FIBROBLAST GROWTH FACTOR 23 AND CAROTID ARTERY INTIMAL MEDIUM THICKNESS IN CHILDREN WITH CHRONIC KIDNEY DISEASE

By

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ABSTRACT

Background*: The cause of early-accelerated atherosclerosis development observed in chronic kidney disease (CKD) is not fully understood. The determination of the relationship between the levels of fibroblast growth factor 23 (FGF-23) and carotid artery intimal medium thicknesses (CIMT) and development of endothelial dysfunction lends support to the possibility that FGF-23 plays a role in the development of atherosclerosis in CKD.*

Objectives*: to assess the circulating FGF23 as a marker of vascular stiffness in children with CKD regardless the etiology and to correlate its level with carotid artery intimal medium thickness as detected by Doppler U/S.*

Patients and Methods: *This is a prospective case control study was done on forty cases with CKD on conservative treatment (stage II - III and IV) regardless the etiology. Also twenty of apparently healthy children age and sex matched with cases were included as a control group. After complete clinical evaluation of cases and controls including weight, height, body mass index, blood pressure and all body systems, FGF23 was measured and carotid artery intimal medium thicknesses (CIMT) was measured as a marker of atherosclerosis using Doppler U/S.*

Results: *CIMT measurements and FGF-23 levels were significantly higher in patients than controls. There was positive correlation between FGF23 and CIMT, CKD stage. On the other hand there was negative correlation between FGF23 and GFR.*

Conclusion: *FGF-23 may be used as a sensitive and non-invasive indicator of subclinical atherosclerosis in patients with chronic kidney disease where our results showed a positive correlation between FGF23 and CIMT.*

Key words: *Chronic Kidney Disease, Fibroblast Growth Factor 23, Carotid Artery Intimal Medium Thicknesses, Glomerular Filtration Rate.*

INTRODUCTION

Chronic kidney disease (CKD) describes the gradual loss of kidney function. Kidneys filter wastes and excess fluids from blood, which are then excreted in urine. When chronic kidney disease reaches an advanced stage, dangerous levels of fluid, electrolytes and wastes can build up in the body **(Goldman, 2016)**.

Structural and functional abnormalities and calcification in the large vessels begin as early as the first decade of life in patients with CKD contributing to higher mortality than the age-matched population **(Shroff and Mitsnefes, 2010)**.

Fibroblast growth factor-23 (FGF-23), a 251-amino acid protein synthesized and secreted by osteoblasts and osteocytes, is a recently discovered potent regulator of serum phosphate levels **(Sarmento-Dias et al., 2016)**.

Examination of the carotid artery intima media thickness (CIMT) gives every clinician an opportunity to evaluate subclinical alterations in wall structure that precede and predict future cardiovascular clinical events. B-mode ultrasonography is a noninvasive, safe, easily performed, sensitive, relatively inexpensive and widely available method for detection of early stages of atherosclerosis and is accepted as one of the best methods for evaluation of CMIT **(Mancia et al., 2013)**.

AIM OF THE WORK

To assess the circulating FGF23 as a marker of vascular stiffness in children with CKD regardless the etiology and to correlate its level with carotid artery intimal medium thickness as detected by Doppler U/S.

PATIENT AND METHODS

This is a prospective case control study was carried out on forty cases with chronic kidney disease regardless the etiology. Those patients were chosen for the study from the Combined Clinic of Pediatric Nephrology & Urology at Al-Hussein university hospital AL-Azher University during the period from October 2016 to April 2018.

Their age ranged from 2 to 17 years with mean age of 9.4 ± 4.7 years. They were 26 males and 14 females. Also twenty of apparently healthy children age and sex matched with the cases were included as a control group.

Inclusion criteria:

● All cases with CKD stages (II-III-IV) regardless the etiology.

● Staging of CKD in our cases based on the level of GFR (according to the classification of (KDIGO, 2013) as follow:

Chronic kidney disease stage		GFR (ml/min/1.73 m2)
I	Kidney damage with normal or increased GFR	> 90
II	Kidney damage with mild decrease in GFR	60–89
III	Moderate	30–59
IV	Severe	15–29
V	ESRD	< 15

Exclusion criteria:

- Cases with other systemic diseases or chronic illnesses (comorbidities) other than CKD.
- End stage renal disease and patients with CKD who are not regular on follow up and not compliant with medication.4. Children received mechanical ventilation for less than 48 hours.
- Patients with neoplastic and metabolic diseases.

ETHICAL CONSIDERATIONS

- An informed consent was obtained from all parents of patients and control group before getting them involved in the study.
- This study was approved by the ethical committee for researches of both pediatric department and Al-Azhar faculty of medicine.
- The steps of the study, the aim, the potential benefits and hazards, all were discussed with the parents of the studied groups.
- Confidentiality of all data was ensured.
- The patient and control groups had the right to withdraw from the study at any time without giving any reasons.
- The authors declared that there was no conflict of interest regarding the research and publication
- The authors received no financial support regarding the research and publication

For all included cases the following was done:

1. Detailed history especially for: urinary symptoms, history of renal diseases in the family and pertinent medications, family history of hypertension, history

of hospital admission due to renal diseases and medications received.

2. **Thorough clinical examination including:** height, weight, body mass index (BMI), blood pressure and all body systems.

3. **Laboratory investigations including:**
   - CBC and CRP.
   - Urine analysis & culture.
   - Blood urea, serum creatinine by ELISA.
   - Serum calcium, phosphorus, alkaline phosphatase and parathormone hormone by ELISA in all cases
   - Lipid profile (serum cholesterol and serum triglycerides) by ELISA.
   - Serum fibroblast growth factor 23 by ELISA.

4. **Imaging studies including:**
   - Carotid artery intimal medium thickness by Doppler U/S for all cases and controls.
   - Renal U/S for all cases and controls.
   - MRU, VCUG and Renal scan in selected cases with significant findings in U/S.

5. **Renal biopsy:** For certain cases (8 cases) (in whom congenital obstruction of the urinary tract, renal stones and nephrocalcinosis has been excluded)

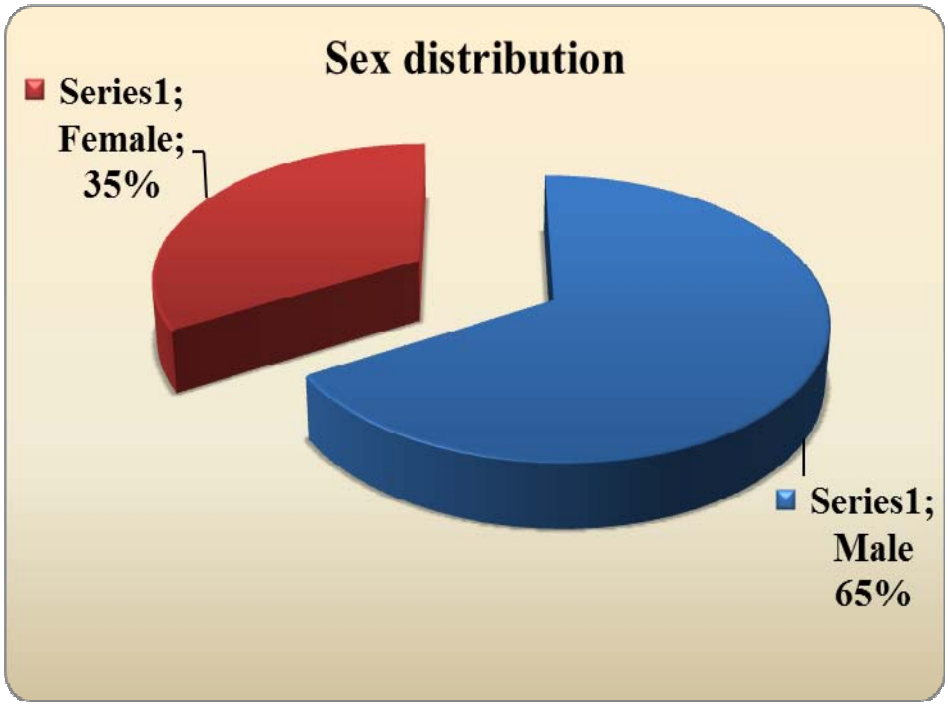
   •**As regard to controls,** they were subjected to history taking, clinical evaluation, routine laboratory investigations, ultrasonography, carotid artery intimal medium thickness and serum fibroblast growth factor 23.

RESULTS

Table (1): Age & sex of the studied cases &controls

	Cases (n=40)	**Controls (n=20)**	**P value**
Sex n, (%)			
Male	26 (65%)	9 (45.0%)	0.17
Female	14 (35%)	11 (55.0%)	NS
Age(years)			
Range	2 – 17	4 – 15	0.9
Mean ± SD	9.4 ± 4.7	8.9 ± 3.5	NS
Median (IQR)	8.5 (6.0 - 14.0)	9.0 (5.3 - 11.8)	

Table (1) and Figure (1) revealed that there was no significant difference between the two groups as regard age and sex and prevalence of CKD in males



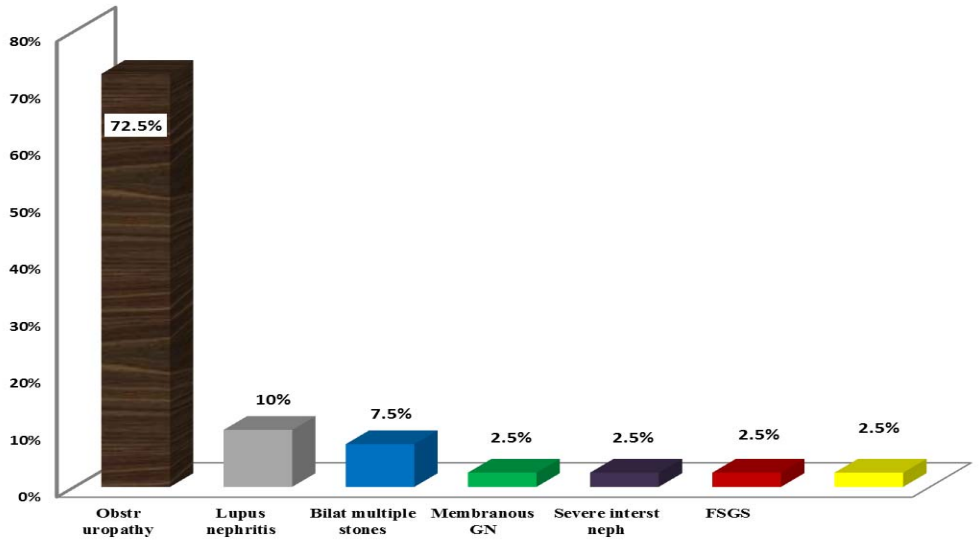
(65%) more than females (35%).

Figure (1): Sex distribution of the studied cases

Table (2): Etiology of CKD in the studied cases

Etiology of CKD	Frequency (n=40)	Percentage (%)
Obstructive uropathy	29	72.5%
Lupus nephritis	4	10%
Bilateral multiple stones	3	7.5%
Membranous glomerulonephritis	1	2.5%
Severe interstitial nephritis	1	2.5%
FSGS (focal segmental glomerulosclerosis)	1	2.5%
Global& segmental glomerulosclerosis, moderate chronic tubulointerstiial nephritis	1	2.5%
Total	**40**	**100%**

Table (2) and Figure (2) revealed that obstructive uropathy is the most



common etiology of CKD in our patients.

Figure (2): Etiology of CKD in the studied cases

Table (4): Stages of chronic kidney disease in the studied cases (according to (KDIGO , 2013)

	Frequency (n=40)	**Percentage**
Stages		
II	15	37.5
III	15	37.5
IV	10	25
Total	**40**	**100%**

Table (5): Results of GFR in the studied cases according to stages of CKD:

Stage of CKD	**NO of cases**	**Level of GFR (ml/min/1.73m2)**	**Percentage (%)**
Stage II	15	85- 62	37.5%
Stage III	15	48- 33	37.5%
Stage IV	10	29- 22	25%

Table (4) and Table (5) revealed that stage II and III represent 37.5% of cases for each stage where stage IV represent 25% of cases.

Table (6): Renal biopsy findings of selected cases (n=8)

Renal biopsy findings	**Frequency (n=8)**	**Percentage**
Lupus nephritis (stage IV)	4	50 %
Membranous glomerulonephritis	1	12.5%
Severe interstitial nephritis	1	12.5%
FSGS(focal segmental glomerulosclerosis)	1	12.5%
Global& segmental glomerulosclerosis, moderate chronic tubulointersticial nephritis	1	12.5%
Total Number	8	100%

This table showed that lupus nephritis represents 50% of cases subjected to renal biopsy.

In those cases: Congenital obstruction, renal stones and nephrocalcinosis have been excluded.

Table (7): Comparison between cases & controls regarding FGF23 and CIMT

	Cases (N=40)	**Controls (N=20)**	**P value**
	Mean ± SD	**Mean ± SD**	
FGF23 (pg/ml)	371 ± 276	34 ± 7	**< 0.001**
CIMT (mm)	0.81 ± 0.30	0.29 ± 0.08	**< 0.001**

Table (7) and Figures (3,4) revealed that there were significant differences between cases and controls as regard to FGF23 and CIMT.

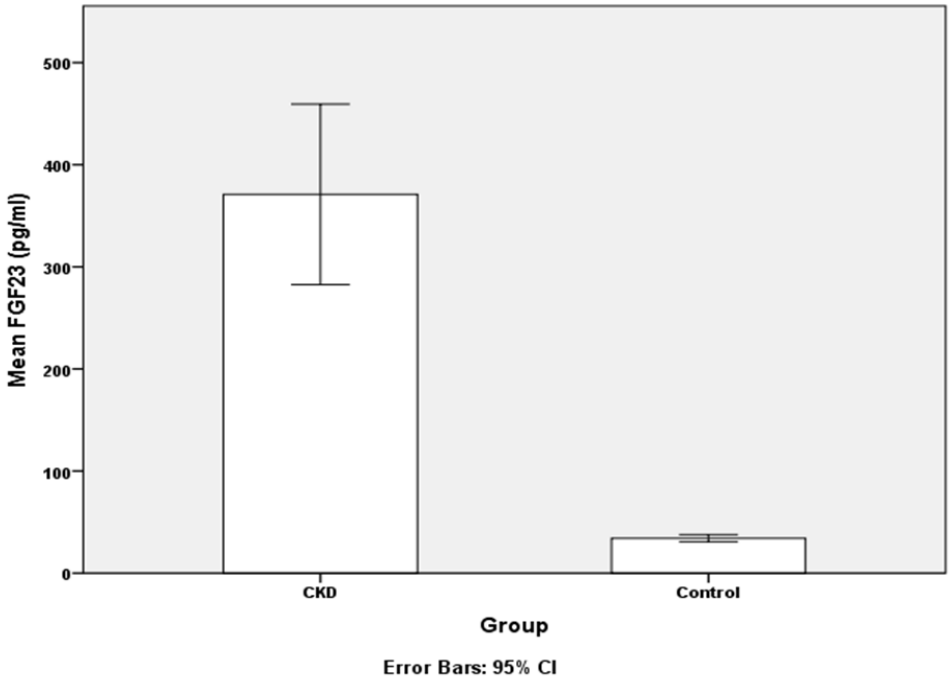


Figure (3): Mean FGF23 level in cases with CKD and controls. Error bars represent the 95% confidence interval (95% CI).

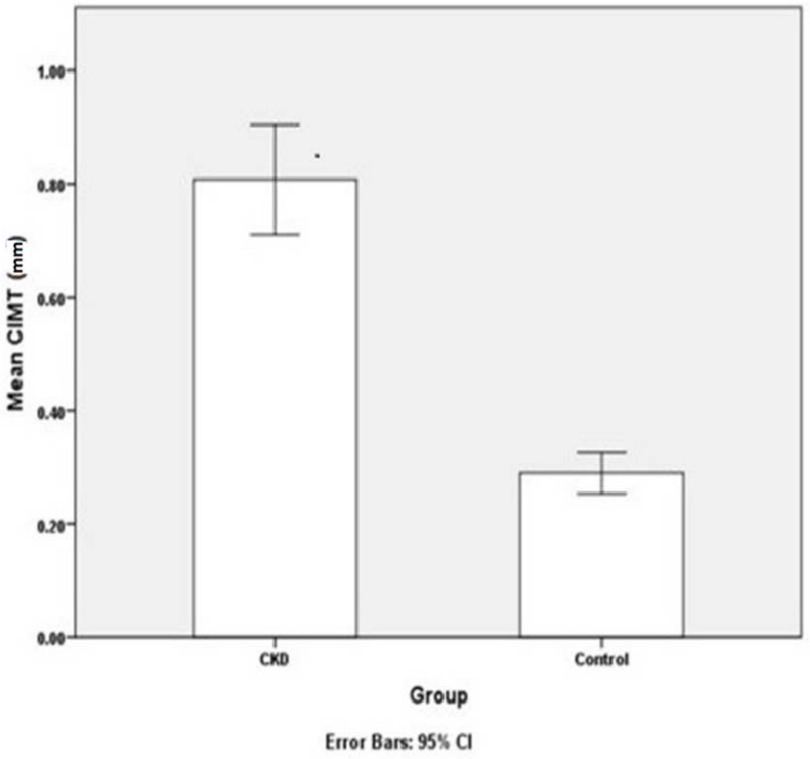


Figure (4): Mean CIMT in cases with CKD and controls. Error bars represent the 95% confidence interval (95% CI).

Table (8): Correlation between FGF23 and other important quantitative variables

	FGF23	
Variable	Pearson's r	P-value
CIMT	0.790	**< 0.001**
GFR	- 0.865	**< 0.001**
CKD stage	0.854	**< 0.001**

Table (8) and Figures (5,6,7) revealed that there were positive correlation between FGF23 and CIMT, CKD and negative correlation between FGF23 and GFR.

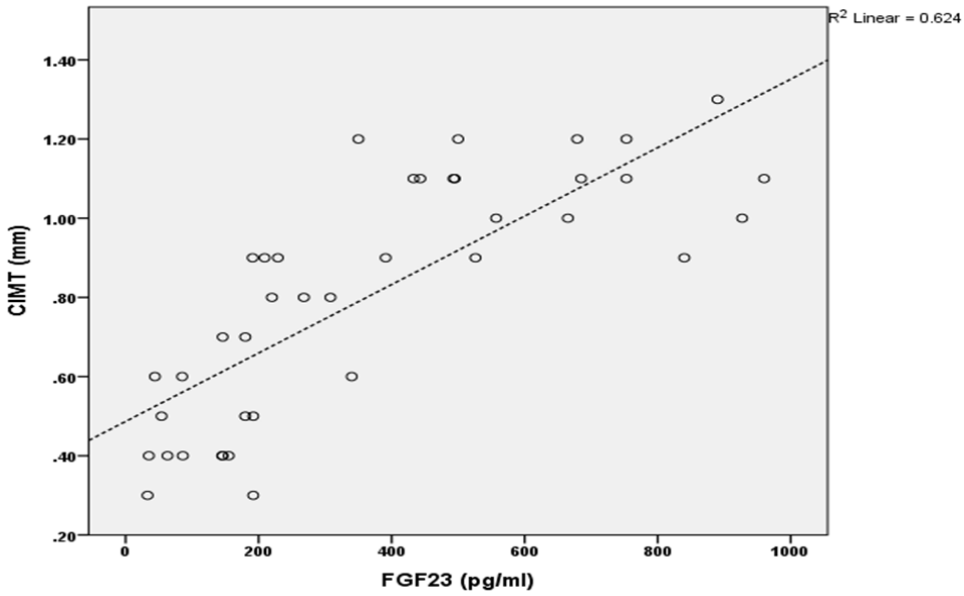


Figure (5): Scatter plot illustrating the correlation between FGF23 and the CIMT. There is strong positive correlation between FGF23 and the CIMT (r = 0.790, p-value < 0.001)

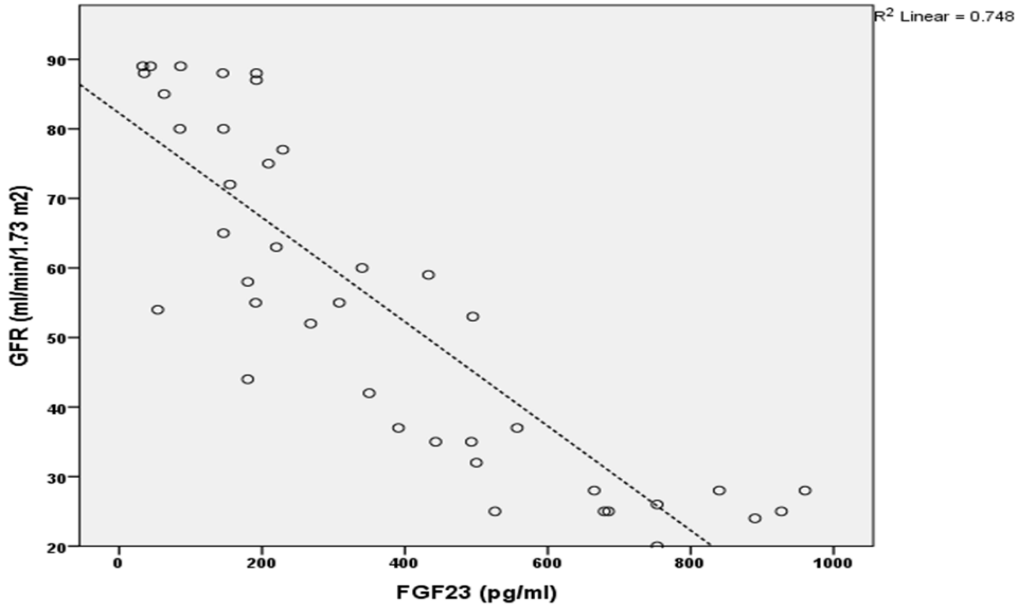


Figure (6): Scatter plot illustrating the correlation between FGF23 and the GFR. There is strong negative correlation between FGF23 and the GFR (r = -0.865, p-value < 0.001).

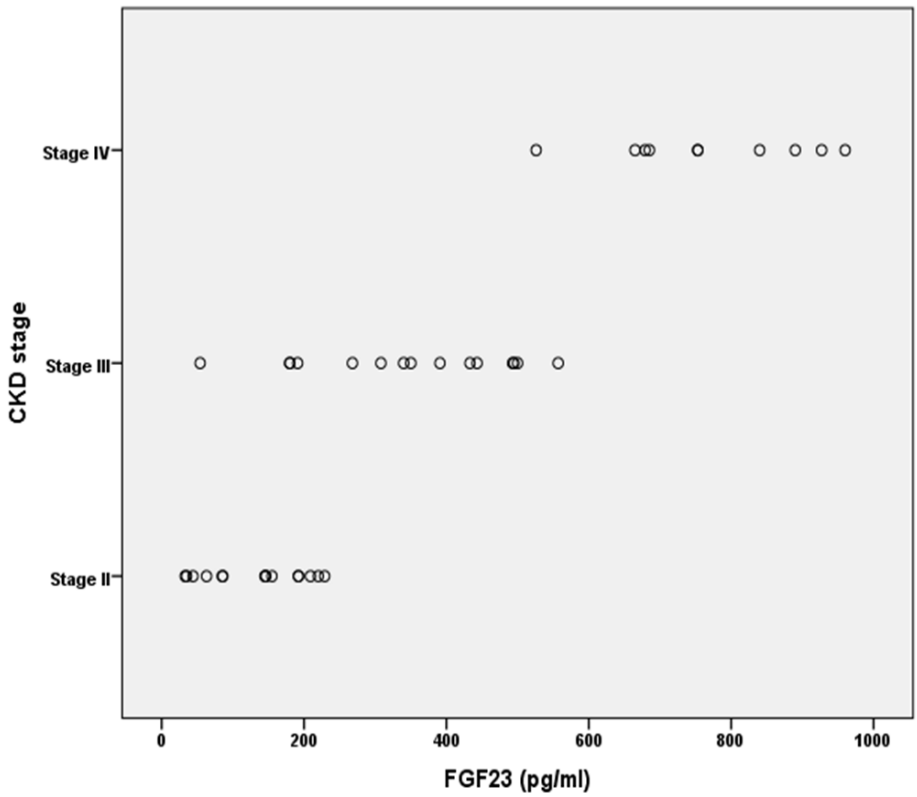


Figure (7): Scatter plot illustrating the correlation between FGF23 and the stage of CKD. There is strong positive correlation between FGF23 and the stage of CKD (rho = 0.854, p-value < 0.001).

DISCUSSION

Chronic kidney disease is associated with increased CIMT. This suggests that even in young children uremia and/or metabolic alterations have a profound impact on the arterial structure and function leading to cardiovascular morbidity and mortality **(Elshafie et al., 2016)**.

Laboratory assessment of FGF23 and Doppler U/S for those patients was the main scope of our study to detect if there is relation between FGF23 and CIMT for detection of preclinical atherosclerosis.

As regard to demographic data, our study revealed that the prevalence of CKD was higher in males **(65%)** than in females **(35%) (Table 1, Figure 1)**. Male preponderance may be explained by higher prevalence of obstructive uropathy in our study

which is generally common in boys.

This agrees with **(Warady and Chadha, 2007)** who reported male preponderance in their studies on CKD children on conservative treatment.

Also **(Harambat et al., 2012)** reported that incidence and prevalence of CKD is greater in males than females because of the higher frequency of congenital abnormalities of the kidney and urinary tract (CAKUT) in males.

Regarding the etiology of CKD, obstructive uropathy was the commonest cause of CKD in our study **(table 2, figure 2)**.This agrees with **(Becherucci et al., 2016)** who reported that the commonest cause of CKD was CAKUT, followed by steroid resistant nephrotic syndrome and glomerulonephritis.

FGF-23 is a 32-kDa protein with 251 amino acids with a mean level of 35 pg /mL, synthesized and secreted by bone cells, mainly osteoblasts. It is composed of an amino-terminal signal peptide (residues 1-24), an "FGF-like sequence" (residues 25-180), and a carboxyl-terminal extended sequence (residues 181-251) **(Sarmento-Dias et al., 2016)**.

Our results showed that a **significant differences** between cases and controls as regard to **FGF 23 (P value > 0.001) (table 7, figure 3)**.This agrees with **(Yilmaz et al., 2015)** study done on 91 patient with chronic kidney disease and found that **FGF 23** was significant higher in patients than controls.

Our results also showed that a **significant differences** between cases and controls as regard CIMT **(P value > 0.001) (table 7, figure 4)**.

This agree with the Egyptian study by **(Elshafie et al., 2016)** on 60 children divided into three groups: 20 children had chronic kidney disease on conservative therapy (group I) (predialysis), 20 children were undergoing hemodialysis (group II), and 20 children constituting the control group (group III) and found that there was a significant increase in CIMT in group I and group II when each group was compared with group III.

There was strong positive correlation between FGF23 and the CIMT **(r = 0.790, p value > 0.001) (table 8, figure 5)**. This agrees with **(Yilmaz et al., 2015)** found that positive correlation between FGF23 and CIMT and monitoring of serum FGF-23 may be useful as a non-invasive indicator of subclinical

atherosclerosis in patients with chronic kidney disease.

There was strong negative correlation between FGF23 and GFR **(r = - 0.865, p value > 0.001) (table 8, figure 6)** and strong positive correlation between FGF23 and the stage of CKD **(rho = 0.854, p value > 0.001) (table 7, figure 7)**.

This agree **with (Liu et al., 2017)** in study done on 141 pediatric CKD patients stage I-V and concluded that FGF23 had strong negative correlation with GFR and FGF23 levels rise as renal function declines.

CONCLUSION

- Children with CKD are prone to the development of vascular calcifications becoming more prevalent with the worsening of kidney function and with CKD duration.
- Fibroblast growth factor 23 is considered as a marker of development of endothelial dysfunction and development of preclinical atherosclerosis.
- Measurement of CIMT has a value to diagnose the atherosclerosis in CKD patients.

RECOMMENDATIONS

- Regular follow up for children with CKD include: clinical and laboratory evaluation as well as imaging studies for early detection of renal dysfunction.
- Early and regular Doppler US for all children with CKD to detect early and preclinical atherosclerosis.
- Measurement of CIMT in CKD children to detect early atherosclerotic changes is of significant value.
- FGF23 is considered as noninvasive marker of vascular calcification.
- Every pediatric nephrologist should be aware about FGF 23 and relation of FGF 23 and vascular calcification, progression of CKD and mortality.

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عامل نمو الخلايا الليفيه ٢٣وسمك الباطنه الوسطي للشريان السباتى في الأطفال الذين يعانون من مرض الكلى المزمن

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الهدف من البحث: تقييم عامل نمو الخلايا الليفيه ٢٣ كعامل مسئول عن تصلب الأوعيه الدمويه وتقييم العلاقه بينه وبين سمك الباطنه الوسطى للشريان السباتى في الأطفال الذين يعانون من مرض الكلى المزمن.

الطريقة والأدوات: أجريت هذه الدراسةِ على اربعون طفلا (سته وعشرون ذكرا واربعة عشره انثي) يعانون من قصور مزمن في وظائف الكلي(من الدرجة الثانية الي الدرجة الرابعة) من المترددين على العيادة المشتركة لكلى ومسالك الأطفال بمستشفى الحسين الجامعى بالقاهرة، بالاضافة لعشرين طفل أصحاء كمجموعة ضابطة والذين تتراوح أعُمارهم ٢ إلى ١٧ سَنَة، وبعد أخذ التاريخ المرضى الكامل والفحص السريري الشامل، والأبحاث الطبية والفحوصات بالأشعه اللازمه تم عمل سونار بالدوبلر علي الشريان السباتي وقياس مستوي عامل نمو الخلايا الليفيه ٢٣ لجميع الحالات التي اخضعت لهذه الدراسه.

النتائج :خلصت النتائج الي ارتفاع مستوي عامل نمو الخلايا الليفيه ٢٣ عند الأطفال الذين يعانون من مرض الكلي المزمن وكذالك ايضا زيادة سمك الباطنه الوسطي للشريان السباتي عند هؤلاء الأطفال وأن هناك ترابط ايجابي بين عامل نمو الخلايا الليفيه ٢٣ وسمك الباطنه الوسطي للشريان السباتي.

الإستنتاج: مستوى عامل نمو الخلايا الليفيه ٢٣ يعتبر علامة هامة للتنبؤ بتصلب الشرايين عند الأطفال الذين يعانون من مرض الكلي المزمن وينصح بالكشف المبكر والمتابعه الدوريه لتصلب الشرايين عن طريق سونار بالدوبلر لجميع الأطفال الذين يعانون من مرض الكلي المزمن.