysis procedure (29). Therefore, another reason may be that we did not measure the dynamic level of FGE 23 hormone during dialysis, as the Wetmore et al. study may have an effect on its slope.

As a result, the mean and median FGF23 were 855.07 ± 43.33 and 762.6 (IQR = 456.6 -1430.3) pg/mL, respectively. In addition, the mean age of the patients was 56.45 ± 13.64 years and the median dialysis time in patients was seven months. In Jeon et al. study, the mean serum FGF23 level was 7060 ± 1350 RU/mL and linear regressions showed a significant correlation between log FGF23 and age. In addition, the mean age was 66.6 ± 14 years (30). In Negishi et al. study, the serum FGF23 level in dialysis patients was 1171 ± 553 pg/mL. The level of FGF23 was significantly higher in hemodialysis patients than in healthy volunteers (1171 vs. 48 pg/mL) (31). Therefore, all studies indicated a higher level of FGF23 in renal disease and CKD patients. Similarly, Chao et al. reported that the mean age of patients was 66.4 ± 13.9 years (17).
Table 1. Distribution of FGF23, Mineral Factors (Ca, P, and Mg), PTH, 25-Hydroxyvitamin D, and Biochemical Markers by Sex Among Hemodialysis Patients

| Variables               | Male (n = 81) | Female (n = 54) | Total (n = 135) | P Value |
|-------------------------|--------------|----------------|-----------------|---------|
| Dialysis time (mo)      | 7 (5 - 10)   | 8 (4 - 11)     | 7 (5 - 10)      | 0.64a   |
| FGF23 (pg/mL)           | 701.5 (423.1 - 1430.3) | 822.5 (475 - 1424) | 762.6 (456.6 - 1430.3) | 0.45b   |
| Ca (mg/mL)              | 8.7 (8.3 - 9.1) | 9 (8.4 - 9.5)  | 8.8 (8.3 - 9.3)  | 0.12b   |
| Vit. D (mg/mL)          | 37 (25.3 - 53.9) | 31 (19 - 57.6) | 37 (21 - 57)    | 0.73    |
| PTH (ng/mL)             | 325 (191 - 575) | 325 (191 - 575) | 325 (191 - 575) | 0.86b   |
| kt/V                    | 1.2 (1.14 - 1.24) | 1.19 (1.18 - 1.22) | 1.2 (1.15 - 1.23) | 0.65c   |
| P (mg/mL)               | 5.38 (1.3)   | 5.16 (1.13)    | 5.29 (1.23)     | 0.29c   |
| Mg (mg/mL)              | 2.16 (0.26)  | 2.26 (0.30)    | 2.20 (0.28)     | 0.03c   |
| Na (meq/L)              | 139.22 (5.9) | 139.05 (4.63)  | 139.15 (4.2)    | 0.82c   |
| K (meq/L)               | 4.58 (0.63)  | 4.77 (0.84)    | 4.66 (0.72)     | 0.15c   |
| Uric acid (mg/dL)       | 6.5 (1.33)   | 6.49 (1.32)    | 6.50 (1.32)     | 0.94c   |

aQ1 - Q3 = Interquartile range.  
bBased on Mann-Whitney test.  
cBased on t-test.

Table 2. The Univariate and Multivariate Association of Mineral Factors (Ca, P, and Mg), PTH, and 25-Hydroxyvitamin D with FGF23 Among Hemodialysis Patients

| Dependent Variables | Univariate | Multivariate |
|---------------------|------------|--------------|
|                     | β          | SE          | P Value | β          | SE          | 95% CI     | P Value | R²       |
| Ca (mg/mL)          | -0.0004    | 0.001       | 0.81    | -0.0002    | 0.002       | -0.005 - 0.005 | 0.93     | 0.02     |
| P (mg/mL)           | 0.002      | 0.002       | 0.32    | 0.003      | 0.002       | -0.001 - 0.007 | 0.11     | 0.08     |
| Mg (mg/mL)          | -0.0002    | 0.0004      | 0.59    | -0.0001    | -0.0005     | -0.001 - 0.009 | 0.84     | 0.11     |
| PTH (ng/mL)         | 0.001      | 0.001       | 0.25    | 0.001      | 0.001       | -0.001 - 0.004 | 0.48     | 0.08     |
| Vit. D (mg/mL)      | 0.04       | 0.03        | 0.29    | 0.06       | 0.03        | 0.002 - 0.11  | 0.04     | 0.16     |

aβ, regression coefficient; CI, confidence interval; R², coefficient of determination; SE, standard error.  
bBased on linear regression and adjusted for: age, sex, dialysis time, uric acid, Na, K, and kt/V.

4.1. Conclusion

According to the results, FGF23 had a linear association with vitamin D and an increase in FGF23 was significantly associated with an increase in vitamin D. In addition, there was no significant association between mineral factors and PTH, and FGF23.

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Footnotes

Authors’ Contribution: Study concept and design: Farzanehsadat Minoo and Elham Ramezanzadeh; analysis and interpretation of data: Mehrzad Mojarad; drafting of the manuscript: Azam Alamdari and Mohammadtaghi Najafi; critical revision of the manuscript for important intellectual content: Farzanehsadat Minoo, Elham Ramezanzadeh, Mehrzad Mojarad, Azam Alamdari and Mohammadtaghi Najafi; statistical analysis: Farzanehsadat Minoo and Elham Ramezanzadeh.

Conflict of Interests: All authors declare that they have no conflict of interest.

Ethical Considerations: All procedures performed in the study involving data extraction from existing information were in accordance with the ethical standards of the Tehran University of Medical Sciences Research Committee.
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