Fibroblast Growth Factor 23 in Children With End Stage Renal Disease on Hemodialysis

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Abstract: Background: Fibroblast growth factor 23 (FGF-23) is a novel regulator of phosphate metabolism. In adults with chronic kidney disease (CKD) FGF-23 is increased; however, comparable studies in children are lacking. **Objective:** To investigate the level of FGF-23 in children with end stage renal disease (ESRD) on maintenance hemodialysis and its relation to serum phosphorus, Ca^{2+} and PTH. **Patients and Methods:** The serum level of FGF-23 was measured in twenty children with ESRD on maintenance hemodialysis and compared to their age- and sex matched healthy children. Biochemical parameters including serum urea, creatinine, hemoglobin, ALP, phosphorus, $Ca^{2+,}$ and PTH were measured in this study to unravel their relationship with circulating FGF-23. **Results:** Levels of FGF-23 were significantly higher in pediatric patients in comparison with healthy control group and positively correlated with PTH and phosphorus. Phosphorus level in the diseased group was significantly high in spite of increasing level of FGF-23. The blood urea, creatinine and PTH increased significantly, concomitant with significant decrease in hemoglobin level and insignificant alteration of calcium levels in the diseased pediatric patients, compared to the healthy control group. **Conclusion:** FGF-23 could represent a promising therapeutic target that might improve the fatal progression of dialysis in children with chronic kidney disease on maintenance hemodialysis.

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Abbreviations: FGF: Fibroblastic growth factor. **PTH:** Parathyroid hormone. **CKD:** Chronic Kidney Disease. **ESRD:** End stage renal disease

1. Introduction

Chronic kidney disease (CKD) is a growing public health hazard that is associated with a markedly increasing risk of cardiovascular and bone diseases. In healthy individuals, kidneys regulate calcium and phosphorus homeostasis through tubular reabsorption mechanism. Patients with CKD have seriously compromised homeostatic mechanism, giving rise to different adaptive changes in calcium(Ca^{2+}), phosphorus (Ph), parathyroid hormone (PTH) and fibroblast growth factor (FGF-23) levels ***(Mejia et al.,2001)***. Hyperphosphatemia is a risk factor for cardiovascular disease and mortality and thus a potential target for interventions to improve clinical outcomes in CKD ***(Isakova et al., 2009).***

Fibroblast growth factor (FGF-23) is a novel bone-derived hormone that inhibits phosphate reabsorption and calcitriol production by the kidney. This phosphaturic hormone, which is made predominately by osteocytes in bone, appears to have a physiologic role as a counter-regulator hormone for vitamin D ***(Stubbs et al., 2007).*** It achieves its cellular specificity in the kidney and parathyroid glands by binding to co-receptor Klotho which increases the affinity of FGF-23 for ubiquitously expressed FGF receptors ***(Urakawa et al., 2006).***

In patients with chronic kidney diseases, FGF-23 level is thought to increase as a compensatory response to maintain normal phosphate balance as the capacity for renal phosphorus excretion declines **(*Gutierrez et al., 2008)*.** As the main physiologic function of FGF-23 is the enhancement of renal phosphate excretion and elevation in FGF-23 precedes the rise of serum phosphate, FGF-23 level is found to be inversely related to renal function in patients with CKD. Thus, serum levels of FGF-23 might be a better prognostic biomarker than serum phosphate level for risk assessment in patient with CKD ***(Shigematsu et al., 2004).***

Control of bone and mineral homeostasis is considered essential in children with CKD to prevent skeletal complications, achieve adequate growth and maintain cardiovascular health ***(Claus and Otto, 2011).*** Thus, the aim of the present study was to assess the levels of FGF-23 and other biochemical variables of bone metabolism in children with chronic renal disease on hemodialysis.

2. Patients and Methods

Prior to initiation, this study received approval by the Ethical Committee of the Faculty of Medicine at Elazhar University. This study investigated twenty children with ESRD who had been on regular

maintenance hemodialysis. These patients were recruited from renal dialysis units at Al-Zahraa University Hospital. Informed consent was taken from the children and their parents. The ratio of male/female is 12/8. Their ages ranged from 10-18 years with mean (13.15 ± 2.91) years. Their mean weight and height was 21.79 ± 4.16 Kg and 120.85 ± 9.29 cm, respectively.. Twenty age and sex matched healthy subjects were enrolled in this study as a control group. Their mean age was 13.42 ± 2.78 years. Their mean weight and height were 32.17 ± 3.07 Kg and 135.83 ± 6.04 cm, respectively.

All patients and controls were subjected to full history taking, thorough clinical examinations, anthropometric measurement and only those children with end stage renal disease who had been on a regular dialysis were included in the study. Three hemodialysis sessions weekly (4 hours/session) were performed. Hemodialysis access was arteriovenous fistula and was performed using a bicarbonate buffered dialysate.

All patients and controls were exposed to laboratory investigations including complete blood count (CBC), renal function tests, Ca^{2+}, Ph, alkaline phosphatase (ALP), intact parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23).

Five mls of peripheral venous blood were withdrawn and divided into two parts. One ml was anticoagulated with EDTA for CBC performance, and from the remaining 4 mls serum was obtained by centrifugation at 4000 xg for 10 minutes. An aliquot of the serum was stored frozen at -20˚c for the assay of FGF-23. The remaining aliquot was used for assessment of renal function tests, Ca^{2} ,Ph, ALP and intact PTH.

CBC was done on Cell Dyn 1800 autoanalyzer **(**Abbott Cell Dyn 1800 Hematology Analyzer, USA). Renal function tests, Ca^{2+},Ph, and ALP were assayed photometrically on Hitachi 911Autoanalyzer using kits purchased from Roche-Diagnostic systems(F. Hoffmann-LaRoche Ltd., Basel, Switzerland)

Intact PTH was assayed by immulite 1000 systems (chemiluminescence method) using kit supplied by Seimens Health care Diagnostics products Ltd (United Kingdom). Intact PTH is a solid-phase, two site chemiluminesent enzyme-labeled immunometric assay. The solid phase was a polystyrene bead enclosed within a test unit containing the coated bead. An alkaline phosphatase conjugated to polyclonal goat anti intact PTH was also added to the test unit. After the wash and incubation steps, chemiluminescent substrate underwent hydrolysis in the presence of alkaline phosphatase. The photon output as measured by the luminometer is related to the presence of intact PTH in the sample ***(Babson, 1991).***

FGF-23 was assayed by ELISA immune assay kit (Glory Science Co., Ltd, USA) according to the manufacture instructions. The microtiter plate provided in this kit has been pre-coated with an antibody specific to FGF-23 ***(Larsson et al., 2003).*** Standards or samples were then added to the appropriate microtiter plate well with a biotin-conjugated antibody preparation specific to FGF-23 and streptavidin conjugated to Horseradish peroxidase (HRP) was added to each microplate well and incubated. Then achromogen solution (A and B) was added to each well. Only those wells that contained FGF-23, biotin-conjugated antibody and enzyme-conjugated streptavidin will exhibited a change in color. The reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at wave length of 450 nm. The concentration of FGF-23 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis:

Means ± SD of all basic and clinical variables were computed to identify sample characteristics. Differences in characteristics between participants were tested with t test for the normally distributed variables. The significance level was set at five percent. Univariate correlation analysis was done for variables that possibly associated with FGF using Pearson correlation. Statistical Package for Social Sciences Version 17.0 was used for these analyses.

3. Results

The clinical and laboratory data of the patients and their control counterparts are shown in table (1). Patients and their healthy control who are selected within the same age range showed a significant difference (P <0.001) regarding their mean weights and heights. The dialysed children showed a 32.26 % decrease in mean body weights and 11% in mean heights.

Statistical analysis of kidney function tests in the diseased group showed significant increase in serum levels of urea (5 times) and creatinine (10.4 times), compared to their control groups at (P<0.01).

The mean hemoglobin of patients was 9.09 ± 0.91 compared to 11.6 ± 0.67 among control subjects, showing 21.6 % decrease. Furthermore, the patients had significantly higher phosphorus and alkaline phosphatase levels (1.7 and 11 times, respectively at P< 0.00).

Concerning the serum calcium level, patients had lower mean level (8.48 ± 0.87 mg/dl), compared to control group(8.99 ± 0.38 mg/dl); however, this difference was statistically insignificant (P<0.06).

The present study revealed a drastic elevation (P<0.00) of serum levels of FGF-23 and iPTH in dialyzed patients (9.84, 23.6 times, at P< 0.001), compared to control subject.

Univariate correlation analysis of FGF-23 with the other parameters revealed that serum FGF-23 was positively correlated with phosphorus (r= + 0.62, *P*= 0.00); iPTH (r= 0.73, *P*= 0.00) and ALP (r= 0.77,*P*=0.00) as shown in table (2) and figures (1) and (2).

Table 1: Clinical and biochemical level in studied group.

Variable	Patient (n =20)	Control (n = 20)	t- test	*P* value
Age (years)	13.15 ± 2.91	13.42 ± 2.78	0.26	0.8
Weight (Kg)	21.79±4.16	32.17±3.07	7.48	0.00*
Height (cm)	120.85±9.29	135.83±6.04	4.97	0.00*
Hemoglobin (g/dl)	9.09 ± 0.91	11.60 ± 0.67	8.25	0.00*
Urea (mg/dl)	113.15±42.01	22.67±8.89	7.32	0.00*
Creatinine (mg/dl)	8.84±2.51	0.85±0.23	10.92	0.00*
Calcium (mg/dl)	8.47±0.8	8.99±0.38	2.3	0.02*
Phosphorus (mg/dl)	6.15±1.68	3.46±0.63	5.29	0.00*
ALP (U/L)	513.40±116.5	57.75±10.54	-17.3	0.00*
PTH (Pg/ml)	357.00±92.9	26.83±4.6	- 15.8	0.00*
FGF 23 (ng/L)	510.25±65.8	53.33±9.5	-30.5	0.00*

Values shown are means (± SD), n = 20 individuals per group. (*)Values shown are significantly different from the normal control group at P < 0.05 (t-test).

Table 2: Correlation between FGF-23 and other parameters

	Pearson correlation "r"	*P* value
Urea	**0.91**	**0.00***
Creatinine	**0.95**	**0.00***
Calcium	**- 0.49**	**0.004***
Phosphorus	**0.62**	**0.00***
ALP	**0.77**	**0.00***
PTH	**0.73**	**0.00***

*Value shown resulted in a significant Pearson Correlation (r) at P < 0.05.

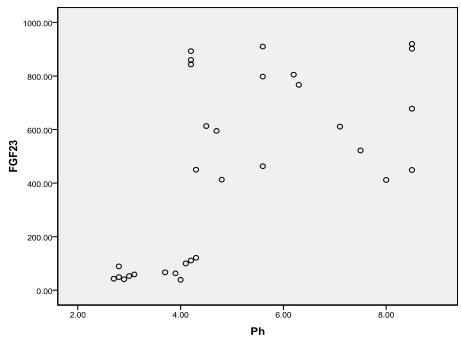

Fig. 1: Correlation between FGF-23 and Phosphorus levels. An r = 0. 62 was determined for the plot shown

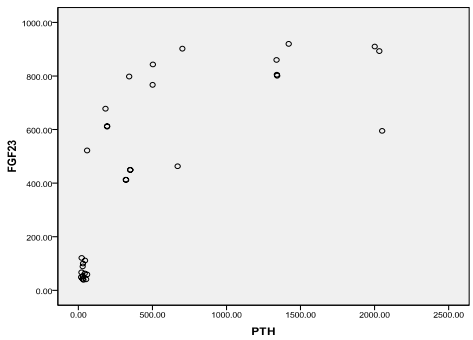

Fig. 2: Correlation between FGF-23 and PTH levels. An r = 0. 73 was determined for the plot shown.

4. Discussion

In patients with CKD, circulating FGF-23 levels are progressively elevated to compensate for persistent phosphate retention ***(Komaba and Fukagawa, 2009).*** In late CKD, FGF-23 cannot reduce serum phosphate levels, and abnormally high FGF-23 concentration appears to exert unwarranted effects, including left ventricular hypertrophy, faster CKD progression and premature mortality ***(Jüppner, 2011).***

Although many studies have been performed in CKD adults, few data are available on FGF-23 metabolism in CKD children ***(Magnusson et al., 2010).***

In the present study, we studied 20 dialysis patients compared to their matched age and sex healthy controls. There was a significant decrease in weight and height of dialysis patients when compared to healthy controls. These findings were consistent with ***Stefandis and Klaus (2007)***who reported that growth failure is a common and significant clinical problem for children on dialysis and often remains a major impediment to their rehabilitation.

In our study, levels of FGF-23 in patients on dialysis were significantly higher in comparison with those in the control group. These increases in the FGF-23 levels are in agreement with ***van Husen et al. (2010)*** who proved that the highest levels of FGF-23 were found in stage 5 compared to stages 1 and 2 CKD.

The increased level of FGF-23 was positively correlated to the elevation of serum creatinine and blood urea, as illustrated in the present study. These findings are in agreement with ***Nakanishi et al.(2005)*** who suggested that serum FGF-23 levels are progressively increased as kidney function declines and are markedly elevated once on dialysis.

Phosphorus is an important mineral for cell structure and energy ***(Giachelli, 2009).*** It is filtered freely in the glomerulus and then reabsorbed in the proximal tubules under the effect of various hormones. The amount of reabsorbed phosphorus is the main regulator of the serum phosphorus levels in subject with normal renal function, or moderately reduced glomerular filtration rate ***(Prie et al., 2009).***

In the current study, the phosphorus levels were significantly elevated in the studied patients and positively correlated with FGF-23 levels. These findings were consistent with ***Fourtounas (2011)]*** who found that, in CKD, the kidneys fail to excrete the phosphorus, resulting in positive phosphorus balance. The skeleton through the disorders of the bone that accompany CKD, contributes to this hyperphospatemia, as it fails to handle the exceeding phosphorus. Furthermore, our findings were in agreement with ***Komaba and Fukagawa (2009)*** who stated that reduced renal function directly affects phosphorus reabsorption. The kidney becomes incapable of filtering enough phosphorus and its high level in blood directly stimulates the parathyroid gland which in turn stimulates FGF-23 synthesis and secretion by the osteocytes.

Sliem et al.(2011) explained this positive correlation providing two explanations; the first is the kidney, which is the principal target of FGF-23, becomes no longer responsive to FGF-23 in CKD. Moreover, Klotho production by the kidney is reduced in end stage renal disease. The second is that, in early stage CKD, serum FGF-23 is elevated to maintain normal serum phosphate levels, by promoting urinary phosphate excretion,. However, in patients at the advanced stage, overt phosphate loading may overcome such compensation for decrease glomerular filtration rate (GFR) despite markedly elevated FGF-23 levels.

Parathyroid gland is the main organ responsible for PTH production and Ca^{2+} homeostasis in the organism. It senses serum Ca^{2+} concentration via the Ca^{2+} receptor (Ca R) and vitamin D receptors (VDR) ***(Duran et al.,2010).*** Extracellular ionic Ca^{2+} is the main parathyroid regulator; low levels stimulate PTH secretion and high levels inhibit hormone release and furthermore, favor its degradation within the parathyroid cells ***(Silver and Levin, 2005).***

The present study revealed marked increase in the parathyroid hormone levels, which was positively correlated with FGF-23 levels. These data were supported by ***Rodrinuez et al. (2006)*** who stated that, in CKD the incorrect control of PTH secretion was attributed to the reduced VDR and CaR expression which occur in parallel to the parathyroid gland growth. Parathyroid gland hyperplasia and the consequent increase in PTH secretion are responsible for hyperparathyroidism observed in CKD.

Komaba and Fukagawa (2010) explained t the failure of increased FGF-23 levels to suppress PTH, by the parathyroid resistance that might be due to the decreased expression of the Klotho-FGF R1 complex in the hyperplastic parathyroid gland. ***Nakanishi et al.(2005)*** pointed out that elevated level of serum FGF-23 is suggested to be an important predictor of secondary hyperparathyroidism in patients who are undergoing dialysis treatment.

The findings of this study were also in agreement with ***van Husen et al. (2010)*** who recorded a positive correlation of the levels of FGF-23 with parathyroid hormone and phosphate concentration. ***Gutierrez et al. (2008)***documented that in CKD, serum FGF-23 levels are increased together with secondary hyperparathyroidism, indicating resistance of the parathyroid to FGF-23. Measurement of FGF-23 seemed to have prognostic significance in the treatment of secondary hyperparathyroidism.

Hemoglobin levels were significantly lowered in the studied patient group. The development of anemia

in patients with CKD was explained by ***Rao et al.(1993)***who reported that high PTH levels directly inhibit the production of RBCs and increase their fragility. Hyperparathyroidism can also cause marrow fibrosis decreasing thereby the production of red blood cells.

Alkaline phosphatase (ALP) is an enzyme measurable in most body fluids and usually originates from the liver or bone. In CKD patients without liver disease, ALP can be elevated in high-turnover bone disease ***(Regidor et al., 2008).*** However, measuring this readily available and inexpensive biomarker has not been singled out as an individual therapeutic target of CKD ***(Eknoyan et al., 2003).***

The present study showed significant elevation of serum ALP in the studied patient group in comparison to the control group; these data are supported by the study of ***Fahrleitner-Pammer et al.(2008)and Kovesdy et al. (2010).***

Concerning calcium levels, a significant decrease was observed in comparison to the control group. This hypocalcemia was supported by the study of ***Levin et al.(2007)*** who reported that, in advanced cases of renal failure, serum calcium levels drop in response to decreased intestinal calcium absorption, resulting from low circulating levels of 1,25(OH)2 D3. On the other hand, ***Wesseling et al.(2008)*** state that, hypocalcemia is quite uncommon in CKD stage 3 and early stage 4, but more often observed in stage 5.

In conclusion FGF-23 could represent a promising therapeutic target that might improve the fatal prognosis of dialysis children with chronic renal failure in regard to management of disordered phosphorus metabolism. Further research is needed to show whether lowering FGF-23 levels improve outcomes in children on maintenance hemodialysis

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