**Original Research Article**

# A study on FGF23 among chronic renal failure patients in Perambluar District

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## Abstract

**Background:** Fibroblast growth factor 23 (FGF23) is a phosphate-regulating hormone primarily secreted by osteocytes. Levels of FGF23 increase as kidney function declines as a physiologic response to maintain normal serum phosphate levels and neutral phosphate balance. Although FGF23 helps to prevent hyperphosphatemia, elevated circulating levels are independently associated with vascular dysfunction, left ventricular hypertrophy, increased risk for ESRD, and death in patients with CKD.

**Aim of the study:** To evaluate the FGF23 and eGFR levels in chronic kidney disease patients to compare them with healthy controls.

**Materials and methods:** Totally 100 patients were included in the study. The study was conducted from June 2018 – November 2018 over a period of 6 months at Nephrology department of DSMCH, Perambalur. Group – I (50) who were in CKD stage - IV. Group - II (50) healthy controls were included in the study. Fibroblast growth factor 23 (FGF23) was estimated by standard techniques and results are analyzed accordingly.

**Results:** The mean value of FGF23 in Group – I was 730.7 ± 492.72 pg/ml was higher than that of the Group – II whose mean value was 39.49 ± 12.47 pg/ml and this difference was statistically significant( $p<0.05$). Group – I had very low mean eGFR levels than Group - II and this difference was statistically significant.

**Conclusion:** Higher FGF23 levels are independently associated with higher levels of inflammatory markers in patients with CKD and with significantly greater odds of severe inflammation. Future studies should evaluate whether inflammation modifies the association between FGF23 and adverse outcomes in CKD.

**Key words**

FGF23, CKD, GFR, Risk for Cardiovascular Disease.

## Introduction

The mortality rates in patients with chronic renal failure are higher than the others. Around 50% of these patients die as a result of cardiovascular disease conditions such as pulmonary edema, chronic heart failure, fatal arrhythmias, pericarditis, valvular disease, and cardiogenic shock are some of the cardiovascular disorders (CVD), as the leading cause of death in these patients [1]. This can be probably due to a higher prevalence of important risk factors such as hypertension, anemia, uremia, disturbances in calcium, phosphorous and sodium, balance, dyslipidemia, atherosclerosis, diabetes mellitus, and smoking [2]. Chronic kidney disease (CKD) has been known as a serious condition which has a significant complication.FGF23 is a 32 kDa protein, predominantly expressed in osteoblasts and osteocytes but also expressed in several other tissues including the spleen, thymus, heart, lung, and muscle [5, 22] and it is a member of the fibroblast growth factors (FGFs) family [3]. While some FGFs (FGF11-14) function as intracellular signaling molecules, the most signal in an autocrine/paracrine manner, except FGF15/19, 21 and 23 that function as endocrine hormones [4]. Early in the course of CKD, fibroblast growth factor 23 (FGF23), a bone-derived protein, is elevated. FGF23 increases renal P excretion by down-regulating the sodium phosphate cotransporter in the proximal tubules, thereby, at least in early CKD, increasing P excretion [5]. It also suppresses 1α-hydroxylase activity and reduces the production of 1,25-dihydroxy vitamin D [1,25(OH)2D] [11]. Since vitamin D is thought to be a pleiotropic hormone with multiple cardioprotective, anti-inflammatory and immunomodulatory properties, the beneficial phosphaturic effects of FGF23 would appear to come with a 'trade-off' of potentially detrimental effects of suppressing 1,25(OH)2D production [6]. Importantly, FGF23 is not simply a biomarker but is biologically active and has been independently associated with left ventricular hypertrophy, impaired left ventricular function, vascular calcification, heart failure, CKD progression and mortality [7] in adult CKD patients. FGF23 acts via its obligate co-receptor klotho, a membrane-bound protein that is expressed in the kidneys and parathyroid glands [8]. Defects in either FGF23 or klotho cause a combination of metabolic disturbances, including hyperphosphatemia, hypercalcemia, and hypervitaminosis D. To date, the actions of klotho in CKD have focused on its role as a cofactor for FGF23 signaling in regulating P and vitamin D metabolism [9].

## Materials and methods

Totally 100 patients were included in the study. The study was conducted from June 2018 – November 2018 over a period of 6 months at Nephrology department of DSMCH, Perambalur. Group –I (50) who were in CKD stage - IV. Group-II (50) healthy controls were included in the study. Fibroblast growth factor 23 (FGF23) was estimated by standard techniques and results were analyzed accordingly.

**Inclusion criteria**

- Patients with an established diagnosis of CKD.
- Age between 20 – 75 years.

**Exclusion criteria**

- 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.
- Patients who underwent renal transplantation.

Informed consent was obtained from all subjects prior to the study. Under aseptic precautions, 5ml 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 2000rpm for 15 minutes for separation of serum. An aliquot of the serum was taken for the estimation of FGF23 and stored at -20°C in the deep freezer.

**Estimation of serum fibroblast growth factor 23 (FGF23)**

**Method:** Enzyme Immuno Assay.

**Principle:** The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human fibroblast growth factor 23(FGF23) in samples. The human FGF-23monoclonal antibody is precoated onto a microtiter plate. After a blocking step and incubation of the plate with an anti-FGF23 antibody, Biotinylated FGF23 and standard FGF23 (or) samples are added to all wells. There is a competitive binding between biotinylated FGF23 and standard (or) serum FGF23 with anti-FGF23 antibody. Streptavidin-Horseradish Peroxidase (HRP) was added to the well which reacts with the uncompleted (or) free biotinylated FGF23 to produce a color. The Intensity of the color is directly proportional to the number of biotinylated FGF23 and inversely proportional to the amount of FGF23 peptide in the standard or sample. The concentration of FGF23 in the serum is calculated from a standard curve of different FGF23 concentrations accordingly.

**eGFR calculation using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation**

eGFR = 141 x min $(SCr/k,1)^{\alpha}$ x max $(SCr/k,1)^{-1.209}$ x $0.993^{Age}$.

1.multiply by 1.018 for females.
2.multiply by 1.159 for blacks.
3.SCr - serum creatinine (mg/dL).
4.k is 0.7 for females and 0.9 for males.
5.α is -0.329 for females and -0.411 for males.
6.min indicates the minimum of SCr/k or 1.
7.max indicates the maximum of SCr/k or 1

## Results

The age group of cases ranged from 34-75 years with a mean age of 3.9±10.8 years and the median age of 55 years. The age group of controls ranged from 25-65 years, with the mean age of 46.4±9.6 years and the median age of 46 years. The Chi-square value was 7.715 and the p value between cases and controls was 0.103 and so the difference in the age distribution of cases and controls was not statistically significant and hence they are comparable (**Table – 1**).

**Table - 1:** Age wise distribution of the study group (n=100).

| Age group | Cases n (%) | Controls n (%) | Total n (%) |
|---|---|---|---|
| 21- 30 years | 0 (0) | 3 (6.7) | 3 (3.3) |
| 31 - 40 years | 8 (17.8) | 11 (24.4) | 19 (21.1) |
| 41 – 50 years | 12 (26.7) | 17 (37.8) | 29 (32.2) |
| 51 – 60 years | 15 (26.7) | 8 (17.8) | 23 (22.2) |
| Above 60 years | 20 (28.9) | 6 (13.3) | 26(21.1) |
| Total | 50 (100) | 50 (100) | 100 (100) |

**Table – 2:** Comparison of serum FGF23 levels in the study group (n=100).

| Serum FGF-23 | Cases (n=50) | Controls (n=50) | Student "t" Test p Value |
|---|---|---|---|
| Mean | 730.70 | 39.49 | <0.001* |
| Standard deviation | 492.72 | 12.47 | |

***Significant at 0.05 level**

**Table – 3:** Comparison of eGFR levels among cases and controls (n=100).

| eGFR levels | Cases (n=45) | Controls (n=45) | Student "t" test p value |
|---|---|---|---|
| Mean | 23.769 | 113.80 | <0.001* |
| SD | 16.97 | 10.21 | |

*** Significant at 0.05 level**

The mean value of FGF23 in cases was 730.7 ± 492.72 pg/ml was higher than that of the control group whose mean value was 39.49 ± 12.47 pg/ml and this difference was statistically significant ( $p<0.05$) as per **Table - 2**.

Cases had very low mean eGFR levels than controls and this difference was statistically significant as per **Table – 3**.

## Discussion

Vitamin D and PTH have traditionally believed as the main factors in bone and mineral hemostasis, however, recent findings regarding FGF23 and bone-kidney have produced a new point of view in CKD and mineral bone disease [10]. The most important function of vitamin D and PTH is stabilization of serum calcium levels in a suitable range through induction of 1,25-dihydroxy vitamin D [1,25 (OH)2 D3] synthesis, leading to reabsorption of calcium and reducing urinary excretion of calcium by kidneys [11]. On the other hand, PTH also increases calcium uptake from bones. PTH induces production of 1, 25 $(OH)_2 D_3$ through increasing CYP27b1, increasing reuptake of calcium in proximal tubules and in distal tubules by regulation of TRPV5 [12]. Furthermore, PTH increases the uptake of calcium and phosphate in bones through inducing RANKL by osteoblasts (which in turn stimulate osteoclasts that increase uptake from bones). Additionally, an increase of 1,25 $(OH)_2 D_3$ production by kidneys, causes an increase in phosphate and calcium reabsorption by the small intestine [13]. Overall, uptake of calcium from bones decreases in urinary excretion of calcium and increases in its absorption by intestine results to normalizing serum calcium levels [14]. Increasing the uptake of phosphate from bones and by intestines is regulated by PTH and reduces the reuptake of phosphate in renal tubules to balance the levels of phosphate. In fact, FGF23 and bone-kidney axis act as a biological route which has recently been discovered as the main players in CKD-mineral and bone disorder (CKD-MBD) [15]. Recent findings showed that osteoblasts and osteocytes are important sites for FGF23 production. FGF23 is excreted by bone, targets kidneys, and regulates phosphate and also vitamin D metabolism. In the present study serum, FGF23 concentrations were found to be significantly increased in patients with CKD (mean 730.70 ± 492.72) when compared to the control group (mean 39.49 ± 12.47) [16]. 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 an increase in serum FGF23 develops relatively in the early stages of CKD [17]. Two opposing hypotheses were raised: either kidney injury itself may induce the production of a—yet to be defined—factor that stimulates FGF-23 section (or alternatively, decreased the production of a tonic inhibitor). This hypothesis is partly supported by animal data that demonstrate an early increase in FGF-23 after kidney injury [18]. Alternatively, increased levels of FGF-23 may reflect dampened Klotho expression in target organs. In line with this hypothesis, decreased renal Klotho expression has been detected in kidney biopsy studies [19]. As FGF-23 requires Klotho for its phosphate-regulating effects in kidneys and in parathyroid glands, higher FGF-23 might physiologically be needed to overcome Klotho deficiency and to maintain normophosphataemia in states of low Klotho expression. If this second hypothesis held true, hyperphosphataemia and hypervitaminosis D should at least transiently

occur in early CKD as a reflection of such initial Klotho deficiency. Of note, large epidemiological data stand against this hypothesis, as low rather than high serum phosphate values are found in very early CKD. As FGF-23 itself down-regulates Klotho expression, Klotho deficiency in early CKD might thus reflect a primary increase in FGF-23 [20].

## Conclusion

FGF23 is involved in the regulation of calcium-phosphate metabolism and thus it is also involved in the mineral metabolic disorders implicated in CKD-MBD. Serum FGF23 may be considered as an early marker of progression of CKD. Higher the serum FGF23 levels in CKD, more severe is the disease.

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