Original Research Article

Serum fibroblast growth factor 23 levels in chronic kidney disease and its correlation with bio-chemical parameters in chronic kidney disease: a cross sectional comparative study

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ABSTRACT

Background: Globally, chronic kidney disease (CKD) is a major public health problem. Fibroblast growth factor (FGF)-23 is a newly recognized phosphatonin secreted by the osteocytes which acts as a key regulator of serum phosphate levels in CKD. In the present study, we aimed to estimate the levels of serum FGF-23 in patients with CKD and to compare them with healthy controls. Also, we aimed to compare the levels of FGF-23 with creatinine clearance and kidney size in various stages of CKD.

Methods: A cross sectional comparative study was conducted at Thiruvarur Government Medical College hospital in Tamil Nadu. Patients aged between 20 and 65 years with an established diagnosis of CKD and healthy controls were included in the study. Enzyme Immuno-Assay method was followed for the estimation of FGF-23. Spot Urine sample was collected to determine the presence of albumin. Serum levels of glucose, Urea, Creatinine, Electrolytes (Sodium, Potassium), Albumin, Calcium, Phosphorus and Alkaline Phosphatase were measured. Information on kidney size, cortical echogenicity, parenchymal thickness and cortico-medullary differentiation were assessed based on ultrasound abdomen.

Results: A total of 45 CKD cases and 45 healthy controls were studied. Mean (SD) age of CKD cases was 54(11) years and that of controls was 46(9.6) years. The mean value of FGF23 in cases was 730.7±492.7 pg/ml and this was higher than that of the control group whose mean value was 39.49±12.47 pg/ml (P<0.05). Mean GFR levels in cases and controls were 23.8 and 113.8 and this difference was statistically significant (P value<0.0001). Among cases, Pearson correlation between serum FGF-23 levels and eGFR, serum albumin was statistically significant and had a negative inverse correlation.

Conclusions: The present study demonstrated that serum FGF23 levels were significantly increased in patients with CKD. This increase in serum FGF23 levels were progressive from the early stages to the late stages of CKD.

Keywords: Chronic kidney disease, Fibroblast growth factor, GFR, Serum phosphate

INTRODUCTION

Globally, chronic kidney disease (CKD) is a major public health problem. CKD is one of the leading causes of death and disability worldwide.⁴ CKD leads to poor outcomes like End Stage Renal Disease (ESRD), Cardio Vascular Disease (CVD) and Premature Deaths.⁵ Glomerular filtration rate (GFR) is the primary measure of kidney function. Normal GFR is 120 ml/min and it varies with age, sex and race. Patients with CKD have a
decreased GFR and they develop mineral metabolic disturbance such as hyperphosphatemia even before the diagnosis of CKD is made.

Fibroblast growth factor (FGF)-23 is a newly recognized phosphatonin secreted by the osteocytes which acts as a key regulator of serum phosphate levels in CKD. It initiates secondary hyperparathyroidism in patients with CKD. FGF-23 together with its co-receptor Klotho binds to specific FGF Receptor (FGFR) in the target organs-kidneys and parathyroid glands. FGF-23 induces phosphaturia and causes suppression of Vitamin D production in the kidney.\textsuperscript{3}

Serum FGF-23 levels rise progressively as renal function decreases and increased levels of FGF-23 is an independent predictor of CKD progression, cardiovascular morbidity and mortality.\textsuperscript{5} FGF-23 levels are increased even in the early stages of CKD while creatinine-based estimations of GFR which are used to assess the kidney function do not detect early CKD.\textsuperscript{4} So, measurement of serum FGF-23 levels could therefore have a definitive advantage in the early diagnosis and in predicting the progression of CKD and therapeutic strategies to reduce serum FGF-23 levels could be of great benefit to the patient in terms of improved survival.\textsuperscript{3}

In the present study, we aimed to estimate the levels of serum FGF-23 in patients with CKD and to compare them with healthy controls. Also, we aimed to compare the levels of FGF-23 levels with creatinine clearance and kidney size in various stages of CKD.

**METHODS**

A cross sectional comparative study was conducted at Thiruvarur Government Medical College hospital in Tamil Nadu. Patients aged between 20 and 65 years with an established diagnosis of CKD and healthy controls were included in the study. A total of 45 CKD patients and 45 controls were studied. Patients with any of the following conditions were excluded: acute/chronic inflammatory diseases (sepsis, infection, malignancy and liver disease), previous history of coronary artery bypass graft surgery, acute kidney injury, patients on immunotherapy, previous history of cerebrovascular diseases and patients who underwent renal transplantation.

Informed consent was obtained from all subjects prior to the study. Under aseptic precautions, 5 ml of venous blood sample was collected after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000 rpm for 15 minutes for separation of serum. An aliquot of the serum was taken for the estimation of FGF-23 and stored at -20°C in the deep freezer. The remaining serum was used for the estimation of Glucose, Urea, Creatinine, Electrolytes (Sodium, Potassium), Albumin, Calcium, Phosphorus, Alkaline Phosphatase. Enzyme Immuno-Assay method was followed for estimation of FGF-23. Spot Urine sample was collected to determine the presence of albumin. Information on kidney size, cortical echogenicity, parenchymal thickness, cortico-medullary differentiation, presence of cysts (simple, complex), solid lesions and the state of urinary tract were assessed based on ultrasound abdomen.

Data was entered in Microsoft Excel and statistical analysis was done in SPSS version 17.0. Descriptive statistics were done. Mean and standard deviation (SD) was calculated for continuous variables and proportions or percentages for qualitative variables. Student’s t-test, Chi-square test and one way ANOVA were used for comparison of parameters between the cases and controls. A ‘P’ value less than 0.05 was taken as the significant value. Correlation between the measured parameters were assessed using Pearson’s correlation coefficient.

**RESULTS**

A total of 45 CKD cases and 45 health controls were studied. Mean (SD) age of CKD cases was 54 (11) years and that of controls was 46 (9.6) years. Out of 45 CKD cases, 27 (60%) were males and among controls 25 (56%) were males. The mean value of FGF23 in cases was 730.7±492.7 pg/ml and this was higher than that of the control group whose mean value was 39.49±12.47 pg/ml and this difference was statistically significant (p<0.05). Mean FGF levels in cases and controls were 23.8 and 113.8 and this difference was statistically significant (P value<0.0001). Albuminuria was common and severe in cases compared to controls (Table 1). Out of total 45 cases, 28 (62%) had urine albumin more than 1+.

**Table 1: Comparison of urine albumin between cases and controls.**

| Urine Albumin | Cases n (%) | Controls n (%) | Total n (%) |
|---------------|-------------|----------------|-------------|
| NIL           | 9 (20)      | 38 (84.4)      | 47 (52.2)   |
| TRACE         | 8 (17.8)    | 6 (13.3)       | 14 (15.6)   |
| 1+            | 22 (48.9)   | 1 (2.2)        | 23 (25.6)   |
| 2+            | 5 (11.1)    | 0 (0)          | 5 (5.6)     |
| 3+            | 1 (2.2)     | 0 (0)          | 1 (1.1)     |
| Total         | 45 (100)    | 45 (100)       | 90 (100)    |
| Chi-square value: 43.535, P value: <0.001 |

There was no statistical difference in fasting blood sugar values between the two groups. Mean FBS in cases and controls were 103.4 (38.6) mg% and 101.7 (49.7) mg% respectively. Difference in serum calcium, phosphate, alkaline phosphatase. Albumin, sodium and potassium between the two groups are shown in Table 2. Controls had significantly higher levels of serum calcium, phosphate and albumin. Comparison of FGF-23 in relation to eGFR between cases and controls was
described in Table-3. The severity of CKD increases with increase in FGF-23 levels (P value=0.014).

Table 2: Comparison of various serum biochemical parameters among cases and controls.

| Parameter                  | Cases (n=45) | Controls (n=45) | Student ‘t’ test P value |
|----------------------------|--------------|-----------------|-------------------------|
| Serum calcium (mg%)        | Mean 9.849   | 10.164          | 0.024*                  |
|                           | Standard deviation 0.779 | 0.492           |                         |
| Serum Phosphate (mg%)      | Mean 5.064   | 4.196           | <0.001*                 |
|                           | Standard deviation 1.1786 | 0.8331          |                         |
| Serum Alkaline phosphatase (U/L) | Mean 116.58 | 74.47           | <0.001*                 |
|                           | Standard deviation 46.97 | 17.10           |                         |
| Serum albumin (gm %)       | Mean 2.967   | 3.747           | <0.001*                 |
|                           | Standard deviation 0.569 | 0.445           |                         |
| Serum Sodium (mEq/L)       | Mean 145.05  | 139.98          | <0.001*                 |
|                           | Standard deviation 6.328 | 3.588           |                         |
| Serum Potassium (mEq/L)    | Mean 3.987   | 4.282           | 0.022*                  |
|                           | Standard deviation 0.628 | 0.572           |                         |

Table 3: Comparison of FGF23 in relation to eGFR among cases and controls.

| Study group | eGFR (ml/min) | FGF-23 levels Mean | SD | 95% Confidence Interval for Mean Lower | Upper Bound | Statistical Inference |
|-------------|---------------|---------------------|----|---------------------------------------|-------------|-----------------------|
| Control (n=45) | ≥90            | 39.5                | 12.5 | 35.7                                | 43.2        | P=0.014                |
| Cases (n=45)     | 60-89 (n=4)  | 196.7               | 128.4 | -7.5                                | 400.9       |                      |
|                 | 30-59 (n=6)  | 447.4               | 420.9 | 5.7                                 | 889.1       |                      |
|                 | 15-29 (n=16) | 726.4               | 421.3 | 501.9                                | 950.9       |                      |
|                 | <15 (n=19)   | 936.2               | 508.8 | 691                                 | 1181.5      |                      |

Table 4: Pearson correlation matrix between serum FGF-23 and various parameters among cases and controls.

| Parameters            | Cases (n=45) | Controls (n=45) |
|-----------------------|--------------|-----------------|
|                       | Pearson Correlation | P value | Pearson Correlation | P value |
| eGFR                  | -0.484       | 0.001*          | -0.048               | 0.752   |
| Total Kidney volume   | -0.046       | 0.765           | -0.010               | 0.948   |
| Serum Sodium          | 0.171        | 0.262           | 0.260                | 0.084   |
| Serum Potassium       | -0.104       | 0.495           | -0.147               | 0.337   |
| Blood urea            | -0.005       | 0.972           | -0.049               | 0.750   |
| Serum creatinine      | 0.416        | 0.004*          | 0.090                | 0.556   |
| Serum calcium         | 0.035        | 0.822           | 0.050                | 0.743   |
| Serum phosphate       | 0.348        | 0.019*          | 0.200                | 0.187   |
| Serum alkaline phosphatase | 0.063   | 0.682           | 0.071                | 0.643   |
| Serum albumin         | -0.342       | 0.022*          | 0.234                | 0.121   |
| Serum uric acid       | 0.054        | 0.727           | 0.054                | 0.727   |
| Urine albumin         | 0.291        | 0.052           | -0.145               | 0.341   |

Correlation between FGF-23 and various parameters among cases and controls is shown in Table 4. Among cases, Pearson correlation between serum FGF-23 levels and eGFR, serum albumin was statistically significant and had a negative inverse correlation i.e. rise in serum FGF-23 levels had a corresponding fall in eGFR and serum albumin. Among cases, correlation between serum FGF-23 levels and serum creatinine, serum phosphate was statistically significant and had a positive direct correlation.

**DISCUSSION**

In the present study serum FGF23 concentrations were found to be significantly increased in patients with CKD compared to the control group. When patients in different
stages of CKD were compared, serum FGF23 levels were found to be progressively increased from stage 2 to stage 5 in comparison with the control group. Serum FGF23 levels were inversely correlated with eGFR. This observation showed that increase in serum FGF23 develops relatively in the early stages of CKD. These findings are in accordance with the study of Larsson et al which reported a progressive increase in serum FGF23 levels over the spectrum of CKD63. Also, a strong negative correlation was observed between serum FGF23 and eGFR levels (r=−0.484, P<0.001 significant) which shows that serum FGF23 levels increase with decreasing eGFR levels; similar to findings reported by Takayuki Hamano et al.

The higher levels of serum FGF23 in CKD may be due to hyperphosphatemia, or a novel molecule that stimulates FGF23 secretion or due to low Klotho expression states as in Klotho deficiency.6 The mineral metabolic changes in CKD are described as CKD-MBD which includes hyperphosphatemia, hypercalcemia, increased PTH levels and low calcitriol levels.7 The severity of CKD-MBD is linked with an increased mortality in these patients.

FGF23 acts on FGF-R-Klotho receptor complex to cause phosphaturia and decreases calcitriol synthesis. Both high phosphate and 1, 25-dihydroxy Vitamin D causes stimulation of FGF23 production. So, lack of FGF23 leads to hyperphosphatemia and high calcitriol levels with a risk of extraosseous calcification.8

In relation to FGF23, PTH directly modifies phosphate and calcitriol levels which eventually leads to an effect on FGF23 secretion. Also, serum FGF23 levels increase early in the course of CKD even before PTH is increased. In CKD, as there is a progressive loss of nephrons, both PTH and FGF23 becomes non-operative and it is then, serum phosphate levels begin to rise. When FGF23 is elevated, it indicates an already existing inadequate phosphate control.9

Serum FGF23 levels increase much earlier in the course of CKD. Therefore, FGF23 would be a more reliable indicator of phosphate burden rather than a single measurement of serum phosphate. As of now, creatinine-based estimations of GFR, used for the assessment of kidney function do not always predict CKD at an early stage.10

These results of the present study are in accordance with that of the previous studies suggesting increased serum FGF23 levels which occur even in early stages of CKD even before a rise in serum phosphate. Serum phosphate begins to rise from stage 4 of CKD (Mean 5.11±1.088 SD), whereas serum FGF23 levels start increasing as early as Stage 2 of CKD (Mean 196.7±128.4 SD).

Among cases, Pearson correlation matrix between serum FGF-23 levels and serum phosphate were statistically significant and had a positive direct correlation i.e. Rise in serum FGF-23 levels had a corresponding rise in serum phosphate (r=0.348, P value=0.019). These results are similar to the work done by Thomas J Weber et al.11

Phosphate, even in the upper limit of normal range, is a potential toxin in CKD patients leading to adverse outcome with increased cardiovascular morbidity and mortality.12 Excessive dietary phosphate directly impairs renal function by inflicting tubulointerstitial damage.13 High phosphate forms insoluble crystals in the tubular fluid together with calcium, and these crystals lead to tubular injury and progression of CKD. Calcium-phosphate crystals also affect vascular smooth muscle cell function and cause vascular calcification.14

Taken together the results of the present study suggest that serum FGF23 is an early marker of progression of CKD and its increased levels in serum helps in the early identification of CKD-MBD in patients with CKD. The study also suggests that higher the level of serum FGF23, higher is the severity of CKD. Hence serum FGF23 could be considered as an early marker of progression in CKD patients and in the prevention of complications such as CKD-MBD in the initial stages of CKD.

CONCLUSION

The present study demonstrated that serum FGF23 levels were significantly increased in patients with CKD. This increase in serum FGF23 levels were progressive from the early stages to the late stages of CKD.

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