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High Fibroblast Growth Factor 23 Levels Associated With Low Hemoglobin Levels in Patients With Chronic Kidney Disease Stages 3 and 4

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Abstract: In chronic kidney disease (CKD), decreased erythropoietin production, low serum active vitamin D levels, and high renin-angiotensin-aldosterone activities had been regarded as major causes of renal anemia. At present, no clinical data are available to elucidate the association between renal anemia and fibroblast growth factor 23 (FGF23) levels in CKD. This study aimed to access whether FGF23 is involved in the pathogenesis of renal anemia.

This cross-sectional observational study included 53 stable outpatients with CKD stages 3 and 4. Our primary predictor was serum FGF23 levels and outcome was hemoglobin levels. Measurements contained hemoglobin, FGF23, 25-hydroxyvitamin D, intact parathyroid hormone, plasma renin, serum aldosterone, HbA1C levels, lipid and iron profiles, and serum and urine electrolytes.

Mean age of our patients was 66.4 ± 12.8 (SD) years, mean estimated glomerular filtration rate 33.5 ± 13.9 mL/min/1.73 m^2, median FGF23 level 200 (25th–75th percentile, 124–303) pg/mL, vitamin D level 19.5 (25th–75th percentile, 14.0–25.9) ng/mL, and hemoglobin level 12.7 (25th–75th percentile, 10.7–13.75) g/dL. Even after adjusting multiple variables, lower hemoglobin levels correlated significantly with FGF23 levels that were higher than the median value (>200 pg/mL). Moreover, after adjusting for aldosterone, but not 25-hydroxyvitamin D, it decreased the association with FGF23 that higher than median level and

hemoglobin levels. We also observed a significant decrease of hemoglobin level in the higher FGF23 group who had a diabetes history.

High FGF23 levels were observed to be associated with low hemoglobin levels, which may be partially mediated through the effects of serum aldosterone levels in our patients with CKD stages 3 and 4. Furthermore, we also presumed that diabetes itself may have an impact on the loop among FGF23, hemoglobin, and aldosterone levels in these CKD patients.

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Abbreviations: 25(OH)D = 25-hyroxyvitamin D, alk-p = alkaline phosphate, BMIb = ody mass index, BUN = blood nitrogen, CAD = coronary artery disease, CI = confidence interval, CKD = chronic kidney disease, Cr = creatinine, CVD = cardiovascular disease, DM = diabetes mellitus, eGFR = estimated glomerular filtration rates, FGF23 = fibroblast growth factor 23, Hb = hemoglobin, iCa = ionized calcium, K = potassium, Mg = magnesium, Na = sodium, OR = odds ratio, P = phosphate, RAA = renin-angiotensin-aldosterone activity, SD = standard deviation, TC = total cholesterol, TG = triglyceride, UA = uric acid, UPCR = urine protein-creatinine ratio.

INTRODUCTION

Chronic kidney disease (CKD), a clinical syndrome related to decreased glomerular filtration rate, is a growing and international public health problem.[1–3] With deterioration of renal function, patients suffer from various complications such as fluid-electrolyte imbalance, acid-base abnormalities, aberrant calcium-phosphate homeostasis, and decreased red blood cell production. A recent study revealed that the prevalence of anemia in individuals with CKD was 15.4%, and this prevalence increased with more advanced stages of CKD, from 8.4% to 53.4%, by analyzing the data from the National Health and Nutrition Examination Survey.[3] Renal anemia is one of the established risk factors for cardiovascular disease (CVD) and congestive heart failure in CKD patients.[4,5] The causes for this anemia are multifactorial in origin, which included decreased erythropoietin production, iron deficiency, malnutrition-inflammation, vitamin D insufficiency, and uremic toxin accumulation.[6–8] In addition, the renin-angiotensin-aldosterone activity (RAA) was also depicted to have a direct effect on erythropoiesis.[9–11]

Fibroblast growth factor 23 (FGF23), primarily secreted by osteocytes, is a phosphaturic hormone to increase urinary phosphate excretion and to suppress renal vitamin D synthesis.[12,13] Serum FGF23 level was found to increase once estimated glomerular filtration rate (eGFR) is lower than 90 mL/min/1.73 m^2,[14–16] and was found to be associated with an increase of CVD, left ventricular hypertrophy, and mortality rate in both nondialysis and dialysis CKD patients.[17,18] Not only a direct effect of FGF23 on both pre and postnatal erythropoiesis inhibitions was recently reported in an animal study,[19] but also a

positive correlation between FGF23 and hemoglobin (Hb) levels was portrayed in hemodialysis patients by Akalin et al.[20] Meanwhile, a negative relationship had disclosed between serum FGF23 and aldosterone levels in another animal study, both of which may had an impact on renal anemia.[21] However, at present, this relationship between Hb and FGF23 levels is not available in nondialysis CKD patients. Accordingly, we sought to examine the correlation between anemia, RAA, FGF23, and vitamin D levels in patients with moderate to advanced CKD.

METHODS

Study Population

Fifty-three patients who were firstly diagnosed as CKD were recruited from outpatient nephrology clinics at the Shin-Kong Wu Ho-Su Memorial Hospital Medical Center for this cross-sectional observational study between May 2013 and December 2014. Our inclusion criteria were an age $\geq$20 years and a sustained ($\geq$3 months) decrease in eGFR $\leq$60 mL/min/1.73 m2 based on the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation.[22] Exclusion criteria included CKD stage 5 (eGFR $\leq$15 mL/min/1.73 m2), renal replacement therapy (dialysis or kidney transplant), history of gastrointestinal bleeding within 90 days of enrollment, or history of malignancy. Patients with hematological disorders and malnutrition (body mass index [BMI] <17) were also excluded. This study was approved by the Institutional Review Boards of the Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan. All patients provided written informed consent.

Clinical and Laboratory Data Collection

Vital signs, demographic data, comorbid conditions, and current medications were recorded at enrollment. Fasting blood samples were collected, centrifuged, separated into aliquots, and stored at -20°C for future batched analyses. The testing for different parameters was conducted with standard commercial assays and automated test machine (Beckman AU), including glucose (mg/dL), Hb (g/dL), blood urea nitrogen (BUN, mg/dL), creatinine (Cr, mg/dL), sodium (Na, mEq/L), potassium (K, mEq/L), magnesium (Mg, mg/dL), ionized calcium

(iCa, mg/dL), phosphate (P, mg/dL), albumin (g/dL), uric acid (mg/dL), alkaline phosphate (alk-p, IU/L), HbA1c (%), total cholesterol (TC, mg/dL), triglyceride (TG, mg/dL), ferritin (ng/mL), and iron saturation (iron/iron binding capacity, %) levels. Intact parathyroid hormone (iPTH, pg/mL) concentration was measured by using the Roche Elecsys assay (Roche Diagnostics, www.roche.com). Plasma renin concentration (pg/mL) and aldosterone (pg/mL) levels were measured by the immunoradiometric kit (Cisbio, www.cisbio.com). We used a chemiluminescent immunoassay technology for the quantitative determination of 25-hydroxyvitamin D (25[OH]D, ng/mL; Diasorin, diasorin.com]) levels. FGF23 level was measured in duplicate with a 2-site enzyme-linked immunosorbent assay that detects 2 epitopes in the carboxyl-terminal portion of FGF23 (Immutopics, immutopicsintl.com). Spot urine samples were obtained and K, Ca, P, Mg, and protein levels were determined.

Statistical Analyses

Clinical characteristics were expressed with standard descriptive statistics. We used the Pearson correlation coefficient to examine the correlation between variables, and Spearman rank correlation coefficient was adopted when the data deviated from the normal distribution assumption. We also determined the basic difference in those with FGF23 levels lower and higher than the median by chi-square test for categorical data and Mann–Whitney U test for continuous data due to the normal assumption deviation. A logistic regression was used to determine the risks of FGF23 >200 pg/mL. Next, we performed multivariable linear regression analyses to investigate the independent association between FGF23 and Hb levels. We divided FGF23 into 2 groups by median levels, and continuous FGF23 were natural log-transformed (ln) to approximate a normal distribution. A separate regression model was used to model the change of Hb as a function of an FGF23 >200 pg/mL or ln(FGF23) using a modified stepwise procedure with 5 modeling steps (Table 1). We also performed subgroup analysis by the factors of DM, age, sex, and eGFR. A P value $\leq$0.05 was considered statistically significant. All statistical analyses were performed using the statistical package for social sciences statistical software (SPSS version 16; IBM Inc, Chicago).

TABLE 1. Multivariate Regression Analysis of Risk Factors for Hemoglobin Level (FGF23 Entered as a Dichotomic and Continuous Form)

	Difference in Hemoglobin Between FGF23 >200 vs $\leq$200 pg/mL		Difference in Hemoglobin Per Increase of ln(FGF23)	
	Estimate (95% CI)	P	Estimate (95% CI)	P
Unadjusted	−1.707 (−2.827, −0.587)	0.004	−0.940 (−1.813, −0.067)	0.03
Model 1	−1.488 (−2.615, −0.361)	0.01	−0.792 (−1.622, 0.039)	0.06
Model 2	−1.180 (−2.119, −0.240)	0.01	−0494 (−1.253, 0.265)	0.19
Model 3	−1.096 (−2.152, −0.041)	0.04	−0.433 (−1.251, 0.385)	0.29
Model 4	−1.084 (−2.160, −0.008)	0.04	−0.426 (−0.086, 0.060)	0.30
Model 5	−0.990 (−2.066, 0.085)	0.07	−0.366 (−1.189, 0.457)	0.37

Multivariate model 1 is adjusted for age, sex, and diabetes mellitus. Multivariate model 2 comprises model 1, and also albumin, estimated glomerular filtration rate, urine total protein creatinine ratio, ferritin, and iron saturation. Multivariate model 3 comprises model 2, and also ionized calcium, phosphate, and intact parathyroid hormone. Multivariate model 4 comprises model 3, and also 25-hydroxyvitamin D. Multivariate model 5 comprises model 4 and aldosterone.
CI = confidence interval, FGF23 = fibroblast growth factor 23.

RESULT

Patient Characteristics

The mean age and eGFR in these 53 incident CKD patients were 66.4 ± 12.8 years and 33.5 ± 13.9 mL/min/1.73 m^2, respectively. Among them, 21% were diabetic and 72% were male. Median FGF23 level was 200 (25th–75th percentile, 124–303) pg/mL and with a right skewed distribution (Figure 1). Demographic and clinical data for the participants who were stratified according to the median FGF23 level were listed in Table 2. Those patients with FGF23 levels greater than the median level had significantly higher BUN, P, and K levels, lower eGFR and aldosterone levels, and were more diabetic ($P < 0.05$ for all). No differences were found not only in 25-hyroxyvitamin D (25[OH]D), BMI, iron profile, albumin, lipid profile, urine protein-creatinine ratio (UPCR), and urine electrolyte concentrations but also in renin-angiotensin system-blockade use among subgroups stratified by the median FGF23 level. The median 25(OH)D level was 19.5 (25th–75th percentile, 14.0–25.9) ng/mL, and median Hb level was 12.7 (25th–75th percentile, 10.7–13.75) g/dL.

The Association Between Hemoglobin and FGF23, Aldosterone, and 25(OH)D Levels

Figure 2 shows that Hb levels in CKD patients revealed a negative correlation with ln(FGF23) ($r = -0.290$, $P = 0.03$), a positive correlation with aldosterone ($r = 0.207$, $P = 0.04$) level, and no significant correlation with 25(OH)D levels in crude analysis. This correlation between Hb and ln(FGF23) levels, however, became insignificant after a multivariate adjustment. Besides, a significant difference in Hb concentrations was observed in patients whose FGF23 levels were below and above the median level (13.1 ± 2.0 vs 11.4 ± 1.98 g/dL; $P = 0.004$). A significant correlation was observed between Hb and high median FGF23 levels (>200 pg/mL) in our unadjusted analysis. Moreover, this correlation remained unchanged after adjustment for demographic characteristics, eGFR, and other anemia-specific risk factors (Table 1).
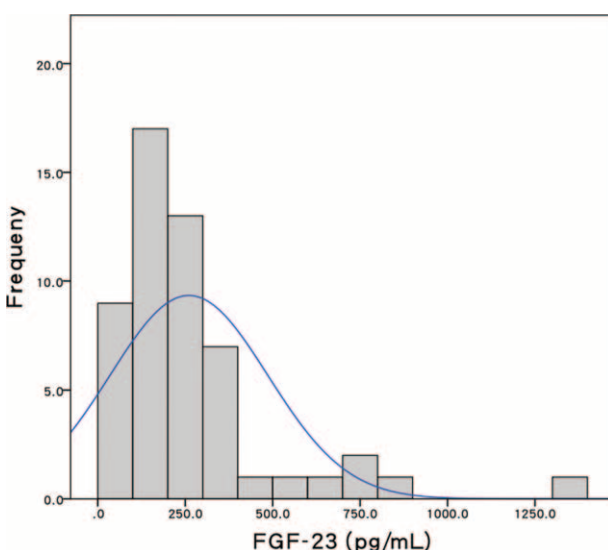

FIGURE 1. Distribution of fibroblast growth factor 23 (FGF23) levels in 53 patients with chronic kidney disease (CKD) stage 3 and 4.

However, this correlation became insignificant ($\beta = -0.990$; 95% confidence interval [CI], -2.066 to 0.085) after further adjusting for serum aldosterone level. Ln(FGF23) has a significant, negative correlation with eGFR ($r = -0.493$; $P < 0.001$), but has no correlation with serum 25(OH)D, aldosterone, and albumin levels (all $P > 0.05$) in our CKD patients (Figure 3).

Determinants of Higher FGF23 Level

The determinants of a FGF23 level greater than the median level (>200 pg/mL) in our patients were delineated in Table 3. In a univariate regression analysis, a FGF23 level >200 pg/mL was found to be significantly correlated with diabetes, low eGFR, low aldosterone, and high P levels (all $P < 0.05$). In a stepwise multivariate analysis, only diabetes (odds ratio [OR] 4.175; 95% CI, 0.146–15.207) and eGFR (OR 0.941; 95% CI, 0.896–0.987) were independently correlated with a FGF23 level >200 pg/mL.

Subgroup Analysis

We also investigated the association between Hb and FGF23 levels by performing analyses in subgroup of patients who were stratified according to age (>65 and ≤ 65 years), sex, eGFR (>30 or ≤ 30 mL/min/1.73 m^2), and history of diabetes. After the multivariate adjustment, there is a significant exacerbation of Hb decrease in the higher FGF23 group with a diabetes history (Table 4). Furthermore, FGF23 that greater than 200 pg/mL in the male sex had a nearly significant effect on the decline of Hb levels.

Further analysis showed that median FGF23 and aldosterone levels significantly correlated with Hb level only in the diabetes group ($r = 0.621$,; $P = 0.003$). However, there was no significant correlation between Hb, continuous FGF23, median FGF23, aldosterone, and 25(OH)D levels in the nondiabetes group (Table 5).

DISCUSSION

Enhanced secretion of FGF23 is regarded as a major cause for the elevation of serum FGF23 levels in CKD patients.[23] Higher FGF23 levels are related to higher mortality, exacerbated CKD progression, and more cardiovascular events in dialysis patients.[17,24] In our cross-sectional study of 53 incident CKD participants, FGF23 that higher than the median level were independently correlated with Hb levels, and this finding is consistent with the results from an animal study.[19] The correlation between FGF23 and Hb levels was independent of traditional anemia risk factors and was unchanged after adjusting for 25(OH)D levels, suggesting that the relationship was not mediated by vitamin D. In contrast, the strength of the correlation between FGF23 and Hb levels was weakening after adjusting for aldosterone levels, suggesting a partial mediation by aldosterone. The higher FGF23 levels (>200 pg/mL) were as an independent risk factor for Hb levels after the multivariable adjustment, indicating that the effects of FGF23 on Hb levels had a threshold.

It is reasonable that higher FGF23 levels correlated with decreasing eGFR, which would also coexist with higher BUN, P, and K levels.[14,16] FGF23 is not only a key regulator of renal sodium reabsorption and plasma volume expansion by direct up-regulation of distal tubular Na$^+$:Cl$^-$ cotransporters (NCC), but also directly suppress the serum aldosterone levels, which showed a synergic effect on NCC activation.[21] Our data supported this finding because we observed a negative correlation

TABLE 2. Demographic and Clinical Data Stratified by Median FGF23 Levels

Variables	All (n = 53)	FGF23 ≤200 pg/mL (n = 26)	FGF23 >200 pg/mL (n = 27)	*P*
Age, years	66.4 ± 12.8	66.2 ± 12.4	68.4 ± 11.4	0.58
Male sex (n, %)	38 (71.6)	20 (76.9)	18 (66.6)	0.54
Diabetes mellitus (n, %)	21 (39.6)	6 (23.0)	15 (55.5)	0.02
Body mass index, kg/m^2	26.0 ± 3.9	25.9 ± 3.7	26.1 ± 4.2	0.94
Systolic BP, mm Hg	129.9 ± 13.7	129.3 ± 10.5	130.5 ± 16.4	0.92
Diastolic BP, mm Hg	70.4 ± 9.8	70.1 ± 9.2	70.6 ± 10.4	0.76
RAS blockade use (n, %)	32 (60.3)	15 (57.6)	17 (62.9)	0.78
25 (OH)D, ng/mL	19.73 ± 7.99	20.13 ± 5.53	18.79 ± 9.68	0.27
iPTH, pg/mL	64.73 ± 36.37	60.50 ± 29.99	70.99 ± 41.96	0.37
Renin, pg/mL	105.4 ± 154.0	83.1 ± 111.5	131.1 ± 189.7	0.74
Aldosterone, pg/mL	135.1 ± 93.1	161.0 ± 93.8	112.28 ± 86.6	0.01
Blood nitrogen, mg/dL	35.26 ± 15.99	29.56 ± 13.95	39.04 ± 13.94	0.002
Creatinine, mg/dL	2.20 ± 0.99	1.91 ± 0.85	2.40 ± 1.00	0.009
eGFR, mL/min/1.73 m^2	33.50 ± 13.9	38.61 ± 14.00	27.55 ± 11.79	0.006
Sodium, mEq/L	138.6 ± 2.5	138.7 ± 2.0	138.5 ± 2.9	0.67
Potassium, mEq/L	4.57 ± 0.54	4.43 ± 0.50	4.72 ± 0.53	0.03
Ionized calcium, mg/dL	4.70 ± 0.18	4.66 ± 0.19	4.73 ± 0.18	0.42
Phosphate, mg/dL	3.75 ± 0.57	3.54 ± 0.51	3.93 ± 0.56	0.02
Magnesium, mg/dL	2.14 ± 0.21	2.12 ± 0.17	2.14 ± 0.24	0.91
Ferritin, ng/dL	222.4 ± 199.5	236.5 ± 175.1	214.8 ± 228.7	0.47
Iron saturation, %	0.30 ± 0.10	0.31 ± 0.12	0.29 ± 0.09	0.75
Alkaline phosphate, mg/dL	59.25 ± 16.99	58.32 ± 14.04	59.96 ± 19.94	0.79
Uric acid, mg/dL	6.84 ± 1.69	6.56 ± 1.46	7.00 ± 1.92	0.40
Glucose, mg/dL	110.9 ± 36.6	108.2 ± 23.1	114.2 ± 46.8	0.90
HbA1C, %	6.70 ± 1.47	6.82 ± 1.50	6.60 ± 1.49	0.78
Total cholesterol, mg/dL	168.3 ± 36.2	173.9 ± 33.6	162.1 ± 38.8	0.25
Triglyceride, mg/dL	178.2 ± 125.5	167.5 ± 98.3	188.1 ± 150.5	0.41
Albumin, g/dL	4.19 ± 0.41	4.32 ± 0.29	4.07 ± 0.48	0.06
Spot urine				
Potassium, mEq/L	30.5 ± 17.0	35 ± 19.9	27.7 ± 13.73	0.49
Calcium, mg/dL	3.33 ± 4.52	3.85 ± 3.68	2.96 ± 5.25	0.17
Phosphate, mg/dL	42.6 ± 28.6	46.5 ± 32.8	39.8 ± 24.9	0.52
Magnesium, mg/dL	4.64 ± 2.25	4.41 ± 1.86	5.11 ± 2.41	0.19
UPCR, g/mg	1.13 ± 1.81	0.72 ± 1.0	1.55 ± 2.29	0.28

25 (OH)D = 25-hyroxyvitamin D, BP = blood pressure, eGFR = estimated glomerular filtration rate, iPTH = intact parathyroid hormone, RAS = renin-angiotensin system, UPCR = urine total protein creatinine ratio.

between FGF23 >200 pg/mL and aldosterone concentrations. However, Imazu et al[25] found a positive correlation between aldosterone and FGF23 in early CKD patients (eGFR >40 mL/min/1.73 m^2). The explanation for this discrepancy may be that the volume status was not high enough to suppress the serum aldosterone level in their earlier CKD patients compared with that in our late CKD patients. Winther et al[26] had reported that hyperinsulinemia could induce FGF23 elevation in diabetic patients, which may explain our finding that patients with higher FGF23 levels showed a greater likelihood of diabetes history. A possible link for this phenomenon is that insulin could increase renal reabsorption of phosphate and consequently lead to hyperphosphatemia development,[27] thus inducing an elevation of FGF23 levels. Our data showed that higher FGF23 levels had more significant effects on decreasing Hb levels only in the diabetic CKD patients.

The RAA played a pivotal role in regulating blood pressure, decreasing proteinuria, and delaying renal function deterioration in CKD patients.[28] However, various clinical and animal studies had reported an association between RAA

and erythropoiesis.[9–11] Severe anemia is thought to be mediated by suppressing the action of angiotensin II, both as a growth factor of erythroid progenitors and as a regulator of erythropoietin secretion, therefore, can impair erythropoiesis.[29,30] The higher FGF23 levels were associated with lower aldosterone levels, indicating a lower RAA, which may be through the action of volume expansion. Previous studies found that aldosterone was associated with vascular calcification by inducing osteoinductive signaling in vascular smooth muscle cells,[31,32] and FGF23 may ameliorate this effect via klotho.[33] Moreover, serum aldosterone levels had been demonstrated to play a role in the correlation between FGF23 and Hb levels in our study. Therefore, we hypothesized that FGF23 may a negative impact on erythropoiesis either directly through a suppressive effect or indirectly through low RAA. However, this hypothesis warranted to be examined. Meanwhile, aldosterone was also shown to have a significant correlation with Hb in our diabetic patients only. It is plausible to presume that diabetes itself may interplay in the intricate loop among FGF23, Hb, and aldosterone levels in CKD.
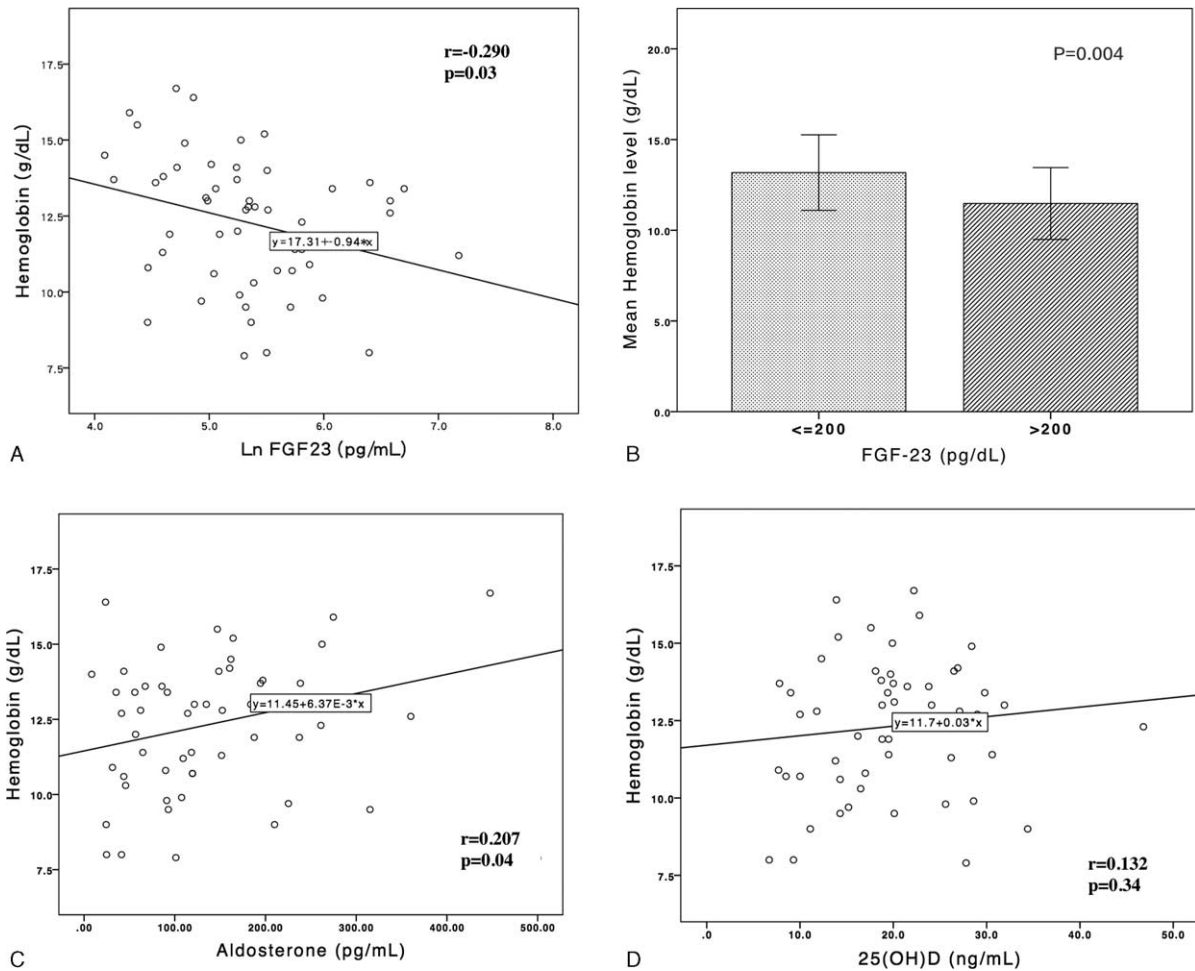
FIGURE 2. Hemoglobin versus serum (A) fibroblast growth factor 23 (FGF23), (B) median FGF23 level. (C) Aldosterone and (D) 25(OH)D levels in patients with CKD. Hemoglobin levels were significantly different between the groups whose serum FGF23 level were above and below median value (13.1 ± 2.0 vs 11.4 ± 1.98 g/dL; *P* = 0.004). Lines indicate best-fit regression lines derived from the least mean square method. 25(OH)D = 25-hyroxyvitamin D, CKD = chronic kidney disease.

Several studies had indicated that vitamin D supplements increased erythropoietin-receptor expression and proliferation of erythroid precursors in vitro and in vivo.[34–36] Not only a reverse correlation between vitamin D and Hb concentrations was observed in a large cross-sectional cohort of early CKD patients,[37] but also aggressive supplements of nutritional vitamin D can increase Hb concentrations in chronic dialysis patients.[38–40] However, our data showed that serum 25(OH)D levels were not correlated with Hb levels and also not related to the effect of FGF23 on decreasing Hb levels. We believed that other factors, beyond serum 25(OH)D concentration, may be relevant to Hb level in patients with CKD stages 3 and 4.

Iron deficiency can stimulate FGF23 transcription in osteocytes[41] and would have an inverse correlation with serum C-terminal FGF23 level in humans.[42] Conversely, rapid intravenous iron loading can decrease the C-terminal FGF23 levels[43]; however, the exact mechanism remains unclear. The iron seems to have a mediating role between the FGF23 and Hb levels. To preclude the effect of iron deficiency in anemia, we adjusted the iron parameter in the regression model. Our

findings showed that the correlation between FGF23 and Hb was independent of iron status in CKD patients.

Our study had some limitations. First, it was a cross-sectional design. Therefore, the causation between FGF23 and anemia could not be inferred. However, our data added to growing evidences that FGF23 has a central role in renal anemia in individuals with CKD and are consistent with results from animal studies.[19] Second, the patient population was small; nevertheless, this concern may be less important because there was a significant correlation between FGF23 and Hb levels. Third, the present data are limited to a single center, which may not be representative of all CKD populations. Therefore, further large-scale and multicenter studies are needed to verify our findings. Finally, 1,25(OH)2D levels were absent in our study, which is an active form of vitamin D. However, we adopted the total 25(OH)D level to alleviate this drawback.

CONCLUSIONS

A negative correlation was found between serum FGF23 and Hb levels in our patients with CKD stages 3 and
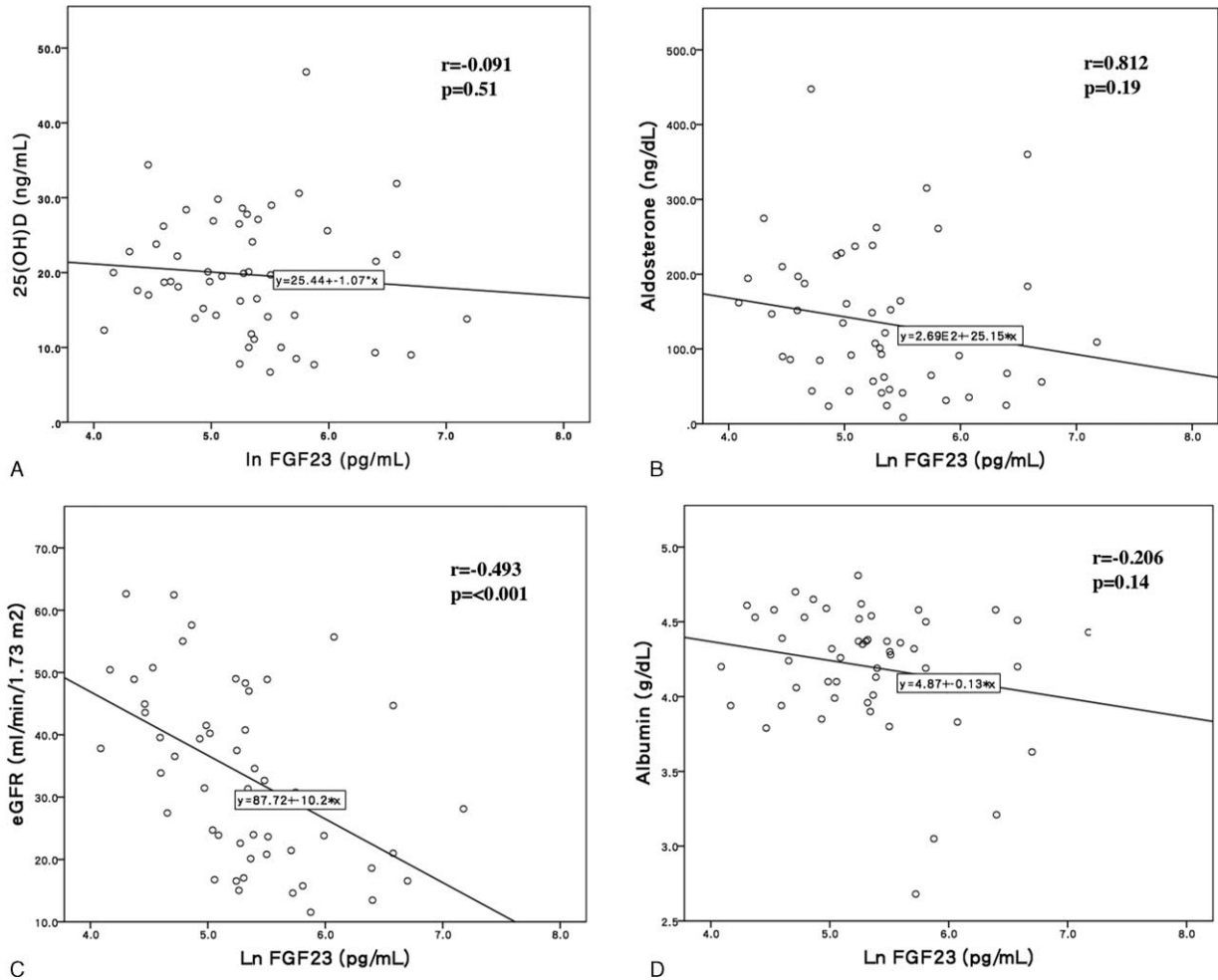
FIGURE 3. Scatter plots of (A) serum 25-hydroxyvitamin D, (B) serum aldosterone, (C) estimated glomerular filtrate rate (eGFR), and (D) albumin versus fibroblast growth factor 23 (FGF23) in patients with CKD. Lines indicate best-fit regression lines derived from the least mean square method. CKD = chronic kidney disease.

TABLE 3. The Determinants of FGF23 >200 pg/mL

	Crude		Stepwise Multiple Regression	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, years	1.006 (0.965, 1.050)	0.76		
Male sex (n, %)	1.667 (0.495, 5.609)	0.40		
Diabetes mellitus	4.167 (1.272,13.65)	0.01	4.175 (1.146, 15.20)	0.03
Systolic BP, mm Hg	1.007 (0.968, 1.048)	0.73		
Diastolic BP, mm Hg	1.005 (0.950, 1.062)	0.87		
Body mass index, kg/m^2	1.014 (0.884, 1.163)	0.84		
RAS blockade use	1.247 (0.414, 3.754)	0.69		
25 (OH)D, ng/mL	0.970 (0.905, 1.040)	0.39		
iPTH, pg/mL	1.008 (0.993, 1.024)	0.38		
Renin, pg/mL	1.002 (0.998, 1.006)	0.29		
Aldosterone, pg/mL	0.993 (0.986, 1.000)	0.04		
eGFR, mL/min/1.73 m^2	0.941 (0.899, 0.985)	0.009	0.941 (0.896, 0.987)	0.01
Ionized calcium, mg/dL	5.983 (0.299, 119.5)	0.24		
Phosphate, mg/dL	3.389 (1.126, 10.20)	0.03		
Magnesium, mg/dL	1.045 (0.084, 13.04)	0.97		

	Crude		Stepwise Multiple Regression	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Ferritin, ng/dL	1.000 (0.997, 1.002)	0.76		
Iron saturation, %	0.997 (0.982, 1.028)	0.37		
Uric acid, mg/dL	1.166 (0.839 1.620)	0.36		
HbA1C, %	0.915 (.626, 1.339)	0.64		
Total cholesterol, mg/dL	0.991 (0.976, 1.007)	0.28		
Triglyceride, mg/dL	1.001 (0.997, 1.006)	0.55		
Albumin, g/dL	0.178 (0.301, 1.027)	0.05		
UPCR, g/mg	1.422 (0.920, 2.196)	0.11		

25 (OH)D = 25-hyroxyvitamin D, BP = blood pressure, CI = confidence interval, eGFR = estimated glomerular filtration rate, iPTH = intact parathyroid hormone, OR = odds ratio, RAS = renin-angiotensin system, UPCR = urine total protein creatinine ratio.

TABLE 4. Subgroup Analysis of the Effect of FGF23 >200 pg/dL on Hemoglobin Level

	FGF23 >200 vs ≤200 pg/dL		
	N	Estimate (95% CI)	*P*
Sex[*]			
Male	38	−1.440 (−3.069, 0.189)	0.08
Female	15	−0.273 (−7.610, 7.046)	0.88
Age, years			
≤65	23	<0.001 (−3.190, 3.190)	0.99
>65	30	−0.534 (−1.857, 0.790)	0.40
eGFR			
>30	30	−0.643 (−2.050, 0.763)	0.34
≤30	23	−0.644 (−3.146, 1.859)	0.57
DM[†]			
Yes	21	−3.720 (−7.340, −0.100)	0.04
No	32	−0.449 (−1.581, 0.683)	0.41

The full model comprised adjusted variables, including age, sex, diabetes, albumin, estimated glomerular filtration rate, urine total protein creatinine ratio, ferritin, iron saturation, ionized calcium, phosphate, intact parathyroid hormone, and 25-hydroxyvitamin D.

CI = confidence interval, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, FGF23 = fibroblast growth factor 23.
[*] The variable of sex was removed from the full model.
[†] The variable of DM was removed from the full model.

TABLE 5. The Correlation With Hemoglobin Levels in Groups With Diabetes and Nondiabetes

	Diabetes (n = 21)		Nondiabetes (n = 32)	
	r	*P*	*r*	*P*
Continuous FGF23	−0.360	0.10	−0.214	0.24
Median FGF23	−0.627	0.002	−0.273	0.13
25 (OH)D	0.280	0.21	0.147	0.42
Aldosterone	0.621	0.003	−0.019	0.91

25(OH)D = 25-hyroxyvitamin D, FGF23 = fibroblast growth factor 23, *r* = correlation coefficient.

4, which may be partially regulated through serum aldosterone levels, but not through serum vitamin D. In addition, we delineated that diabetes may own an impact on the correlation between FGF23 and renal anemia. However, large-cohort studies are required to re-examine these issues in CKD.

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