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Fibroblast Growth Factor 23 Measurement in the Serum of Patients on Hemodialysis in Erbil Kidney Diseases and Dialysis Department

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Abstract

Background and objectives: Fibroblast growth factor 23 and para thyroid hormone in serum rise early during the course of chronic kidney disease in parallel with a decline in vitamin D. Elevations in fibroblast growth factor23 levels are one of the earliest manifestations of disordered bone-mineral metabolism in chronic kidney disease. Our aim was to assess the serum level of fibroblast growth factor23 among hemodialysis patients and comparing them with control group, and their relationships with parathyroid hormone, phosphate and calcium. **Methods:** This is a case – control design where 80 patients of end stage renal disease on regular hemodialysis were enrolled. While the 80 control participants were relatives of the patients who had no medical illnesses. Serum calcium, phosphorus, parathyroid hormone, and fibroblast growth factor23 levels were measured for both groups. **Results:** Diabetes mellitus was the most prevalent etiological factor for development of end stage renal disease (41.25%), hypertension was contributing to 23.75% of end stage renal disease cases. We found that whenever the value of parathyroid hormone, phosphate or calcium increase the level of fibroblast growth factor23 increases too, this increase was statistically significant and this finding was clear among all study samples including control group and also among patients group separately. **Conclusions:** Sufficient hemodialysis has an important role in serum measures particularly parathyroid hormone, calcium, and phosphate and fibroblast growth factor 23.

Keywords: Fibroblast Growth Factor 23; Phosphate; Calcium; Parathyroid hormone, End stage renal disease.

Introduction

The kidney is known as the major regulator of phosphate homeostasis[1]. The main site of phosphate reabsorption is the proximal tubule[2], were phosphate ions enter from the tubular lumen across the apical brush-border membrane (BBM) and leave across the basolateral membrane[1]. To date, three distinct transporters have been identified to be expressed in the BBM of the proximal tubule cells: NaPi-IIa, NaPi-IIc and PiT-2[3]. NaPi-IIa and PiT-2 mediate the electrogenic transport of phosphate coupled to three and two Na+ respectively, whereas NaPi- IIc transports phosphate together with two Na+ in an electroneutral fashion [1-4]. A plethora of factors affects renal phosphate reabsorption, among them PTH, dopamine, dietary phosphate intake, glucocorticoids, acid-base status, growth factors, insulin and Fibroblast Growth Factor 23 (FGF23)[1-3]. FGF23 is a 32 kDa (kilo Dalton) protein, predominantly expressed in osteoblasts and osteocytes[5–13], but also expressed in several other tissues including the spleen, thymus, heart, lung, and muscle[4-15] and it is a member of the fibroblast growth factors (FGFs) family[5-6, 8]. While some FGFs (FGF[11-14]) function as intracellular signaling molecules, most signal in an autocrine/paracrine manner[6, 16], except FGF15/19, 21 and 23 that function as endocrine hormones[8,14]. A unique structural feature of these endocrine FGFs is their lack of a heparin- binding domain that is conserved in all autocrine/ paracrine FGFs.

PTH and FGF23 in serum rise early during the course of chronic kidney disease (CKD) in parallel with a decline in vitamin D. Known causes of these hormonal changes include alterations in circulating calcium and phosphate concentrations and inadequate production of vitamin D by the failing kidneys[12, 17]. Numerous epidemiological studies have reported a robust association between higher plasma FGF23 concentrations and poor patient outcome in CKD populations[15], because it leads to progression to end stage renal disease (ESRD), cardiovascular disease and death[13]. Elevations in FGF23 levels are one of the

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earliest manifestations of disordered bone-mineral metabolism in CKD, with deteriorating renal function; even before phosphate and PTH concentrations had become abnormal[14–15, 18]. PTH secretion is predominantly regulated by the calcium-sensing receptor (CASR) located in the parathyroid gland, which responds to decrements in serum ionized calcium by increasing secretion of PTH[25]. PTH targets PTHR1 G protein-coupled receptors that are highly expressed in the renal tubules and osteoblasts/ osteocytes in bone[1, 19]. PTH acts to increase serum calcium by stimulating osteoclasts to resorb bone; in addition and similarly to FGF23 it inhibits renal phosphate reabsorption by decreasing NaPi-IIa, NaPi-IIc and Pit2 expression[20]. The aim of this study was to assess the serum level of fibroblast growth factor 23 among hemodialysis patients and comparing them with control group, and their relationships with parathyroid hormone, phosphate and calcium.

Patients and methods

This study was planned to evaluate the serum measurements of FGF-23 and relate it to different epidemiological factors, using case-control design. The 80 cases are chronic kidney disease- end stage renal disease (CKD-ESRD) patients who are on regular hemodialysis (HD) enrolled in Erbil teaching hospital-dialysis center, West Erbil hospital-dialysis center 1, West Erbil hospital-dialysis center 2 in Kurdistan region/ Iraq while the 80 control participants were relatives of the patients with negative medical illnesses. Serum measurement of FGF-23 was performed for every participant in each group for the period of February 2017 till August 2017. The cases and controls were matched for gender i.e. approximate numbers of male and females were distributed among cases and control group.

Inclusion criteria for cases were: patients with ESRD on regular scheduled HD while for control group were any patient visiting the hospital for purposes not involving renal or kidney complaint. For both groups the age was set at —18 years. There were no patients less than 18 years on regular hemodialysis in that specific center during the period of the study. Exclusion criteria for cases were agreed to be: acute kidney injury, previous renal transplantation, parathyroidectomy, pregnancy or lactation, use of calcimimetics, and medications known to affect bone metabolism such as glucocorticoids (except vitamin D), and life-threatening comorbid conditions such as, malignancy, active infection, and viral hepatitis. The reason for excluding acute renal failure cases was that all of them are ESRD cases in which stage five renal failure (GFR less than 15 ml/min) and the acute renal failure cases are excluded from the start of the study as they have not been nominated for HD. Early renal impairment also means that GFR is above the indication for putting the patients on regular HD.

Serum calcium and phosphorus levels were measured by automated techniques. PTH was measured by immunoassay method, normal range: 15 – 65 pg/ml; intra-assay coefficient of variation: < 5%. Serum intact FGF-23 levels were measured using a two-site ELISA kit, Erbil teaching hospital - Central Lab.

Data were collected and analyzed using Statistical Package for Social Sciences version 22 and the results will be compared between patients with different variables, with a statistical significance level of < 0.05. The results presented as rates, ratio, frequencies, percentages in tables and figures and analyzed using T-test, Chi square and correlational tests.

This study was submitted to the scientific council of Internal Medicine of the Kurdistan Board of Medical Specialties for scientific and ethical approval. This study was explained for each patient and a written consent was obtained from each patient or his/her guardian (escort). Confidentiality of data was totally ensured.

Results

A total of 80 cases and controls were enrolled in this study. 93 of the participants were male, 67 of them were female. The male: female ratio was 1.5:1. Among all participants, 34.4% of them were from Erbil city, 33.1% outside Erbil city yet within Erbil governorate and 32.5% of them were residing other Iraqi governorates outside Kurdistan Region. The mean ± S.D of age of participants was 48.31 ± 14.33. Among all participants 37.5% of them were at the 40-59 years age group, followed by 32.5% from 20-39 years age group, Table 1.

Table (1): Descriptive data of participants.

Variables	Categories	No.	%
Age	< 20 years	2	1.3
	20 – 39 years	52	32.5
	40 – 59 years	60	37.5
	≥ 60 years	46	28.8
Residence	Outside Erbil city	53	33.1
	Outside Kurdistan	52	32.5
	Inside Erbil city	55	34.4
	Total	160	100

There was statistically significant strong correlation between S.PTH and FGF23 i.e. when the level of S.PTH increases the FGF23 will also increase among all participants. The correlation between s. phosphate and calcium with FGF23 was weak yet statistically significant for all study sample, Table 2.

Table (2): Correlation of S.PTH, phosphate, calcium with FGF23 among all participants.

	FGF 23	
Measures	Correlation coefficient (r)	p-value
S.PTH	0.82	0.001
Phosphate	0.08	0.04
Calcium	0.20	0.008

According to Figure 1 diabetes mellitus was the most prevalent etiological factor for development of ESRD 33cases (41.25%), hypertension was 18 patients and contributing to 23.75% of ESRD cases, followed by glomerular diseases, unknown factors then cystic kidney disease.

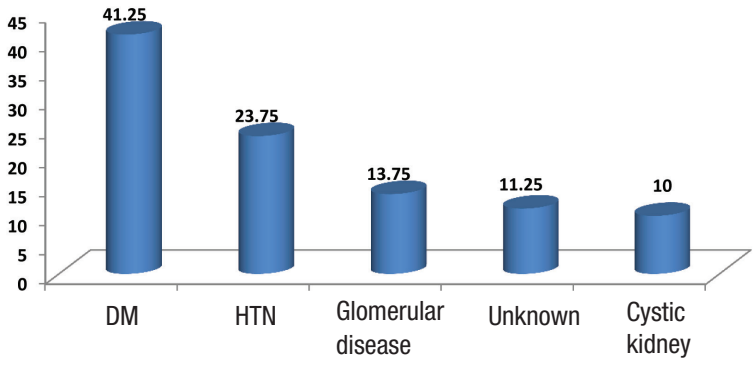

Figure (1): Prevalence of different ESRD etiologies among cases.

The data of Table 3 show significant statistical variations (differences) in all measures between cases and controls. In all conditions the cases had higher measures than control participants including FGF23, serum phosphate, calcium and PTH. T- test was done to compare between them and p-values were less than 0.05.

Table (3): Association between study groups and age and laboratory indices.

Measures	Study groups	No.	Mean	S.D	p-value	T-test
S. FGF 23	Case	80	634.6	261.3	< 0.001	Significant
	Control	80	60.6	26.5		
S. Phosphate	Case	80	6.62	1.51	< 0.001	Significant
	Control	80	3.54	0.49		
S. Calcium	Case	80	10.35	1.35	< 0.001	Significant
	Control	80	9.12	0.39		
S. PTH	Case	80	425.2	222.7	< 0.001	Significant
	Control	80	35.2	13.2		

The data of Table 4 reveal a moderate positive correlation between S.PTH and calcium with S. FGF23. It shows that when the levels of S.PTH or calcium increase the S. FGF23 will also increase among CKD patients these findings were statistically significant, while the correlation between s. phosphate and with S. FGF23 was weak negative and non-significant, which means whenever the level of s. phosphate rises the level of S. FGF23 diminishes, and this finding was statistically non-significant.

Table (4): Correlation of S.PTH, phosphate, calcium with FGF23 among CKD cases.

	S. FGF 23	
Measures	Correlation coefficient (r)	p-value
S. PTH	0.48	0.001
S. Phosphate	- 0.04	0.66
S. Calcium	0.14	0.04

The analyzed findings from Table 5 illustrate that there was a statistically significant difference between subgroups of cases regarding different laboratory and dialysis measures. In all conditions cases of more than six months had higher measures than cases of less than six months. T- test was performed to compare between the averages of the two subgroups and p-values were less than 0.05.

Table (5): Relationship between subgroups of Cases and different measures.

Measures	Sub groups	No.	Mean	S.D	p-value
FGF 23	< 6 months	40	492.7	233.1	< 0.001
	> 6 months	40	776.5	206.6	
S. Phosphate	< 6 months	40	5.86	1.22	< 0.001
	> 6 months	40	7.37	1.39	
S. Calcium	< 6 months	40	9.92	1.17	0.03
	> 6 months	40	10.7	1.39	
S. PTH	< 6 months	40	289.3	170.4	< 0.001
	> 6 months	40	561.1	183.3	
Dialysis / week	< 6 months	40	2.58	0.50	0.02
	> 6 months	40	3.00	1.03	
Hours / week	< 6 months	40	7.75	1.42	0.04
	> 6 months	40	8.85	1.86	

Discussion

This study found that diabetes mellitus alone was contributing to approximately 41% of ESRD cases. This finding is in line with results of other studies namely Cairns HS, et al which states that "in the United States, diabetes has long been the most common attributed cause of ESRD, accounting for 36% of incident cases in 1992[21].

Even in a neighboring country like Iran; Krzesinski found results close to this study "hypertension and diabetes mellitus were the most common causes of ESRD in the Fars province of Iran[22].

The results showed that ESRD patients had higher measures than control participants including FGF23, serum phosphate, calcium and PTH. There are other studies supporting this outcome; Weber et al also found "FGF23 levels are elevated in ESRD, consistent with impaired renal clearance of inactive FGF23 fragments23

The increase ESRD is in line with Larsson who stated that "in CKD, circulating FGF-23 levels gradually increase with declining renal function such that by the time patients reach end-stage renal disease, FGF23 levels can be up to 1000-fold above the normal range. The increase in FGF-23 begins at a very early stage of CKD as a physiological compensation to stabilize serum phosphate levels as the number of intact nephrons declines[24].

The study proves that the levels of FGF23 increases with time for that reason ESRD cases of more than six months have higher FGF23 levels if compared with patients of less than six months duration. This finding is supported by other studies in particular Nakanishi and Kazama who observed that "serum FGF23 levels are progressively increased as kidney function declines and are markedly elevated once on dialysis therapy"[25-26]. Such high levels of FGF23 may be due to persistent phosphate retention or hyperphosphatemia,

There was positive correlation between FGF23 and PTH levels. A finding that is supported by Hu et al who observed that "in dialysis patients serum FGF23 levels are markedly increased and they are positively correlated with serum PTH levels and with serum levels of phosphate"[8]. One may assume that the sustained accumulation of phosphate is the cause of a direct correlation between PTH and FGF23. Nevertheless, given the fact that FGF23 inhibits parathyroid function it is unexpected to observe a parallel increase

in the serum concentrations of FGF23 and PTH. This is explained by the fact that FGF23 receptors responsiveness on parathyroid glands is declined in ESRD patients.

Weber et al also discovered that "a significant correlation was found between FGF23 and serum calcium, serum phosphate and dialysis vintage in dialysis patients. Level of FGF23 was significantly high in hemodialysis patients". In ESRD subjects, he observed a significant positive correlation of serum phosphorus with FGF23 levels[23].

In this study the researcher observed that S. PTH, phosphate, calcium and FGF23 levels are much higher in ESRD patients of more than six months than patients of less than six months duration and this difference was statistically significant which could be explained by the fact that most of the patients were on calcium and vitamin D supplements in addition to development of tertiary hyperparathyroidism.

This observation is in contrary to Melamed et al findings who discovered that "After 6 months of follow-up, mean serum calcium, phosphate, and CaP remained stable. Serum PTH levels fell after the initiation of dialysis and continued to fall for the next year[27]. Zaritsky et al also noted that "FGF23 levels are significantly lower in short daily hemodialysis patients[28].

The justification behind this is that the adequacy of hemodialysis has a paramount effect in controlling those parameters which could explain that ESRD patients were under dialyzed (insufficient hemodialysis) in centers in which the study was done.

Conclusions

Fibroblast growth factors23 levels are significantly elevated in ESRD patients in comparison with control group. Moreover, there is a statistically positive correlation between S.PTH, S. calcium, S. phosphate and FGF23 i.e. whenever the value of S.PTH, S. phosphate or S. calcium increase the level of FGF23 increases too, this finding was clear among all study sample and among patients group separately. In all conditions cases of more than six months had higher measures than cases of less than six months. Hemodialysis has very limited role in decreasing serum measures particularly PTH, calcium, phosphate and FGF23 apart from renal function parameters which are measured more frequently and the main focus is no these measures. Diabetes and hypertension were the most predominant cause for developing ESRD.

Conflict of interest declaration:There is no conflict of interest.

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