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Association of Fibroblast Growth Factor-23 and Tumor Necrosis Factor-α with Autosomal Dominant Polycystic Kidney Disease in Iraqi Patients

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Abstract: Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease and leads to end-stage renal disease. And it is the reason for hemodialysis in 7 to 10 percent of patients. Our study aimed to make assessment of the non-genetic factors on disease prognosis which are Tumor necrosis factor-alpha (TNF-α) and Fibroblast growth factor 23(FGF23). Blood samples were obtained from Nephrology and transplantation center/medical city and Dialysis center/lmamain Al Kadhemain Medical Teaching Hospital from ADPKD patients who have diagnosed with ADPKD according to ultrasonography diagnostic criteria and positive family history through the period from February 2021 to January 2022. The study was conducted to evaluate the serum immunological markers FGF23 and TNF-α in Iraqi ADPKD by using Enzyme Linked Immunosorbent Assay (ELISA). Result showed significant differences between ADPKD patients and apparently heathy control.

Keywords: TNF-α , FGF23, ADPKD Iraqi patients ,Kidney disease .

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease and leads to end-stage renal disease (1). Most ADPKD cases caused by mutations in *PKD1gene* (85%) and *PKD2* gene (15%) (2), which are likely affected by environmental and other genetic factors (3). Disease prevalence ranged from low to high, prevalence estimated between 1:1000 and 1:2500 (4)while , Willey *et al.*, revealed that ADPKD occurs in 1 of 800 live births and it affects 500,000 persons in the United States and 4 million to 6 million worldwide and it is the reason for hemodialysis in 7 to 10 percent of patients(5).

Formation of tremendous bilateral renal fluid filed cyst is the characteristic of autosomal dominant polycystic kidney disease(6).In ADPKD there are extra renal manifestations such as liver cysts, intracranial aneurysms, hypertension and cardiovascular symptoms (7).

Patients with mutations in *PKD1*gene have a more severe renal cystic disease than mutations in *PKD2*(8). In ADPKD patients in their adult life and earlier renal function remains normal while cysts form and expand progressively and cause an irreversible decline in kidney function (9). High frequency of cysts development in human ADPKD suggests that nongenetic factors may also contribute to cyst formation (2).While GFR decline occurs late in ADPKD patients and can be preserved for several decades, possibly because of the compensatory hyperfiltration (10) . Pei *et al.*, find that FGF23 was elevated in ADPKD patients compared to other CKD patients matched for glomerular filtration rate and normal renal function (11). Fibroblast growth factor 23 (FGF23) regulates phosphate homeostasis, elevation in FGF23 levels in ADPKD serum out of proportion to the decrease in overall GFR may be due to FGF23 is a prognostic biomarker for loss of functioning nephrons that is masked early on by hyperfiltration (12). El Tears *et al* ., in their study they found that TNF-α was the only inflammatory cytokine that independently and positively correlated with plasma FGF23(13).

Tumor Necrosis Factor-α an inflammatory cytokine, it is one of the possible physiological factors that may causes syctogensis , TNF-α present in cystic fluid of humans with ADPKD, it was noticed that treated mouse with TNF-α result in formation of cysts, due to the disruption of the polycystin-2 locus on plasma membrane and primary cilia through a scaffold protein, FIP2, which is induced by TNF-α(14)

The present study aimed to make assessment of the non-genetic factors which are TNF-α, FGF23 on disease prognosis

Material and methods

Samples

This study included one hundred patients, 50 individuals (Fourteen family and 8 unrelated patients) Autosomal dominant polycystic kidney disease Iraqi patients collected form Nephrology and kidney transplantation center/medical city and Dialysis center/lmamain Al Kadhemain Medical Teaching Hospital. Fifty individuals were control. Diagnosis of ADKPD patients depending on examination performed using abdominal ultrasound and family history. Ultrasound assessment showed numerous cysts of variable sizes bilateral kidneys according to the diagnosis criteria (11). All patients and control were distributed according to age, gender, hypertension and age of diagnosis, also for patients were distributed according to cyst occurrence or absence.

Estimated GFR was calculated by simple software programs in IOS software, using CKD-EPI Creatinine equation (2021) for ages 18-80 and Revised Bedside Schwartz Formula For ages 1-17 according to

(https://www.kidney.org/apps/professionals/egfr-calculator).

Elisa analysis: Sandwich Eliza technique was used to measure TNF-α and FGF23 serum following company instruction, for Human FGF23 (Sunlog, China) and Human TNF-α (sunlog, china). Using plot software, plot the standard curve, absorbance of standard on the Y axis, and standard concentration on the X axis. The linear equation was used to calculate the values of the samples based on the standard curve with OD.

Statistical analysis

Data analysis was carried out using SPSS-28 (Statistical Packages for Social Sciences- version 28). The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ^2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05. Correlation between two quantitative variables using Pearson correlation. Receiver Operating Characteristic "ROC" curve technique was used in order to determine the use of any parameter as diagnostic or screening tool for disease and the ability to determine the "cut-off value" Cutoff point test with present Sensitivity & Specificity for TNF-α and FGF-23 parameters.

Results and discussion

Results of the present study revealed that there were no significant differences between different ages or gender in the prevalence of the disease. Table 1 showed the result with mean age (33.7±16.7)

Few studies have been carried out in Iraq about the prevalence of ADPKD patients. The present study designed to demonstrate the personal characteristics of polycystic kidney disease patients influence with incidence of the disease. Results revealed a wide range of age for the disease occurrence from 18 months – to 78 years, accordingly we can conclude that this disease is not age dependent. Although the highest ratio of patients at the age 20-29 years, which could be attributed to the biological mechanisms associated with disease development and renal function decline. These results are disagree with Willey *et al.,* who found significant differences in the incidence and prevalence of ADPKD by gender and age (15). All affected ADPKD patients have the potential for early diagnosis in life since it's a hereditary disease.

Study results demonstrate no striking findings for the gender in the development of the disease probably due to small sample population. Understanding of sex and gender-specific differences in the etiology, mechanisms and epidemiology of chronic kidney disease (CKD) could help nephrologists better address the needs of their patients. It is controversial issue if gender correlate with disease incidence, In spite of evident population-based studies indicate that CKD epidemiology differs by sex, affecting more women than men, especially with regard to stage G3 CKD. (16).while Alsaedi *et al* ., found that the

frequency in male was higher than female which is in contrast to Romão *et al.*, who find that higher prevalence in women (17; 18).

Analysis of data related to disease criteria (Table 2) showed that the mean age of diagnosis were (23.8±15.3) .According to family history information, results showed from 50 patients , 10 were with no family history, 40 with family history, divided in to 17 inherited from father side and 23 from mother side. However from the 40 family history patients there were with multi cyst and with no cyst.

Although the ultrasonography is the current radiological method of choice for screening in adults(11;19 ;20) . Study results indicated that not all the ADPKD patients were positive for cyst occurrence by sonography, but according their positive family history they were considered as patients. As referred by Gimpel *et al.*, (21) that sonographic detection of one or more cysts in an at-risk child is highly suggestive of ADPKD, but a negative scan cannot rule out ADPKD in childhood. Genetic testing is recommended for children with very-early-onset symptomatic disease even with negative family history. Diagnostic criteria were based on patients who had a family history of PKD1. In 2009, the criteria were modified to include patients with a family history of PKD2 who began cyst development at a later age and with a lower number of cysts and at-risk adults of unknown gene type(11 ; 22)

Evaluation of estimated GFR (eGFR), results showed that eGFR was decreased significantly in ADPKD disease patients with mean (94.470±50.371) (Figure 1).

The GFR is considered the optimal way to measure kidney function. In patients with kidney cysts the progressive growth of cysts results in loss of functioning nephrons and progressive deterioration of renal function. However, a decrease in GFR may also be a marker of kidney disease and precedes the onset of kidney failure; therefore a persistently reduced GFR is a specific diagnostic criterion for CKD. Present marked decrease in GFR of studied patients indicate Loss of kidney function. Although loss of kidney function is not always linear and rapid; prolonged stabilization of GFR can occur even in advanced disease, many individuals with ADPKD have a linear decline in eGFR (23). Study results did not include a definite description of the GFR decline linearity but it may considered it a linear decline, since GFR decline in patients in linear pattern with time according to (24).GFR stays constant during childhood and early adulthood as a result of compensatory glomerular hyper filtration followed, once the limit of compensation is exceeded, by a constant, rapid decline. A linear progressive decline in GFR was thought to be a general feature of progressive chronic kidney diseases (25). However, the time course of decline in glomerular filtration rate (GFR) is poorly defined. The serum parameters of FGF-23 and TNF-α were elevated significantly in ADPKD patients when compare with healthy control (Figures 2 and 3).

Table -1. Distribution of ADPKD and control according to age and gender

		Polycystic Kidney		Apparently Healthy		P value
		No	%	No	%	
Age (years)	<10years	3	6.0	4	8.0	0.105
	10---19	8	16.0	2	4.0	
	20---29	13	26.0	16	32.0	
	30---39	10	20.0	19	38.0	
	40---49	7	14.0	5	10.0	
	=>50years	9	18.0	4	8.0	
	Mean±SD (Range)	33.7±16.7 (1.5-78)		30.1±12.8 (1.5-62)		0.228
Gender	Male	23	46.0	15	30.0	0.099
	Female	27	54.0	35	70.0	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.						
#Significant difference between two independent means using Students-t-test at 0.05 level.						

Table 2. Distribution of ADPKD patients according to age of diagnosis, cyst occurrence, and family history.

		Polycystic Kidney	
		No	%
Age at diagnosis (years)	<10years	9	18.0
	10---19	10	20.0
	20---29	16	32.0
	30---39	10	20.0
	=>40years	5	10.0
	Mean±SD (Range)	23.8±15.3 (18M-76Y)	
Cyst occurrence	Multi	36	72.0
	None	14	28.0
Family history	Father side	13	26.0
	Mother side	27	54.0
	No	10	20.0

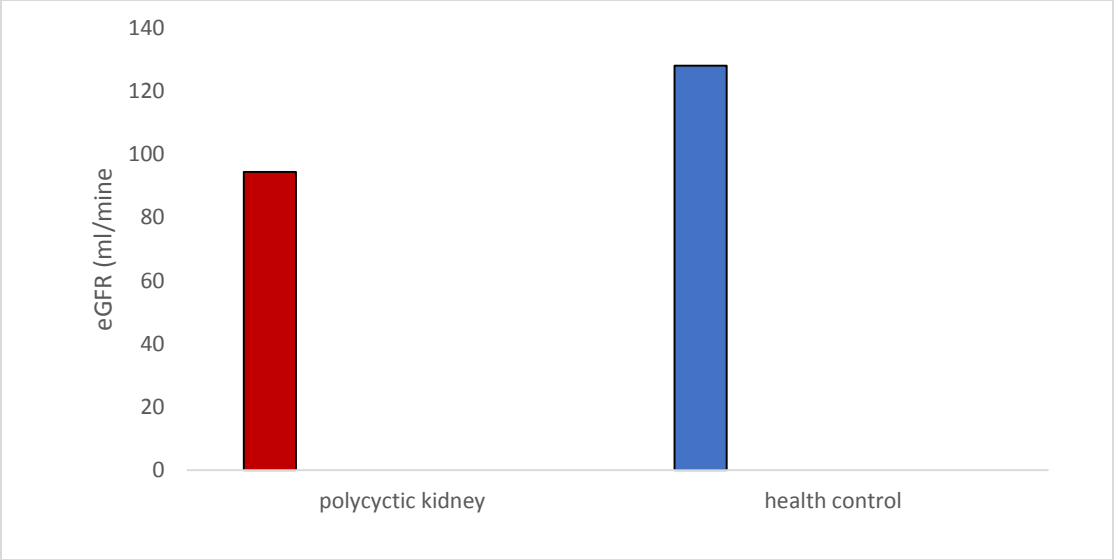

Figure 1. Estimated GFR in ADPKD patients and control

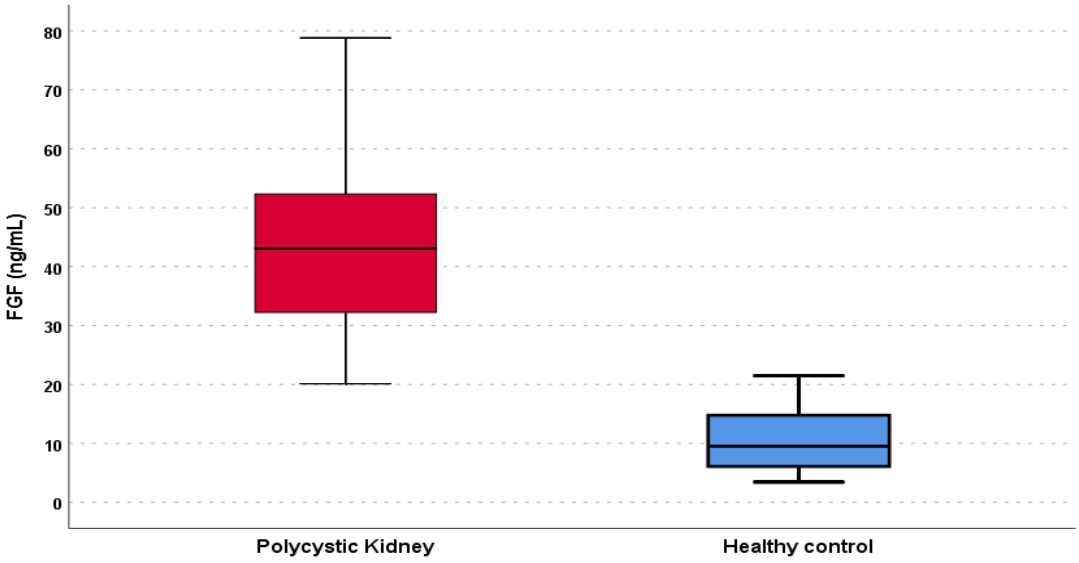

Figure 2. FGF-23 in ADPKD and control Iraqi population

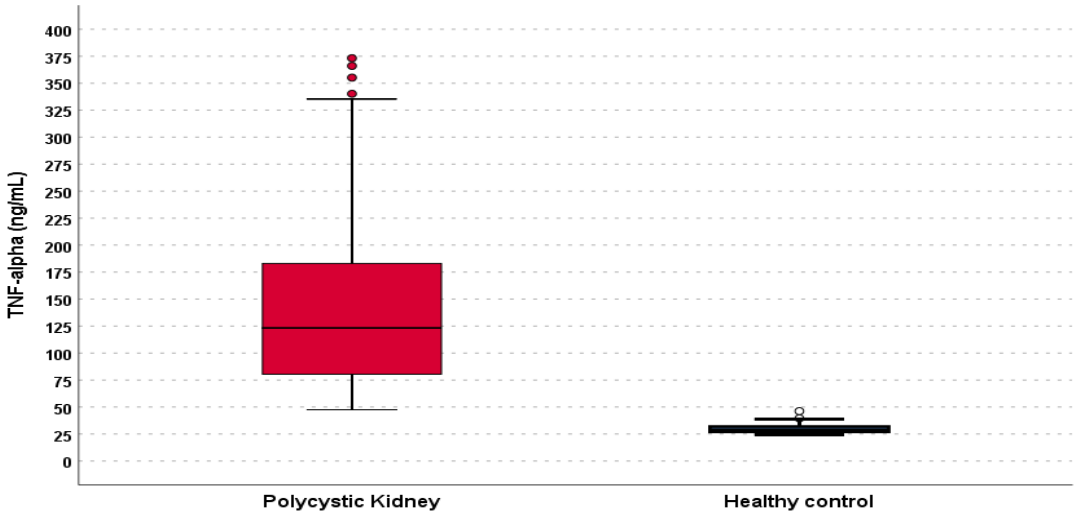

Figure 3. TNF-α in ADPKD patients and control

The references values for the parameters using Roc analysis at 95% confidence interval represented in (Table 3 and figure 4). According to the type of data distribution (patients and control) data analyzed by converting data to Box-Cox conversion to gate more accurate analysis. Results revealed that the lower and upper limit for FGF-23 was 0.993 to 1.000, and for TNF-alpha was 1.000 to 1.000.

Table 3. Reference intervals values and distribution of FGF-23 and TNF-α in the autosomal dominant polycystic kidney disease patients and control individuals at 95% confidence interval.

Test Result Variables	Area Under the Curve (AUC)	Std. Error	P value	95% Confidence Interval	
				Lower limit	Upper limit
FGF (ng/mL)	0.998	0.003	0.0001	3.92550	43.06800
TNF-alpha (ng/mL)	1.000	0.001	0.0001	24.76300	47.96000

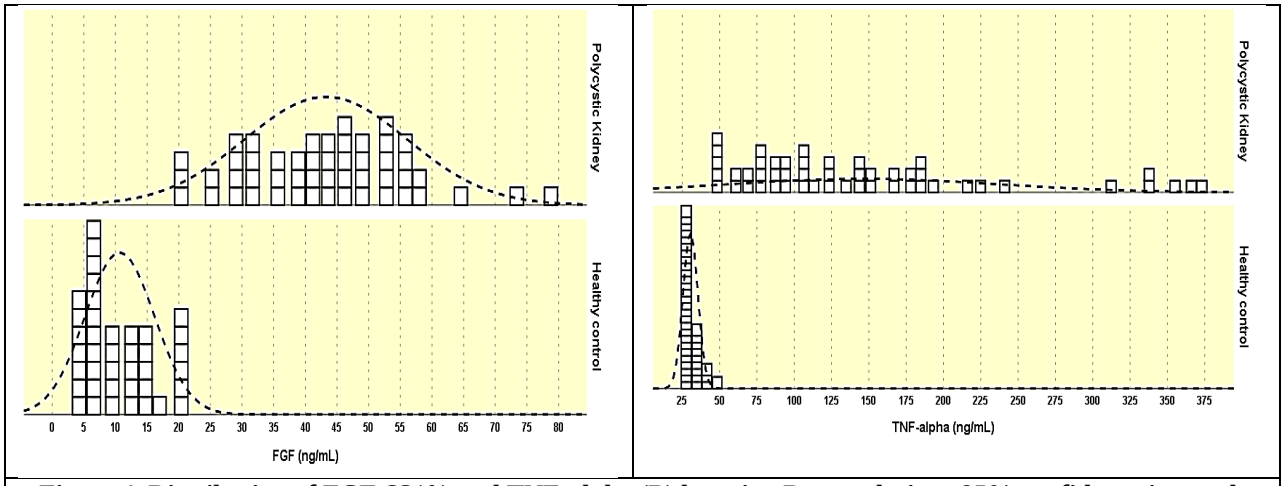

Figure 4. Distribution of FGE-23(A) and TNF-alpha (B) by using Roc analysis at 95% confidence interval.

Results in table 4 and figure 4, revealed that the cutoff point between sensitivity (98.0) and specificity (92.5) for FGE23 was 20.409 and for TNF-α the cutoff point between sensitivity (100) and specificity (1100) was 46.85

Table 4 Estimation of cutoff point test between sensitivity and specificity for FGF-23 and TNF-α parameters that uses a main test for diagnosis of ADPKD in Iraqi patients

parameters	Sensitivity	Specificity	Cutoff point	95% CI
FGF-23 (ng/mL)	98.0	92.5	20.409	39.590 - 46.899
TNF-α (ng/mL)	100	100	46.85400	121.143 - 172.159

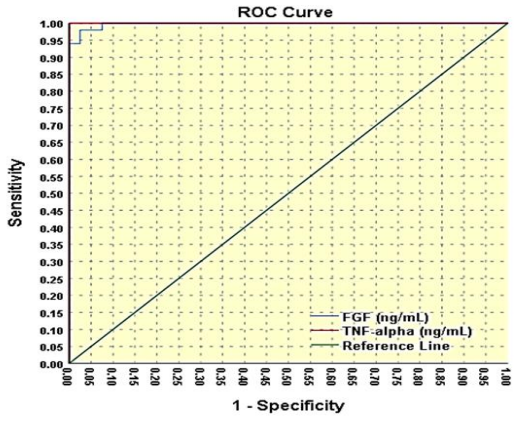

Figure 4. Cutoff point test with present Sensitivity and Specificity for FGF-23 and TNF-α parameters

Fibroblast growth factor is secreted primarily by the bone, followed by the thymus, heart, and other tissues in low levels, but in polycystic kidney disease it expression in the cyst-lining epithelium of kidneys (13). Increases in fibroblast growth factor 23 precede kidney function decline in autosomal dominant polycystic kidney disease; however, the role of fibroblast growth factor 23 in autosomal dominant polycystic kidney disease has not been well characterized. However, fibroblast growth factor 23 did not substantially improve prediction of rapid kidney function decline (26). Recent researches direct to consider FGF23 as a predictor of ADPKD Progression (27,28). Higher serum fibroblast growth factor 23 concentration was associated with kidney function decline, height-adjusted total kidney volume percentage increase, and death in patients with autosomal dominant polycystic kidney disease. In patients with ADPKD, as the disease stage advanced, serum FGF-23 levels increased(29). Tumor necrosis factor alpha, a primary proinflammatory cytokine, is considered to be a potential mediator involved in several kidney diseases, such as PKD (14). The expression of TNFα mRNA is upregulated in Pkd1 mutant renal epithelial cells and kidney tissues from Pkd1 knockout mice(12) . The present increased serum TNF in serum of studied patients in agreement with Zhou*., et al* who explained the prognosis of the disease with mechanism for TNFα signaling in regulating cystic renal epithelial cell proliferation in ADPKD (30). Inflammation plays an important role in polycystic kidney disease (PKD), increased release of inflammatory cytokines including tumor necrosis factor alpha were upregulated and inflammation-related pathways were activated (31,32).

Regardless of the importance of reference interval (RI) for suggested biomarkers in diagnosis of disease in Iraqi patients, there are a few reports on this field which were published. For this reason the current study in our knowledge represented the first work to establish the reference interval for Iraqi ADPDK patients FGF23 and TNF-α and assists the clinician to reach a definitive diagnosis. Results of the ROC analysis for subjects included in the present study, might be the first of kind in ADPKD patients, it outcomes anew sight on consider the serum FGF23 a predictor for the disease incidence, if we compare estimated values with the predicted values obtained from the present results.

Conclusion

Analysis of patient claims data allowed us to generate an overall diagnosed prevalence and to provide insight into age and gender differences in ADPKD incidence and prevalence even using a small sample, Most importantly result reveled that FGF23 and TNF-α could be used as non-genetic biomarker for early detection for the loss of functioning nephrons.

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