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Relationship between cFGF23/Klotho ratio and phosphate levels in patients with chronic kidney disease

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Abstract

Purpose To characterize the relationship between the cFGF23/Klotho ratio and phosphate level in patients with chronic kidney disease (CKD).

Methods A total of 152 patients with CKD stage 3–5 (CKD stage 3: $n=74$; CKD stage 4: $n=60$; CKD stage 5: $n=18$) were included in the study. Thirty healthy volunteers served as controls. Intact-FGF23, cFGF23, Klotho, serum calcium, serum phosphate, and serum creatinine were measured, and estimated glomerular filtration rate (eGFR) was calculated. The Kruskal–Wallis H test was used for comparison between groups, and the Spearman test was used for correlation analysis.

Results In CKD stage 3–5, creatinine and iFGF23 levels, as well as the cFGF23/Klotho ratio, were higher ($P<0.01$), phosphate levels were higher ($P<0.05$), and Klotho levels were lower ($P<0.01$), compared with controls. C-terminal-FGF23 levels were higher in CKD phase 4–5 ($P<0.05$). In CKD stage 4–5, creatinine, iFGF23, and phosphate levels, as well as the cFGF23/Klotho ratio, were higher ($P<0.01$), cFGF23 levels were higher ($P<0.05$), and Klotho levels were lower ($P<0.05$), compared with CKD stage 3. In CKD stage 5, creatinine and cFGF23 levels, as well as the cFGF23/Klotho ratio, were higher ($P<0.01$), phosphate and iFGF23 levels were higher ($P<0.05$), and Klotho levels were lower ($P<0.01$), compared with CKD stage 4. Phosphate was positively correlated with the cFGF23/Klotho ratio ($r=0.235$, $P<0.01$).

Conclusions EGFR reduction was associated with an increased cFGF23/Klotho ratio, and the cFGF23/Klotho ratio was positively correlated with phosphate. This suggests that the phosphate level can be controlled by modifying the cFGF23/Klotho ratio.

Keywords CKD · Serum phosphate · Klotho · iFGF23–Klotho signaling axis · cFGF23/Klotho ratio

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Introduction

Hyperphosphatemia is a significant complication of chronic kidney disease (CKD) and it increases the risk of, and mortality from, cardiovascular diseases in CKD patients [1]. Phosphate levels in CKD patients are mainly regulated by diet, fibroblast growth factor 23 (FGF23), Klotho, PTH, and $1,25(OH)_2D_3$. FGF23 exists in the blood in three forms: mature full-length FGF23 (intact FGF23; iFGF23), the amino terminal peptide segment (nFGF23), and the carboxyl terminal peptide segment (cFGF23). As CKD progresses, increased phosphate levels stimulate the secretion of iFGF23, which promotes urinary phosphate excretion by binding to the FGF23 receptor (FGFR)–Klotho complex; this regulates phosphate metabolism by inhibiting $1,25(OH)_2D_3$ synthesis and PTH secretion. However, cFGF23 competitively binds to the binary FGFR–Klotho complex to inhibit the iFGF23–Klotho signaling axis and urinary phosphate excretion [2]. There are no available reports regarding the effect of cFGF23 on phosphate metabolism in CKD. As CKD progresses, iFGF23 and cFGF23 levels increase; both iFGF23 and cFGF23 regulate phosphate metabolism through Klotho. Therefore, we investigated the effect of the cFGF23/Klotho ratio on phosphate metabolism in different stages of CKD.

Patients and methods

This observational study was approved by the Medical Ethics Committee of Fujian Provincial People's Hospital (Fujian, China).

Patients

A total of 152 patients were selected randomly from the Nephrology Department of Fujian Provincial People's Hospital between October 2013 and April 2015, including 78 males and 74 females, aged 25–86 years (average, 60.82 ± 16.08 years). These included 70 patients with chronic glomerulonephritis, 42 patients with diabetic nephropathy, 24 patients with hypertensive nephropathy, 6 patients with high uric acid nephropathy, and 10 patients with IgA nephropathy. Thirty healthy volunteers were selected as the control group from the same hospital during the same period, including 14 males and 16 females, aged 50–87 years (average age, 60.93 ± 6.38 years). There were no statistically significant differences between the two groups regarding sex, age, or disease ($P > 0.05$).

Diagnostic criteria

Patients were diagnosed in accordance with the diagnostic criteria of the Clinical Practice Guidelines for Chronic Kidney Disease (2002), set by the National Kidney Foundation (Tables 1, 2).

Inclusion criteria

With reference to the diagnostic criteria above, patients with CKD stage 3–5 were included in the study.

Exclusion criteria

Patients were excluded from the study for the following reasons: infection, surgery, or tumors within 1 month; CKD caused by systemic lupus erythematosus, Sjogren's syndrome, and other connective tissue diseases; acute renal failure; treatment with anti-inflammatory drugs, antioxidants,

Table 1 Definition of CKD

1. Kidney damage (structural or functional abnormalities of the kidney) for ≥ 3 months, with or without decreased glomerular filtration rate (GFR), manifest by either: pathological abnormalities; or markers of kidney damage: including abnormalities in blood or urine tests or imaging studies
2. GFR < 60 mL/min/1.73 m^2 for ≥ 3 months, with or without kidney damage

Table 2 Stages of CKD

Stage	Description	GFR (mL/min/1.73 m^2)
G1	Kidney damage with normal or high GFR	≥ 90
G2	Kidney damage with mildly decreased GFR	60–89
G3	Moderately decreased in GFR	30–59
G4	Severely decreased in GFR	15–29
G5	Kidney failure	< 15 (or dialysis)

or aspirin; presence of factors that can cause a transitory reversible reduction of renal function, such as uncontrolled hypertension, severe infection, trauma, the use of drugs for renal failure, and a lack of blood volume; treatment with hemodialysis, peritoneal dialysis, or renal transplantation; pregnancy or lactation; or drug allergies.

Treatments

All patients with CKD received conventional treatments before and after enrollment: general treatment included correcting water and electrolyte acid–base disorders, high-quality low-protein diet concomitant with high calorie intake supplemented with α-keto acid tablets (four tablets tid, Beijing Fresenius Kabi Pharmaceutical Co., Ltd., Beijing, China) and oral calcitriol (0.5 μg/day, Kunming Beck Norton Pharmaceutical Co., Ltd., Kunming, Yunnan Province, China) to regulate calcium and phosphate metabolism. In addition to general treatments, patients with hypertensive nephropathy received antihypertensive drugs to maintain blood pressure between 140 and 160 mmHg [serum creatinine (Scr) < 265 μmol/L, angiotensin-converting enzyme inhibitors or angiotensin receptor blocker antihypertensive drugs selected; Scr > 265 μmol/L, calcium channel blocker antihypertensive drugs selected]. Patients with diabetic nephropathy received hypoglycemic drugs to maintain HbA1c between 7 and 8%.

Observation and method indexes

All patients provided fasting blood samples the morning after enrollment. A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect Hepcidin, iFGF23, and cFGF23. A BECKMAN-C800 automatic biochemical analyzer was used to detect serum iron (Fe), phosphate (P), urea nitrogen (BUN), and Scr. The estimated eGFR was calculated based on the simplified Modification of Diet in Renal Disease Trial (MDRD) formula.

Statistical methods

Skewed distribution data are expressed as median ± Q1;Q3. Normally distributed data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using the SPSS20.0 software system (International Business Machines Corporation, Armonk, NY, USA). The Kruskal–Wallis H test was used for comparisons between groups, and the Spearman test was used for correlation analysis. $P < 0.05$ was considered significant.

Results

Indicators for each group

Compared with the control group, the Scr, iFGF23 levels, and the cFGF23/Klotho ratio were higher in patients with CKD stage 3–5 ($P < 0.01$). Serum klotho and calcium levels were lower ($P < 0.01$, $P < 0.05$, respectively) and serum phosphate levels were higher ($P < 0.05$) in CKD stage 3–5 compared with control. Serum cFGF23 levels were higher in CKD stage 4–5 compared with the control group ($P < 0.05$).

Compared with CKD stage 3, Scr, iFGF23, phosphate levels, and the cFGF23/Klotho ratio were higher in patients with CKD stage 4–5 ($P < 0.01$); cFGF23 levels were higher ($P < 0.05$) and serum klotho levels were lower ($P < 0.05$) in CKD stage 4–5 compared with CKD stage 3. Serum calcium levels were lower in CKD stage 5 compared with CKD stage 3 ($P < 0.01$).

Compared with CKD stage 4, Scr, cFGF23 levels, and the cFGF23/Klotho ratio were higher in patients with CKD stage 5 ($P < 0.01$). Serum phosphate and iFGF23 levels were higher ($P < 0.05$), serum klotho levels were lower ($P < 0.01$), and serum calcium levels were higher ($P < 0.01$) in CKD stage 5 compared with CKD stage 4 (Tables 3, 4).

Simple correlation analysis between the indicators

As shown in Table 5, eGFR was negatively correlated with iFGF23 ($r = -0.381$, $P < 0.01$), cFGF23 ($r = -0.346$, $P < 0.01$), and serum phosphate ($r = -0.505$, $P < 0.01$), and eGFR was positively correlated with serum calcium ($r = 0.246$, $P < 0.01$). Phosphate was positively correlated with iFGF23 ($r = 0.312$, $P < 0.01$), cFGF23 ($r = 0.206$, $P < 0.05$), and the cFGF23/Klotho ratio ($r = 0.235$, $P < 0.01$).

Table 3 Comparison of the levels of iFGF23, cFGF23, Klotho, Ca, Scr and eGFR in each group (mean ± SD;median ± Q1;Q3)

Group	*n*	iFGF23 (pg/mL)	cFGF23 (pg/mL)	Klotho (pg/mL)	Scr (mmol/L)	eGFR (mL/min)	Ca (mmol/L)
Control	0	330.67 ± 221.12;380.35	192.04 ± 83.96	125.83 ± 21.62	55.28 ± 48.60;65.08	109.28 ± 10.34	2.20 ± 2.10;2.40
CKD3	4	641.26 ± 445.99;926.13[1]	181.24 ± 87.83;361.42	55.07 ± 45.02;73.79[1]	158.89 ± 48.77[1]	51.19 ± 20.63[1]	2.14 ± 0.15[2]
CKD4	0	840.41 ± 607.75;1476.22[1,3]	247.10 ± 152.76;475.93[2,4]	48.00 ± 31.49;69.59[1,4]	337.62 ± 294.64;475.44[1,3]	26.44 ± 58.45[1,3]	2.09 ± 0.19[1]
CKD5	8	1461.55 ± 1045.41;1851.07[1,3,6]	433.59 ± 247.37;957.41[1,3,5]	30.78 ± 25.17;52.31[1,3,5]	792.08 ± 243.99[1,3,5]	6.70 ± 5.69;8.53[1,3,5]	1.92 ± 0.18[1,3,5]

[1] $P < 0.01$ and [2] $P < 0.05$ versus the normal group; [3] $P < 0.01$ and [4] $P < 0.05$ versus the CKD stage 3; [5] $P < 0.01$ and [6] $P < 0.05$ versus CKD stage 4

Table 4 Comparison of the levels of cFGF23/Klotho ratio and phosphate in each group (mean ± SD;median ± Q1;Q3)

Group	*n*	cFGF23/Klotho ratio	*P* (mmol/L)
Control	30	1.56 ± 0.69	1.11 ± 0.09
CKD3	74	2.88 ± 1.39;6.87[1]	1.20 ± 0.23[2]
CKD4	60	5.87 ± 2.83;14.80[1,3]	1.34 ± 1.22;1.58[1,3]
CKD5	18	13.18 ± 6.46;33.99[1,3,5]	1.95 ± 0.68[1,3,6]

[1]$P<0.01$ and [2]$P<0.05$ versus the normal group; [3]$P<0.01$ and [4]$P<0.05$ versus the CKD stage 3; [5]$P<0.01$ and [6]$P<0.05$ versus CKD stage4

iFGF23 was positively correlated with cFGF23 ($r=0.415$, $P<0.01$).

Discussion

FGF23 is the main phosphate-regulating factor. Elevated serum phosphate and $1,25(OH)_2D_3$ levels causes FGF23 secretion [3]. The FGF23 receptor consists of a complex formed by the combination of Klotho and FGFR1. The C-terminal of iFGF23 binds to Klotho, and the N-terminal binds to FGFR1, activating the classical Klotho-dependent pathway [4]. Upon a reduction in the GFR, iFGF23 levels in the blood increase gradually [5]. Increased iFGF23 levels downregulate the apical membrane expression of the sodium phosphate cotransporters type 2a (NaPi-2a) through a Klotho-dependent pathway, promoting urinary phosphate excretion in the proximal renal tubule [6]. Additionally, it downregulates the expression of 1-alpha-hydroxylase in the proximal renal tubule to reduce the production of endogenous $1,25(OH)_2D_3$ [7], thereby indirectly inhibiting intestinal phosphate uptake. iFGF23 can be cleaved into nFGF23 and cFGF23 by the enzyme furin [8]. Goetz et al. [2] found that injection of the FGF23 C-terminal tail peptide into healthy rats inhibited renal phosphate excretion and induced hyperphosphatemia; cFGF23 also inhibited the Klotho-dependent pathway by competitively binding to the binary FGFR–Klotho complex. We found that the cFGF23/Klotho ratio increased gradually with a reduction in eGFR ($P<0.01$); the cFGF23/Klotho ratio was positively correlated with phosphate ($r=0.235$, $P<0.01$). This suggests that the level of phosphate can be controlled by maintaining the cFGF23/Klotho ratio in a suitable range.

Klotho is mainly expressed in the kidney. A lack of vitamin D, high-phosphate diet, activation of endogenous renin–angiotensin system, pathological urinary toxins, inflammatory response, and oxidative stress can all downregulate Klotho expression [9–12]. Active vitamin D treatment, a low-phosphate diet, and application of angiotensin antagonists can increase Klotho expression [13–15]. Asai et al. [16] analyzed renal biopsy specimens and showed that the Klotho expression levels decreased with the decrease in GFR despite the different causes of CKD. Upon reduction of GFR in CKD patients, cFGF23 and iFGF23 expression increased. Klotho binds to iFGF23 to promote phosphate excretion, while Klotho binds to cFGF23 to inhibit phosphate excretion. Our results suggested that a higher cFGF23/Klotho ratio was associated with a greater inhibitory effect of cFGF23 on the iFGF23–Klotho signaling axis. Thus, the cFGF23/Klotho ratio reflects the effects of cFGF23 on serum phosphate levels in CKD patients.

Compared with the control group, iFGF23 levels were higher ($P<0.01$), cFGF23 levels were not significantly changed ($P>0.05$), Klotho levels were lower ($P<0.01$), the cFGF23/Klotho ratio was higher ($P<0.01$), serum phosphate levels were higher ($P<0.05$; in the normal range of 0.85–1.51 mmol/L), and serum calcium levels were lower ($P<0.05$) in CKD stage 3. In early CKD, serum phosphate levels begin to rise, iFGF23 physiological compensatory secretion increases, and iFGF23 binds to the FGFR–Klotho complex, promoting proximal tubular urinary phosphate excretion and distal renal tubular calcium absorption. In this study, Klotho levels decreased and the cFGF23/Klotho ratio increased in CKD stage 3 compared with the control group, both of which inhibited the iFGF23–Klotho signaling axis. However, the phosphate levels remained within the normal range, indicating that the effect on the iFGF23–Klotho signaling axis is weak. When the ratio of cFGF23/Klotho was 2.88, the serum phosphate levels remained within the normal range. C-terminal–FGF23 levels may not have significantly increased in CKD stage 3 because iFGF23 cleavage produces less cFGF23 as a result of the reduced furin activity

Table 5 eGFR, iFGF23, cFGF23, Klotho, cFGF23/Klotho ratio, Ca, and phosphate correlation matrix

	eGFR	iFGF23	cFGF23	Klotho	Ca	*P*
eGFR	–	− 0.381**	− 0.346**		0.246**	− 0.505**
iFGF23		–	0.415**			0.312**
cFGF23			–		− 0.2*	0.206*
Klotho				–		
cFGF23/Klotho ratio						0.235**

**$P<0.01$; *$P<0.05$

in the early stage, and because some cFGF23 binds to the FGFR–Klotho complex.

Compared with patients in CKD stage 3, iFGF23 and cFGF23 levels in CKD stages 4–5 were higher ($P < 0.05$). Klotho levels were lower ($P < 0.05$), the cFGF23/Klotho ratio was higher ($P < 0.01$), and serum phosphate levels were higher ($P < 0.01$) in CKD stage 4–5 compared with CKD stage 3. In later stages of CKD, urinary phosphate excretion barriers keep stimulating iFGF23 production as the GFR levels decrease further. However, there is a concurrent reduction in Klotho, while cFGF23 levels and the cFGF23/Klotho ratio increase further, resulting in the inhibition of the iFGF23–Klotho signaling axis and increased serum phosphate levels. We found that serum phosphate levels were positively correlated with the cFGF23/Klotho ratio ($r = 0.235$, $P < 0.01$). When the cFGF23/Klotho ratio was greater than 2.88, the regulatory effect of iFGF23 on phosphate was gradually reduced.

Upon reduction of GFR, the cFGF23/Klotho ratio increased and the inhibitory effect of cFGF23 on the iFGF23–Klotho signaling axis was enhanced, which led to further aggravation of the urinary phosphate excretion disorder. We found that the regulatory effect of iFGF23 on phosphate is gradually reduced when the cFGF23/Klotho ratio is greater than 2.88. Maintaining the cFGF23/Klotho ratio in a suitable range by increasing Klotho expression and inhibiting iFGF23 cleavage may improve phosphate metabolism in patients with CKD. However, this is a small sample study. And some of the drugs taken by patients may affect the results of indicators. A large sample size study to test the observations is still needed.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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