

## The Effect of Yishen Jiangzhuo Granules on Serum IFGF23 and CFGF23 in Patients with Chronic Kidney Disease 3-5 Stage

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### Abstract

**Aim:** The objective is to investigate the changes of serum IFGF23 and CFGF23 levels in patients with chronic kidney disease of 3-5 stage before and after treatment by Yishen Jiangzhuo Granules and its clinical significance.

**Methods:** A subset of samples (n=76) from a cohort of patients with CKD of renal function between stage three and five. (Stage 3: n=37; stage 4: n=30; stage 5: n=9). The patients were treated in hospital or in clinic between October 2013 and April 2015 at the nephrology department of Fujian Provincial People's Hospital. Additionally, a total of 30 healthy volunteers were selected in this hospital during the same period as the normal control group. IFGF23, CFGF23 were detected by enzyme-linked immunosorbent assay (ELISA). Calcium (Ca), phosphorus (P), urea nitrogen (BUN), serum creatinine (Scr) was assessed by BECKMAN-C800 automatic biochemical analyzer. Urine creatinine was determined by Reversed Phase High Performance Liquid Chromatography. The endogenous creatinine clearance rate (Ccr) was calculated according to the simplified MDRD formula.

**Result:** The expression levels of serum IFGF23 and CFGF23 did not change significantly before and after drug treatment. But the serum IFGF23 and CFGF23 expression level has been significantly increased in the CKD3 stage, while serum phosphorus levels has not yet increased. Compared with normal group, the expression level of serum IFGF23 and CFGF23 was significantly higher than that of the normal group before and after treatment.

Both of them showed a tendency to increase gradually with the decrease of renal function, and the degree of increase of serum IFGF23 expression level was significantly higher than that of CFGF23. Scr and P levels were decreased in CKD 3-5 stage after treatment (P<0.05). In CKD 4-5 stage, after treatment, the serum calcium level was higher than that before treatment (P<0.05). In CKD 3-5 stage, after treatment, the Ccr was higher than that before treatment (P<0.05).

**Conclusion:** IFGF23 increases rapidly with the decrease of Ccr and CFGF23 increases slowly with the decrease of Ccr. In the short term, Yishenjiangzhuo Granules cannot improve serum IFGF23 and CFGF23 levels in CKD 3-5 stage in patients, but it improves the renal function and the metabolism of calcium and phosphorus, and reduces the level of serum creatinine.

**Keywords:** Yishen Jiangzhuo granules; Chronic kidney disease; IFGF23; CFGF23

### Introduction

Studies have found that elevated serum fibroblast growth factor 23 (FGF23) levels not only are sensitive indexes of disorders of calcium phosphate metabolism and cardiovascular disease in patients with CKD, but are also associated with left ventricular hypertrophy and atherosclerosis, and is an independent risk factor for death in patients with CKD. Therefore, reducing the level of FGF23 may play an important role in reducing the incidence of mineral and bone metabolic disorders and cardiovascular events, and also improve the quality of life and survival of patients with CKD.

FGF23 is a cytokine secreted by osteoblasts and osteocytes, which contribute to reduction of serum phosphorus and active vitamin D,

levels, regulated mainly by serum phosphorus, 1,25 (OH) 2D3 levels [1]. The protein of FGF23 may comprise intact (IFGF23) and amino terminal peptide (nFGF23) and carboxyl terminal peptide (cFGF23) forms' measurement in the circulation. Only IFGF23 has biological activity. IFGF23 can directly reduce blood phosphorus, and C-terminal can be combined with Klotho, and then adjust the balance of serum phosphorus and vitamin D *in vivo* [2]. Most of the current studies have focused on reducing the level of CKD in patients with FGF23, but it rarely pay attention to the levels of IFGF23 and CFGF23 in patients with CKD.

Previous studies have found that Yishen granule has the effect of invigorating spleen, kidney, turbidity and blood stasis. It can improve renal function by antioxidative stress, enhance immunity, anti-inflammatory and anti-oxidation effectively and improve the microcirculation of the kidney. But it can reduce the level of IFGF23 and cFGF23? The aim of this study was to investigate the changes of

serum IFGF23 and CFGF23 levels in patients with chronic kidney disease between three and five stage.

## Materials and Methods

### General information

Randomly select 76 cases of patients with CKD3, the patients in the Department of Nephrology, outpatient department and inpatient department of Fujian Provincial People's Hospital from October 2013 to April 2015 as the treatment group, there were 39 males and 37 females, aged 25 years to 86 years, with an average age of  $60.82 \pm 16.14$  years old. Among them, there were 35 cases of chronic glomerulonephritis, 21 cases of diabetic nephropathy, 12 cases of hypertensive nephropathy, 5 cases of IgA nephropathy, 3 cases of high uric acid nephropathy. There were 30 cases for normal control group, including 14 male and 16 female, aged 50 years to 87 years with an average age of  $60.93 \pm 6.38$  years old. There was no statistically significant difference between the two groups in gender, age and disease ( $P > 0.05$ , comparable).

### Diagnosis, inclusion and exclusion criteria

**Diagnostic criteria:** Reference to the diagnostic criteria of 2002 NKF-KDOQI clinical practice guidelines for chronic kidney disease in the United States.

**Inclusion criteria:** Patients with chronic kidney disease (Chronic kidney, CKD) 3, 4, and 5 patients (both men and women) who met diagnostic criteria.

**Exclusion criteria:** Patient with infection, surgery or tumor within 1 month;

- CKD caused by secondary systemic lupus erythematosus (SLE), Sjogren's syndrome and other connective tissue diseases.
- Acute renal failure; Treatment with anti-inflammatory drugs, antioxidants, aspirin before seeing a doctor.
- There are some factors which can cause the transient reversible decrease of renal function, such as uncontrolled hypertension, severe infection, trauma, the use of drugs for renal failure, and the lack of blood volume.
- Patients who were treated with hemodialysis, peritoneal dialysis and renal transplantation.
- Pregnant or lactating women, who are allergic to drugs.

### Treatment

Symptomatic treatment (correct water electrolyte acid-base disorders) and diet therapy (high quality low protein, high calorie food, supplement essential amino acids), etc. Alfacalcidol (0.5 g/day, Teva Pharmaceutical Industries Ltd) regulates calcium and phosphorus metabolism; patients with hypertensive nephropathy treated with antihypertensive drugs (when  $Scr < 265$  mol/L, with ACEI or ARB class of antihypertensive drugs; when  $Scr > 265$  mol/L, with CCB, according to the situation of blood pressure with other antihypertensive drugs). Edema (urine volume  $< 2000$  mL) plus furosemide diuretics. Patients with renal anemia and taking Yishenjiangzhuo granule (15 g heterophylla, 15 g Poria, Atractylodes 10 g, Astragalus 15 g, mistletoe 15 g, mulberry 15 g, rhubarb 6 g, Salvia 15 g, June snow 15 g, Plantago 15 g, angelica 6 g, motherwort 15 g, Achyranthes 15 g, orange peel 6 g. Drug Manufacturing Room of The people's Hospital affiliated Fujian

Traditional Chinese Medicine University assisted preparation of The Yishenjiangzhuo granule, Fujian medicine word z06106052.

### Preparation method

Decocted the traditional Chinese medicine for two times according to the dose ratio, each time 1.5 h, alcohol extraction to thick paste, add sugar dextrin, granulating, drying, per gram containing crude drug 1 g. Take it after mixing it with water, 30 min after each meal) on the basic treatment of correcting anemia (EPO 1000 U 1 time/week, Shenzhen champion Biological Pharmaceutical Co., Ltd). Disable all drugs that may affect the efficacy of judgment. Half a month of course of treatment.

### Observation indexes and methods

Draw fasting heparin anticoagulant 2 mL before and after treatment with the Yishenjiangzhuo granule in the early morning. IFGF23 and CFGF23 were detected by double antibody sandwich enzyme-linked immunosorbent assay (enzyme-linked immunosorbent assay, ELISA), determination of calcium (Ca), phosphorus (P), urea nitrogen (BUN) and serum creatinine (Scr) by BECKMAN - C800 automatic biochemical analyzer, determination of creatinine in urine by Reversed Phase High Performance Liquid Chromatography. The endogenous creatinine clearance rate (Ccr) was calculated according to the simplified MDRD formula.

### Statistical methods

All data are expressed as mean  $\pm$  standard deviation. Complete statistical processing with SPSS20.0 software system; the indexes before and after treatment in each group were in line with normal distribution, using two paired sample t test, not in normal distribution, using rank sum test; The indexes of each group were in line with the normal distribution, using the analysis of variance, which does not conform to the normal distribution, using a multiple sample comparison rank sum test, With  $P < 0.05$ , the difference was statistically significant.

### Result

**Comparison of IFGF23, CFGF23, Ca, P, Ccr, Scr before and after treatment in the treatment group:** There was significantly no change in serum IFGF23 and CFGF23 levels before and after treatment ( $P > 0.05$ ). After treatment, the levels of Scr and P were lower than those before treatment ( $P < 0.05$ ). The level of  $Ca^{2+}$  in CKD4-5 phase was higher than that before treatment ( $P < 0.05$ ). Ccr was higher than that before treatment ( $P < 0.05$ ) (Table 1).

**Comparison of IFGF23, CFGF23, Ca, P, Ccr, Scr levels in each group before treatment:** Compared with the normal group, the serum IFGF23 and CFGF23 levels in CKD3-5 phase increased before treatment ( $P < 0.05$ ), and with the progress of CKD that increased gradually. The serum creatinine and the phosphorus in CKD3-5 phase increased before treatment ( $P < 0.05$ ), and with the progress of CKD phase that increased gradually. The serum calcium and Ccr in CKD3-5 phase decreased before treatment ( $P < 0.05$ ), and with the progress of CKD phase Ccr decreased gradually ( $P < 0.05$ ) (Table 1).

**Comparison of IFGF23, CFGF23, Ca, P, Ccr, Scr levels of each group after treatment:** Compared with the normal group, the serum IFGF23 and CFGF23 levels in CKD3-5 phase increased after treatment ( $P < 0.05$ ). The serum creatinine in CKD3-5 phase increased after

treatment (P<0.05). The serum calcium in CKD5 phase decreased after treatment (P<0.05). The serum phosphorus in CKD4 phase increased after treatment (P<0.05). The endogenous creatinine clearance rate in CKD3-5 phase decreased after treatment (P<0.05) (Table 1).

Groups	n	IF GF23 (pg/mL)	CFGF23 (pg/mL)	Ca (mmol/l)	P (mmol/l)	Ccr (ml/min)	Scr (mmol/l)
normal	30	354.84 ± 204.93	192.04 ± 83.96	2.25 ± 0.18	1.11 ± 0.09	109.28 ± 10.34	57.13 ± 8.72
CKD337	before	802.77 ± 552.412 <sup>b</sup>	348.33 ± 427.98 <sup>d</sup>	2.13 ± 0.16 <sup>b</sup>	1.28 ± 0.24 <sup>b</sup>	45.16 ± 17.08 <sup>b</sup>	172.8 ± 46.28 <sup>b</sup>
	after	796.32 ± 494.59 <sup>e</sup>	317.11 ± 385.47 <sup>a</sup>	2.14 ± 0.15	1.13 ± 0.19 <sup>a</sup>	57.22 ± 22.29 <sup>a,e</sup>	144.78 ± 47.67 <sup>a,e</sup>
CKD430	before	1159.23 ± 1051.79 <sup>b,c</sup>	408.26 ± 430.31	2.05 ± 0.21 <sup>b</sup>	1.48 ± 0.41 <sup>b</sup>	16.55 ± 6.12 <sup>b,c</sup>	411.23 ± 126.76 <sup>b,c</sup>
	after	1485.3 ± 1469.72 <sup>e,f</sup>	464.92 ± 497.16 <sup>e</sup>	2.13 ± 0.17 <sup>b</sup>	1.34 ± 0.32 <sup>a</sup>	36.34 ± 81.92 <sup>a,e,f</sup>	338.75 ± 109.13 <sup>a,e,f</sup>
CKD59	before	1735.52 ± 1163.40 <sup>b,c</sup>	757.14 ± 620.88 <sup>b,c,d</sup>	1.81 ± 0.09 <sup>b</sup>	2.32 ± 0.57 <sup>b,c</sup>	6.2 ± 1.5 <sup>b,c,d</sup>	874.7 ± 193.48 <sup>b,c,d</sup>
	after	1760.45 ± 1171.1 <sup>e,f</sup>	613.09 ± 517.85 <sup>e,f</sup>	2.02 ± 0.19 <sup>a,e,f</sup>	1.57 ± 0.58 <sup>a</sup>	9.43 ± 6.34 <sup>a,e,g</sup>	709.47 ± 271.49 <sup>a,e,f,g</sup>

Note: Before and after treatment in the treatment group: a)P<0.05; Comparison in each group before treatment: vs. the normal group; b)P<0.05;vs. the CKD3 phase; c)P<0.05;vs. the CKD4 phase; d)P<0.05; Comparison in each group after treatment: vs. the normal group; e)P<0.05;vs. the CKD3 phase; f)P<0.05;vs. the CKD4 phase; g)P<0.05.

**Table 1:** Comparison Of IFGF23, CFGF23, Ca, P, Ccr, Scr levels in each group (x s).

## Discussion

FGF23 is a cytokine secreted by osteoblasts and bone cells, whose relative molecular mass is about 32,000. It belongs to the family of fibroblast growth factor family (FGFs). The main physiological role of FGF23 is to decrease the level of serum phosphorus and vitamin D. The expression level of FGF23 was mainly regulated by phosphorus, 1,25 (OH) 2D3, high phosphorus diet, hyperphosphatemia and high 1,25 (OH) 2D3 levels could promote FGF23 secretion [1]. The FGF23 precursor was first excised into a 24-amino acid signal peptide chain and then transformed into the mature 25-FGF-23-251 protein (IFGF23) by O-glycosylation of the polypeptide-N-acetylgalactosyltransferase 3 (GALNT3) [3], which was secreted into the blood and then degraded into the amino-terminal peptide (NFGF23) and the carboxyl-containing peptide (CFGF23).

The results showed that the expression levels of serum IFGF23 and CFGF23 did not change significantly before and after drug treatment. But the serum IFGF23 and CFGF23 expression level has been significantly increased in the CKD3 stage when a serum phosphorus level has not yet increased. Compared with normal group, the expression level of serum IFGF23 and CFGF23 was significantly higher than that of the normal group before and after treatment. Both of them showed a tendency to increase gradually with the decrease of renal function, and the degree of increase of serum IFGF23 expression level was significantly higher than that of CFGF23. Literature [4] reported an increase in total FGF23 (including IFG F23 and CFGF23) with the decrease of glomerular filtration rate in the early stages of CKD (CKD 2, 3) even before the abnormal changes of serum phosphorus, PTH, 1,25-dihydroxyvitamin D3, serum total FGF23 levels have increased.

What factors have led to an increase of serum IFGF23 and CFGF23 in the early stages of CKD? Hyperphosphatemia often occurs in patients with CKD when GFR falls to 25 ml/min<sup>-1</sup> (1.73 m<sup>2</sup>)<sup>-1</sup> to 30 ml/min<sup>-1</sup> (1.73 m<sup>2</sup>)<sup>-1</sup>, and in the early stage of CKD to maintain serum phosphorus in the normal range of a physiological compensatory, total serum FGF23 physiological compensatory secretion increases. FGF23 can reduce the intestinal reabsorption of phosphorus by reducing the phosphoreduction of renal tubules, down-regulating 1-alpha

hydroxylase and up-regulating 23-hydroxylase, reducing the level of 1,25 (OH) 2D3 in the circulation, and thus maintaining the normal level of normal phosphorus [1]. At the same time, the renal secretion of Klotho protein is reduced, resulting in a compensation mechanism of "FGF23 resistance" [5]. FGF23 can play a physiological role through classical Klotho -dependent and non-dependent pathways, which require receptor FGFR1c or FGFR4 involvement [6]. Klotho provides a binding site for the C-terminus of FGF23 and then binds to the receptor FGFR through the N-terminus of FGF23 to activate the classical Klotho-dependent pathway [7]. However, with the progress of CKD, FGF23-Klotho axislose balance. The serum total FGF23 level increases significantly, while the Klotho protein expression level was reduced significantly. Klotho cannot provide enough adequate binding sites to increase FGF23 gradually anymore, leading to "FGF23 resistance ". For the occurrence of that phenomenon, Klotho protein levels continue to decline as FGF23 increases gradually [8]. Animal experiments have shown that blood FGF23 levels of Klotho gene-deficient mouse increased significantly, suggesting that the lack or fall in Klotho protein expression can cause FGF23 resistance, which stimulate osteoblasts and bone cells to secrete FGF23. In addition, the ability of CKD patients clearing FGF23 has reduced. Large numbers of studies have shown that FGF23 is mainly cleared by the kidneys. Due to damage of renal unit and renal dysfunction, renal ability to remove serum FGF23 has decreased. This may be the main reason which led to a gradual increase in serum FGF23 expression level. However, some scholars believe that FGF23 expression level increases in the early stage of CKD mainly due to increased bone FGF23 synthesis, rather than a decrease in renal clearance [9]. Therefore, FGF23 expression levels started to increase in the early stages of CKD and are evident in CKD stage 3. The total FGF23 expression levels has increased significantly, resulting in increase of IFGF23, CFGF23 (Which produced by decomposition of FGF ) expression levels too.

With the progression of disease, GFR of CKD patients reduces significantly. On one hand, a lack of phosphorus filtration leads to difficulty in excreting phosphorus in urine as well as an increase in blood phosphorus level. On the other hand, due to a fall in Klotho protein expression in kidney, complex binding between FGFR-klotho of CFGF23 and the distal tubule of kidney decreases, resulting in a

reduction and impairment of the proximal tubule sodium-phosphate co transporter capacity, thus urinary phosphorus reabsorption increases and phosphorus excretion decreases. This leads to a negative feedback disorder between the exchange of phosphorus and FGF23, forming a vicious cycle leading to blood phosphorus and total FGF23 to increase continuously. The total increase of FGF23 in ESRD patients is significant and can be up to 100 times more than normal people. Renal excretion of phosphorus is the main reason leading to elevated FGF23 [10]. In patients with chronic renal failure, the content of bioactive FGF23 increases and the proportion of IFGF23 to total FGF23 are significantly higher than that of normal population [11]. Shimada et al. [12] found that FGF23 was predominantly in the form of IFGF23 in patients with long term peritoneal dialysis, presumably due to the decline of renal function. Gradual reduction of IFGF23 degradation leads to the reduction of CFGF23, which causes a degree of inconsistency between the two, but the specific mechanism is not clear. In this study, we found that the levels of IFGF23 and CFGF23 increases gradually with a decrease in renal function, and the level of IFGF23 was significantly higher than that of CFGF23, which is consistent with the above theory. Yishen Jiangzhuo Granule is mainly composed of Astragalus, Radix Pseudostellariae, Atractylodes, Poria, Angelica, Mulberry, *Ramulus taxilli*, Po-lygonatum odoratum, Motherwort, Tangerine peel, *Serissa foetida* Comm, composing of Rhubarb and other drugs. Previous studies have shown that Yishenjiangzhuo granules have a definite effect of relieving symptoms of chronic renal failure, reducing proteinuria, protecting residual renal function, delaying renal function progression, delaying dialysis time and so on. This study observed that Yishenjiangzhuo Granule could decrease the creatinine level in CKD3-5 patients and delay the progression of high renal metabolism, but it could not reduce the levels of IFGF23 and CFGF23 in CKD3-5 patients. We speculated that FGF23 has started to increase in the early stage of CKD, which leads to a significant increase in its decomposition products IFGF23 and CFGF23 in the 3 period. As a result of the use of alfacalcidol and calcium treatment, the total FGF23 Secretion increased further, making Yishenjiangzhuo granules failing to improve the CKD3-5 patients with serum IFGF23 and CFGF23 levels in the short term.

In summary, CKD patients has high level of IFGF23, CFGF23 in the early stage and as the disease progresses, IFGF23 rises even more. Due to the degradation of IFGF23 and klotho protein binding, the rising

trend of CFGF23 becomes more gentle; In addition, the treatment time is short, so Yishenjiangzhuo granules, calcium supplementation and the application of alfacalcidol, are not easy to control CKD patients with increase in IFGF23 and CFGF23.

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