Fibroblast Growth Factor 23 in Children with Chronic Kidney Disease

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Abstract

Background: Decreased of glomerular filtration rate in chronic kidney disease (CKD) changes the calcium and phosphate balance. High phosphate levels in children with CKD stimulate secretion of Fibroblast Growth Factor 23 (FGF23). High FGF23 levels have harmful that potentially increase the morbidity and mortality of children with CKD.

Objective: To analyze the level of FGF23 in children with CKD.

Methods: A cross sectional study was performed in Pediatric Nephrology Ward and Outpatient Clinic of Dr. Soetomo General Hospital Surabaya, during December 2019-March 2020 for children with CKD stage 1-5, aged 3 months to 18 years old. Children on phosphate-binder, vitamin D therapy, or severely ill were excluded. Blood level of FGF23 was measured using ELISA with statistic analysis with SPSS 20.

Results: A total of 52 CKD stage 1-5 children were involved, mean age was 11.44 years old, and 50% were boys. There were 51% children have FGF23 level more than 30 pg/ml. The lowest mean of FGF23 levels was found in the CKD grade 1 (8.94 ± 8.77 pg/mL) and the highest mean at CKD grade 5 (113.30 ± 78.73 pg/mL).

Conclusion: The FGF23 level increasing accordance with increasing in the grade of CKD.

Keywords: *Chronic kidney disease, Children, FGF23, CKD Grade*

Background

Children with Chronic Kidney Disease (CKD) have high morbidity and mortality. Decrease in the Glomerular Filtration Rate (GFR) resulting in an imbalance of calcium and phosphate, resulting in changes of Fibroblast Growth Factor 23 (FGF23) levels, calcitriol, and parathyroid hormone [1]. Fibroblast Growth Factor 23 is a hormone produced by bone which has a direct effect on the heart, trigger left ventricular hypertrophy, and has been associated with consequences, including cardiovascular morbidity [2].

A decrease in GFR leads to a disruption of calcium and phosphate balance. This disorder begins with retention of phosphate in blood. Increased of phosphate levels stimulate bone to increase secretion of FGF23 by osteocytes. Fibroblast Growth Factor 23 secreted in response to increase in phosphate levels as a result of a decrease in LFG. Phosphate retention will activate klotho to increase FGF23. Decrease of GFR will also reduce alpha 1 hydroxylase levels, which in turn increase parathyroid hormone levels, further increasing FGF23

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levels. Increased levels of FGF23 also have contribution either in increasing incidence of cardiovascular disease (CVD) or mortality [3]. In CKD, the presence of systemic inflammation cause an increase in levels of CRP, IL6 and TNF alpha. High levels of FGF23 leads to hypertrophy of myocardial cells and cardiomyocytes [4,5]. High FGF23 is an independent risk factor for CKD progression in children [6]. FGF23 at a concentration >170 pg/ml is an independent predictor of ventricular hypertrophy in CKD children with GFR >45 ml/minute/1.73 m^2 [1]. Nowadays, studies of FGF23 levels in children with CKD in Indonesia are still limited. Therefore, this study conducted to analyze the level of FGF23 in children with CKD.

Methods and Materials

This study was a cross sectional study conducted in Pediatric Nephrology Ward and Outpatient Clinic of Dr. Soetomo General Hospital Surabaya, from December 2019 to March 2020. The inclusion criteria in this study were children aged 3 months - 18 years old, grade I-V of chronic kidney disease, and agreed to participate in the study. Exclusion criteria in this study were consuming phosphate binder and calcitriol, suffering from severe infections, and having a previous history of heart disease. The subject was selected based on random sampling technique. Variables of this study were CKD and FGF23.

Chronic Kidney Disease Criteria

Chronic kidney disease is kidney damage which lasts for at least 3 months with or without a decrease in GFR or GFR of <60 mL/minute/1.73 m^2 for at least 3 months with or without kidney damage (NKF-KDOQI, 2005). The diagnosis was made by the Pediatric Nephrologist. CKD was grouped into: CKD grade 1 (GFR:> 90 mL/minute/1.73 m^2), CKD grade 2 (60-89 mL/minute/1.73 m^2), CKD grade 3 (30-59 mL/minute/1.73 m^2), CKD grade 4 (15-29 mL/minute/1.73 m^2), and CKD grade 5 (<15 mL/minute/1.73 m^2).

Fibroblast Growth Factor 23 Measurement

Fibroblast Growth Factor 23, play a role in regulating phosphate levels homeostasis in circulation, by regulating phosphate reabsorption in kidneys. Blood sample was taken as much as 3 ml, and stored at -80^{0}C until assayed. FGF23 measured in the Clinical Pathology laboratory of Dr. Soetomo General Hospital, using the ELISA kit (Human FGF23 ELISA Kit, Elabscience, WuHan, China) in units of pg/ml.

Result

During the period, 52 children with chronic kidney disease were studied. CKD grade 5 was the largest group with 18 (34.6%) subjects. The next was CKD grade 1 with 12 (23.1%) subjects, followed by CKD grade 4 10 subjects, then CKD grade 2, and 4 with 6 subjects each (Table 1).

Table 1. Frequency distribution of CKD

CKD Grade	N	%
1	12	23.1
2	6	11.5
3	10	19.2
4	6	11.5
5	18	34.6
Total	52	100.0

There were 26 boys, 10 (38.5%) of them have CKD grade 5. As many as 86.5% aged over 5 years with the average age in this study was 11.44 ± 4.44 years. Glomerulonephritis was the most common underlying disease of CKD with 33 subjects, 10 of which were in CKD grade 1 and 10 subjects in CKD grade 5. Tubulopathy and cysts were rare causes of CKD. In this study, 27 subjects had been diagnosed with CKD in less than 12 months and 6 subjects for more than 5 years (Table 2).

Table 2. Demographic characteristics in children with CKD

Variable	CKD Grade					Total
	1	2	3	4	5	
Sex, n (%) Male Female	 5 (19.2) 7 (26.9)	 4 (15.4) 2 (7.7)	 3 (11.5) 7 (26.9)	 4 (15.4) 2 (7.7)	 10 (38.5) 8 (30.8)	 26 (100.0) 26 (100.0)
Age, year `x ±SD	 10.50 ±5.47	 9.50±4.55	 10.70±4.88	 9.17±4.49	 13.89±2.19	 11.44±4.44
Cause, n (%)						
Glomerulonephritis	10 (30.3%)	4 (12.1%)	7 (21.2%)	2 (6.1%)	10 (30.3%)	33 (100.0%)
CAKUT	2 (15.4%)	0 (0.0%)	2 (15.4%)	2 (15.4%)	7 (53.8%)	13 (100.0%)
Stone	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	3 (100.0%)
Tubulopathy	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
Cystic	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (100.0%)
Duration of illness, n (%)						
< 1 year	10 (37.0%)	4 (14.8%)	4 (14.8%)	2 (7.4%)	7 (25.9%)	27 (100.0%)
>1-3 years	1 (7.1%)	0 (0.0%)	4 (28.6%)	1 (7.1%)	8 (57.1%)	14 (100.0%)
>3-5 years	1 (20.0%)	2 (40.0%)	0 (0.0%)	1 (20.0%)	1 (20.0%)	5 (100.0%)
>5 years	0 (0.0%)	0 (0.0%)	2 (33.3%)	2 (33.3%)	2 (33.3%)	6 (100.0%)

CAKUT, *Congenital anomalies of the kidneys and urinary tracts*

BUN and serum creatinin levels were increased in children with CKD with a mean of 39.19 ±27.91 mg/dl and 4.15 ±4.57 ml/min. Slight decrease of calcium were found at a mean of 8.12 ±1.02 mg/dl. Phosphate levels were remains within normal range with mean 4.43 ±1.41 mg/dl (Table 3).

Table 3. Laboratory characteristics in children with CKD

Variable	CKD Grade					Total
	1	2	3	4	5	
BUN, mg/dl	10.00±5.14	33.83±25.55	26.30±8.73	62.67±27.48	59.78±22. 64	39.19±27.91
Serum creatinine, ml/min	0.46±0.16	0.87±0.26	1.58±0.45	3.22±0.60	9.45±3.90	4.15±4.57
Calcium, mg/dl	8.63±0.88	8.28±0.72	8.14±0.66	8.37±0.75	7.64±1.28	8.12±1.02
Phosphate, mg/dl	4.29±0.94	3.58±1.96	5.07±1.43	5.00±1.74	4.25±1.29	4.43±1.41

The increase of the grade of CKD in line with the increase in FGF23 levels (Table 4). The lowest mean of FGF23 levels was found in CKD grade 1 (8.94 ±8.77 pg/mL), and the highest mean at CKD grade 5 (113.30 ±78.73 pg/mL).

Table 4. FGF23 levels in children with CKD

CKD	n	FGF23 (pg/ml)
		`x±SD
1	12	8.94 ±8.77
2	6	10.58 ±6.72
3	10	20.97 ±15.94
4	6	53.26 ±29.71
5	18	113.30 ±78.73
Total	52	52.68 ±66.12

Spearman (r_s) = 0,834 p = 0,000

The grade of CKD with FGF23 level forming a one-way linear line, which describes that the higher the grade of CKD the higher the FGF23 level (Figure 1).

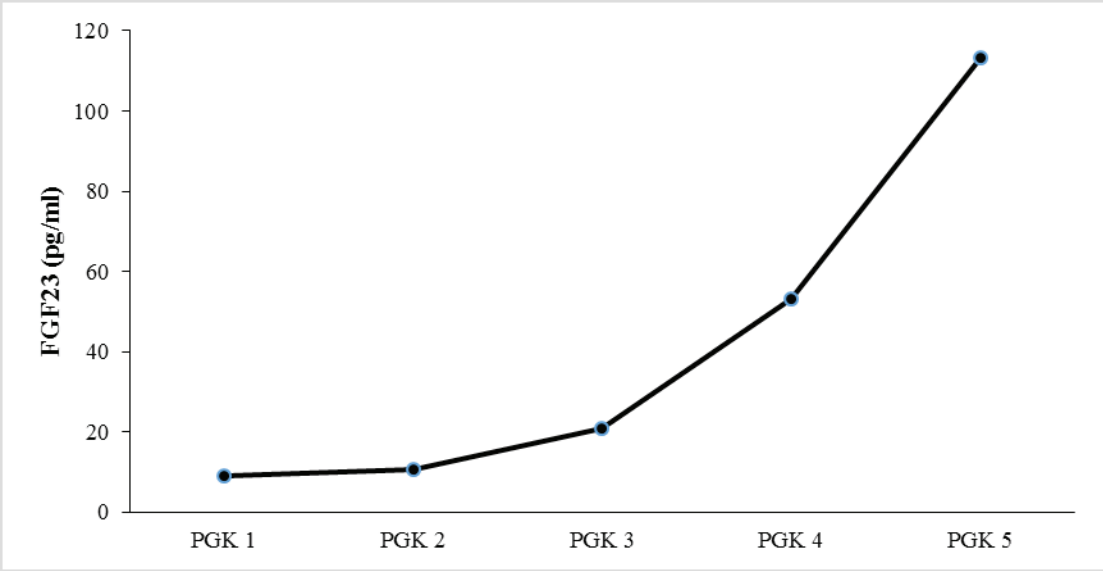

Figure 1. CKD grade and FGF23 level

Discussion

In this study, research subjects were grouped into 5 groups based on the grade of CKD. A total of 12 subjects (23.1%) CKD grade 1, 6 subjects (11.5%) CKD grade 2, 10 subjects (19.2%) CKD grade 3, 6 subjects (22.5%) CKD grade 4, and the largest group of 18 subjects (34.6%) was CKD grade 5. In this study, the distribution of sex for male is the same as for female, with 26 subjects each. Tuttle's research, 2019, which involved 12,591 children with CKD, found a higher prevalence of female than male (56.2% vs 43.8% [6]. The age distribution in this study was almost the same in each group. The number of subjects over 5 years in the study was quite large, namely 45 subjects (86.5%). The mean age was 11.44 ±4.44 years old, with the youngest were 1 year, and the oldest were 17 years old. Tuttle, 2019, in his research, the median age of CKD children is 6 years old (1-13 years). The prevalence of CKD according to age is 20.2% in children less than 1 year old, 17.8% in children 1-3 years, 12% in children 4-6 years, 14.8% in children 7-10 years, 15.2% in children 11-14 years old, and 19.9% in children 15-17 years old [6].

In this study, FGF23 levels measurement was carried out quantitatively by measuring the intact FGF23 levels. FGF23 began to rise above the normal value in CKD grade 3, with a mean of 20.97 ±15.94 pg/ml. Fibroblast growth factor 23 is a hormone that regulate homeostasis of circulating phosphate by regulating phosphate reabsorption in the kidneys. Fibroblast growth factor 23 works by suppressing the expression of the sodium phosphate co-transporter family (NaPi IIa and NaPi IIc) in the proximal tubule of the kidney. Decreased expression of NaPi IIa and NaPi IIc results in decreased renal reabsorption of phosphate. Thus, the excretion of phosphate through urine will increase (phosphaturia) [7].

FGF23 will increase in CKD grade 3 or more. It occurs due to kidneys' ability to excrete phosphate begins to decrease and resulting in hyperphosphatemia. Hyperphosphatemia causes decreased of calcium levels and increased of parathyroid hormone (PTH) secretion. Initially, the serum phosphate level returns to normal and formed a new balance with high PTH and FGF23 levels. As a compensation for hyperparathyroidism and elevated levels of FGF23, which are phosphaturia, are the main mechanisms that maintain phosphate within normal range. This compensatory mechanism will continue to repeat until kidney function worsens and severe hyperparathyroidism occurs, and in the end, there is a marked increase in serum phosphate levels [8].

High levels of FGF23 in CKD patients represents a physiological compensation for maintaining serum phosphate levels, while simultaneously decreasing the number of nephrons. Then FGF23 will increase the excretion of phosphate in urine and decrease the

absorption of phosphate in the gastrointestinal tract through 1-α hydroxylase inhibition, and indirectly cause a decrease in circulating calcitriol levels. Excess FGF23 production will keep the body's phosphate levels within the normal range, until the occurrence of CKD continues. This is supported by a study which showed that in patients with CKD grade 1 to 4, normal serum phosphate levels were obtained. However, an increase in serum phosphate levels began to occur at grade 5 CKD, where the threshold for urinary phosphate excretion capacity has been exceeded [9]. In working to maintain the balance of calcium and phosphate, fibroblast growth factor 23 requires a co transporter, namely Klotho. Klotho is a transmembrane protein which is mainly expressed in the distal tubule of the kidney and in other tissues such as the brain, parathyroid glands and gastrointestinal tract. Klotho is also secreted into blood, urine, and cerebrospinal fluid, therefore there are two forms of klotho, namely soluble klotho that circulates in the circulation and klotho in tissues. In the kidneys, klotho acts as a co-receptor for FGF23, which plays a role in increasing the excretion of phosphate through urine [10]. Klotho together with FGF23 not only increases the amount of phosphate in the urine, but also makes the urine does not contain high calcium, thus preventing supersaturation of urine [11].

Apart from high phosphate in serum, high calcitriol levels will also cause an increase in FGF23. Fibroblast growth factor 23 will inhibit the 1α-hydroxylase enzyme, by inhibiting the conversion of calcidiol to calcitriol. This mechanism is a physiological response to maintain the balance of calcium and phosphate [12,13]. Under normal conditions, FGF23 will provide negative regulation of PTH gene expression, secretion and proliferation of parathyroid cells in vitro and reduce PTH secretion in vivo. Conversely, the elevated PTH levels lead to increased expression of the FGF23 gene in bone. At CKD, the negative regulatory mechanism did not work. It because in patients with CKD there is resistance and down regulation of the FGF23 receptor in the parathyroid glands, so that there will be an increase in FGF23 and PTH [14].

Conclusion

An increase in FGF23 level accordance with the increase in the grade of CKD.

Conflict of Interest : None declared.

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Ethical Clearance : Approved by the Ethics Commission for Biomedical Research on Humans, Dr. Soetomo General Hospital, Surabaya. Ethical number published on December 3rd 2019, 1687/KEPK/XII/2019.

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