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Original Research Article

Association of fibroblast growth factor-23 among chronic kidney disease patients with mineral bone disease

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ABSTRACT

Background: Chronic kidney disease (CKD) is characterized by slow and progressive loss of kidney function over years. Most of the time it goes undetected till severe irreparable damage has occurred. CKD is a worldwide public health problem.
Methods: The present cross sectional study was conducted in nephrology outpatient department (OPD) and medicine wards of Government Medical College and Hospital, Ambikapur, Sarguja, Chhattisgarh, India from June 2020 to June 2021. The study involved 50 adult patients (>18 years) of CKD who were on hemodialysis. Patients who are not willing to participate and comatose patients were excluded from the study.
Results: Among the CKD patients, 10 (20%) had left ventricular hypertrophy, 5 (10%) patients had left ventricular dysfunction with ejection fraction (EF) less than 40. 98% of the study participants were undergoing twice weekly hemodialysis and 2% were on thrice weekly hemodialysis. No significant association was found between left ventricular hypertrophy (LVH), pulmonary arterial hypertension (PAH), left ventricular dysfunction (LVD) with fibroblast growth factor-23 (FGF-23) ($p>0.05$) whereas there was a significant association between phosphate and FGF-23 ($p<0.05$) and between calcium and FGF-23 ($p<0.05$).
Conclusions: CKD mineral and bone metabolism (MBD) is common in patients with CKD especially stage 5. They are also prone for cardiovascular manifestation. FGF23 levels were high in those with elevated phosphorous, left ventricular hypertrophy and left ventricular dysfunction. Levels of calcium, intact parathyroid hormone (iPTH) and ejection fraction did not correlate with FGF-23. Larger population in the study would have helped in actual identification of these patients and also helped in the knowing the predominant role of FGF-23.

Keywords: Chronic kidney disease, CKD-mineral bone disorder, Ejection fraction, Fibroblast, Growth factor-23

INTRODUCTION

Chronic kidney disease (CKD) is characterized by slow and progressive loss of kidney function over years. Most of the time it goes undetected till severe irreparable damage has occurred. CKD is a worldwide public health problem. There is rising incidence and prevalence of patients with kidney failure which is usually associated with poor outcomes. The cost of treatment of this disease is very expensive. It is prevalent in elderly population. Over the age of 65 years, about 30 percent of patients have a stable kidney disease.[1]

While young patients are found to have early progressive loss of renal function. Whatever the underlying etiology, once the loss of nephrons and reduction of functional renal mass reaches a certain point. The remaining nephrons begin a process of irreversible sclerosis that leads to a progressive reduction in the glomerular filtration rate (GFR).[2]

CKD has been staged by kidney disease: improving global outcome (KDIGO) 2012 according to GFR and albuminuria. This essentially helps to treat and control the disease according at various stages. By CKD stages 3 and 4, changes in the body occurs affecting the bone and mineral metabolism. This syndrome has been named 'CKD-mineral bone disorder' (CKD-MBD). The prevalence of various mineral bone disease abnormalities were 70% hyperphosphatemia, 85% hyperparathyroidism, and 100% low levels of 25(OH)D among the patients. But the earliest histological abnormalities of bone in CKD-MBD are seen after a relatively mild reduction in GFR in CKD stage l2.[3]

By CKD stage 5, skeletal abnormalities are found in all patients. It is one of the dreaded complication and if not treated in time can lead to increased morbidity and mortality. Disorders of bone and mineral homeostasis are also recognized to play an important role in cardiovascular complication of CKD. The current understanding of the initial mechanisms involved in the pathogenesis of CKD-MBD is focused on very early rise in the skeletal hormone, fibroblast growth factor-23 (FGF-23), as a sign of disturbed skeletal function, loss of skeletal anabolism, hyperphosphatemia, reduced calcitriol and secondary hyperparathyroidism.

In the clinical practice, we should monitor calcium, phosphorous, vitamin D level, parathormone and FGF-23 status of our dialysis. This prevents further complications. The advances in the basic science of these serum factors and their interactions and changes in CKD stage 5 are a source of great scientific interest. Understanding the changes in the levels of mentioned parameters will go a long way to improve the health and decrease the morbidity and mortality associated with it.

In the present study, the aim was to find out the status of mineral bone disease in CKD patients in relation to FGF-23, Ca^{2+}, phosphorus and intact parathormone (iPTH) in patients and to correlate with left ventricle (LV) function with the help of echocardiographic findings.

METHODS

The present cross sectional study was conducted in nephrology OPD and medicine wards of Government Medical College and Hospital, Ambikapur, Sarguja, Chhattisgarh, India from June 2020 to June 2021. The study involved 50 adult patients (>18 years) of CKD who were on hemodialysis. Patients who are not willing to participate and comatose patients were excluded from the study. Ethical consideration was made through Institutional ethical committee and informed consent was taken from the subjects prior to study.

Study procedure

The kit uses 2 affinity-purified goat antibodies that bind at the carboxy terminal portion of fibroblast growth factor 23 (FGF-23). One antibody is coated onto microtiter wells and the other is biotinylated. Horseradish peroxide conjugated to avidin and 3.3', 5,5'-tetramethylbenzidine (TMB) substrate provide the colored product, which is read in a microtiter plate spectrophotometer. Ca^{2+} ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. The change in absorbance is directly proportional to the Ca^{2+} concentration and is measured photometrically.

The method is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate (without reduction). The addition of an accelerator gives rise to a more rapid rate of reaction.

Sample collection and storage

We will take 5 ml of blood: fasting-overnight (12-14 hours); serum gel tubes should be centrifuged within 2 hours of collection; and red-top tubes should be centrifuged and aliquoted within 2 hours of collection.

Data was recorded in Microsoft excel and checked for its completeness and correctness then it was analysed by using suitable statistical software and p value <0.05 was considered as a statistically significant.

RESULTS

The mean±standard deviation (SD) age of study participants was 49.80±12.03 year's ranges from 20 to 70 years. Male female ratio was 4:1. 16 (32%) of the study subjects had peripheral vascular diseases. Among the CKD patients, 10 (20%) had left ventricular hypertrophy, 5 (10%) patients had LVD with ejection fraction (EF) less than 40. 98% of the study participants were undergoing twice weekly hemodialysis and 2% were on thrice weekly hemodialysis (Table 1).

Among these patients, majority of them had hypocalcemia (64%). Mean calcium level was 8.172 mg/dl and a SD of 873 mg/dl. Mean phosphate level was 6.91 mg/dl with a SD of 1.978 mg/dl. Elevation of parathormone levels were prevalent in study subjects (46%).

Mean level parathyroid hormone level was 93.2 pg/dl and SD of 43.8. 36% of the patient had EF less than 50 and 64% had EF in the range between 50-70. Distribution of FGF-23 in the study population ranges from 19 to 1836.86. Mean FGF-23 was 279.57 pg/ml and a standard deviation of 347.088pg/ml. Among the 50 CKD subjects majority of them had elevated FGF-23 levels (74%) (Table 2).

No significant association was found between LVH, PAH, LVD with FGF-23 ($p>0.05$) whereas there was a significant association between phosphate and FGF-23 ($p<0.05$) and between calcium and FGF-23 ($p<0.05$) (Table 4).

Table 1: Patients profile and history.

Variable	No. (%)
Age group	
20-39	9 (18)
40-59	28 (56)
60-79	13 (26)
Gender	
Male	40 (80)
Female	10 (20)
Peripheral vascular diseases	
Yes	16 (32)
No	34 (68)
CVA	
Yes	14 (28)
No	36 (72)
Frequency of HD	
Thrice weekly	1 (2)
Twice weekly	49 (98)
LVH	
Yes	10 (20)
No	40 (80)
PAH	
Yes	5 (10)
No	45 (90)
LVD	
Yes	5 (10)
No	45 (90)
Total	50 (100)

Table 2: Biochemical parameters of the study subjects.

Variable	No. (%)
Uric acid	
Hypourecemia	4 (8)
Normal	27 (54)
Hyperurecemia	19 (38)
Potassium	
Normal	9 (18)
Hyperkalemia	41 (82)
HCO_3	
Metabolic acidosis	38 (76)
Normal	12 (24)
Calcium	
Hypocalcemia	32 (64)
Normal	18 (36)
Phosphate	
Normal	6 (12)
Hyperphosphatemia	44 (88)
iPTH	
Hypoparathyroid	1 (2)
Normal	26 (52)
Hyperparathyroid	23 (46)
EF	
<50	18 (36)
50-70	32 (64)

Continued.

Variable	No. (%)
>70	0 (0)
FGF-23	
Elevated	37 (74)
Normal	13 (26)
Total	50 (100)

Table 3: Distribution of biochemical parameters.

Variable	Mean	SD	Minimum	Maximum
Urea	126.9	29.1	68	192
Creatinine	10.87	3.39	4.2	18.8
Potassium	5.47	0.64	3.7	6.7
HCO_3	18.6	3.73	9	26
Uric acid	6.56	1.92	2	10
Calcium	8.17	0.87	6	9.6
Phosphorus	6.91	1.98	2.5	11.7
ALP	140.48	40.01	84	230
iPTH	93.24	48.89	12.2	256.6
Hb	8.67	1.89	4.6	14.6
EF	51.54	9.52	27	66
FGF-23	279.57	347.08	19	1836.9

Table 4: Association of phosphate, calcium, LVH, PAH and LVD with FGF-23.

Variables	FGF-23 (%)		Total	Chi-square, df, p value
	Elevated	Normal		
LVH				
Yes	10 (100)	0 (0.0)	10	2.865, 1, 0.090, insignificant
No	27 (67.5)	13 (32.5)	40	
PAH				
Yes	5 (100)	0 (0)	5	0.739, 1, 0.389, insignificant
No	32 (71.1)	13 (28.9)	45	
LVD				
Yes	5 (100)	0 (0)	5	0.739, 1, 0.389, insignificant
No	32 (71.1)	13 (28.9)	45	
Phosphate				
Normal	1 (16.7)	5 (83.3)	6	8.509, 1, 0.003, significant
Hyperphosphatemia	36 (81.8)	8 (18.2)	44	
Calcium				
Normal	11 (61.1)	7 (38.9)	18	4.563, 1, 0.032, significant
Hypocalcemia	29 (90.6)	3 (9.4)	32	
Total	37	13	50	

DISCUSSION

The prevalence of CKD in the southern part of India has been found to be on the increase. The number of patients on renal replacement therapy has also been on the rise. In this study, 50 patients were selected after taking informed consent. All of them were in the CKD stage 5. Though the present study was done, to find out the status of mineral bone disease in patients with CKD irrespective of the different stages of CKD, importance was given to FGF-23, along with other parameters like phosphorous, calcium and iPTH. FGF-23 was selected with the intention of identifying an early biomarker for mineral bone disease in the patients selected for the study. This was also used to study relationship with the cardiovascular system.

At present, no Indian studies have shown its relevance in the status of CKD MBD. The previous studies already done, have not been able to strongly correlate FGF-23 with the various parameters like calcium, phosphorous, parathormone etc.

The age group of study patients ranged from 20 years to 70 years. Here more than 50% were in the age group of 40–59 years. In these 80% were male and essentially the bread winners of the family. All the patients taken up for

the study were uniform in the etiology of CKD and had both diabetes and hypertension. Taking into account the duration of hypertension, out of 50 patients, majority of the patients, 26 (52%) had hypertension for more than 5 years. Similarly 23 subjects were found to have DM for less than 5 years while 27 subjects were found to have DM for more than 5 years.

It the present study, 28% of the patients had previous history of cerebrovascular accidents. It was also observed that 32% had peripheral vascular disease. There was no correlation between gender and cerebrovascular accidents and peripheral vascular disease. This was done to note any disease of major blood vessels as CKD patients are at risk for the same.

Our study shows that these patients are at high risk for CVA (28%) and PVD (32%). This correlates with other studies viz. Iseki and Fukiyama found that the risk of stroke was five times higher in CKD patients than general population.[4,5] FGF-23 level was increased in all study subjects who were having PVD. Similarly Batra et al found that increase in FGF-23 has increased thickness of arterial wall which lead to PVD. Highlights of our study showed correlation of CVA around (28%) in our patients as well.[6]

Study of FGF-23 is a relatively new biomarker in the early detection of CKD MBD. It had been used to study and correlate the involvement in mineral bone disease in CKD patients. So far its correlation with different stages of kidney damages have not been strong. Diniz and Russo in their studies found that increases in FGF-23 was associated with earliest abnormality of mineral metabolism in CKD patients. Similarly this study, showed 64% had hypocalcaemia. Significant association was found between increased levels of FGF-23 and hypocalcaemia which indicates mineral bone disease in this study group.[7,8]

The distribution of haemoglobin and various biochemical parameters were observed along with FGF-23. The levels of FGF-23 ranged from 19 to 1836.86 pg/ml with mean FGF-23 was 279.57 pg/ml and a standard deviation of 347.088 pg/ml. Only two patients had values more than 1000 pg/ml and 2 above 500 pg/ml. One patient had phosphorus 11.7 mg/dl, with serum calcium was 8 mg/dl and iPTH was 146 pg/ml. It is known that only when compensatory mechanisms to prevent phosphorous elevation fails, that phosphorous starts to rise. Here FGF-23 was low and its value was 123 pg/ml. The lowest recorded FGF-23 was 19 pg/ml. This patient had phosphorous of 3.6 mg/dl and calcium 6.6 mg/dl. The highest level of FGF-23 1836.86 pg/ml was noted. This patient had phosphorous of 3.3 mg/dl and calcium 8.8 mg/dl. Mean serum phosphorous level was 6.91 mg/dl with a standard deviation of 1.978 mg/dl. It ranged from 2.5 to 11.7 mg/dl. There was positive correlation with elevated FGF-23 and elevated phosphorous with p value of 0.003. The highest phosphorous noted was 11.7 mg/dl. But this did not hold true when different ranges of phosphorous was considered. This shows that probably FGF-23 increases only with increase in phosphorous. Mean serum phosphorous level was 6.91 mg/dl with a standard deviation of 1.978 mg/dl. It ranged from 2.5 to 11.7 mg/dl. The correlation was statistically significant when phosphorous levels were high.

The iPTH value ranged from 12.2 pg/ml to 256.6 pg/ml with mean of 93.238- 48.885 pg/ml. The levels of FGF23 did not correlate with the levels of iPTH either. The blood urea level and serum creatinine levels ranged from 68 mg/dl to 193 mg/dl and 4.2 mg/dl to 18.8 mg/dl respectively. The mean values were 126.9 mg/dl with standard deviation of 29.104 mg/dl for urea, and mean of 10.874 mg/dl with standard deviation of 3.393 mg/dl for creatinine was observed in these patients. Haemoglobin ranged from 4.6 gm% to 14.6 gm% with mean of 8.67±1.888 gm%. The association with FGF-23 was studied in particular. There was no correlation with the level of haemoglobin.

To study the relationship between FGF-23 and cardiovascular disease, assessment was done using left ventricular hypertrophy and left ventricular systolic dysfunction. The number of patients having LVH were 10 (20%). LVH did not have correlation with age, gender, or vintage of dialysis. The LVH varied with different levels of FGF-23. 10 out of 50 patients had LV dysfunction. Their EF varied from minimum of 27 and maximum of 66. In the study low EF, had shown significant association with high FGF-23 with p value of 0.025. This correlated with different levels of FGF-23. Thus the FGF-23 was positively associated with LVH and different levels of EF.

The present study shows that among the 50 participants, 10 (20%) had left ventricular hypertrophy and FGF-23 level was elevated in all patients with left ventricular hypertrophy. This is similar to study by Amaral et al. The FGF-23 levels and rates of LVH were elevated in CKD and that elevated FGF-23 was independently associated with LVH.[9]

CONCLUSION

CKD mineral bone disorder varies from patient to patient. Regular monitoring of calcium, phosphorus, iPTH is required. Individualized therapy is essential in the management of CKD - MBD. FGF-23 can be utilized as a biomarker not only for identifying bone disease but also for cardiovascular disease as well. Recommended that measurements of FGF-23 may be used for regular monitoring of CKD. With regular monitoring of all patients with CKD, the cost of the investigation should come down and long term expenditure incurred on the patients can be brought down.

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