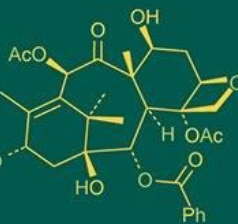
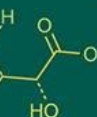
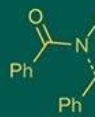


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Study the relationship of erythropoietin and interleukin 1 beta with some biochemical variables in chronic kidney disease patients

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Abstract

Background: Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years. Inflammatory cytokine status of patients with CKD represents a fruitful area of research world-wide. Many hypotheses were mounted in this regards and most of them based on levels of certain inflammatory markers.

Objective: To evaluate the serum levels of some inflammatory markers in patients with CKD and to predict their correlation with the disease severity.

Materials and Methods: This is a cross sectional survey study. It was performed during December, 2023 through April, 2024 at Al- Fallujah teaching hospital. A total of 60 doctor-diagnosed patients with chronic kidney diseases for different causes and in variable two stages of the disease 3A and 3B. The tow staged 3A, 3B level distribution and age range were (60 men; 40-92 years), while 30 healthy persons were chosen as control group. An informed consent was taken from all the participants submitted to the study. Age, gender, neck, waist hip and hypertension were recorded. Then, 5-ml venous blood sample was taken from each participant and sera were isolated via centrifuge.

Results: The serum levels of the following markers were determined using ELISA; Erythropoietin, Interleukin-1 and some biochemical change. Serum levels of all studied Pro and Anti- inflammatory cytokine markers were significantly higher in patients with CKD than those in the control subjects, p value < 0.05. An exclusion is the non-significant Lower in serum level of Zn, Fe, Mg and Na in CKD stages other than. while levels of K are significantly higher in moderate CKD than other stages of renal impairment included in this study (p value < 0.05).

Keywords: Chronic kidney disease, cytokine, GFR, interleukin-1, erythropoietin

Introduction

Chronic kidney disease is a global general medical issue which causes of morbidity and mortality worldwide and increasingly recognized as a global public healthcare challenge, especially in developing countries ^[1]. (CKD) represents a condition with reduced renal function, which is defined by glomerular filtration rate (GFR) < 60 mL/min per 1.73 m² or with present markers of kidney damage or both for more than 3 months and with implications for health. Markers of kidney damage are defined by Kidney Disease Improving Global Outcomes (KDIGO) guidelines from 2012 as albuminuria, urine sediment abnormalities, electrolyte, and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, and history of kidney transplantation. CKD should be categorized into categories according to GFR ranging from G1-G5 (GFR in G1 ≥ 90, G2 60-89, G3a 45-59, G3b 30-44, G4 15-29, and G5 ≤ 15 mL/min/1.73 m²), and albuminuria should be accounted for in categories from A1-A3 (albumin excretion rate in A1 < 30, A2 30-300, and A3 > 300 mg/24 h) ^[2].

Erythropoietin, often abbreviated as EPO, is a glycoprotein hormone primarily produced by the kidneys in response to hypoxia (low oxygen levels in the blood) and an endogenous glycoprotein which stimulates red blood cell production. kidney is responsible to produce Erythropoietin and stimulates the diversion and differentiation of committed erythroid progenitors in the bone marrow ^[3, 4]. Its main function is to stimulate the production of red blood cells (erythrocytes) in the bone marrow, thus playing a crucial role in erythropoiesis (the formation of red blood cells).

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Erythropoietin has important clinical applications, particularly in the treatment of anemia, especially in chronic kidney disease patients, cancer patients undergoing chemotherapy, and those with certain other medical conditions [5, 6]. This study's findings contribute significant insights into the prevalence and characteristics of Erythropoietin Resistance in patients with (CKD). The etiology of erythropoietin resistance is multifactorial, involving factors such as iron deficiency, inflammation, malnutrition, and the accumulation of uremic toxins [7]. Interleukin-1 (IL-1) is a pro-inflammatory cytokine that plays a significant role in the immune response and has been implicated in various diseases, including chronic kidney disease (CKD), which may causing kidney damage when elevated levels of IL-1 contribute to the inflammatory environment [8].

Pathophysiology of CKD in CKD, persistent inflammation leads to fibrosis and progressive loss of kidney function. IL-1 promotes the activation of other inflammatory pathways, including the recruitment of immune cells to the kidneys, which can worsen renal injury. Studies have shown that IL-1 inhibitors can reduce inflammation and improve kidney function in experimental models of CKD. Clinical Implications elevated IL-1 levels have been associated with worse outcomes in CKD patients. Monitoring IL-1 levels may provide insights into disease progression and response to therapy [9, 10, 11]. In this observational study, we focused on patients diagnosed with chronic kidney disease (CKD) accompanied by Inflammatory cytokine, who were visiting Fallujah Teaching Hospital in Anbar Governorate.

Materials and Methods

The study spanned 8 months, from December 2023 to April 2024, and employed purposive sampling to select patients. Supervisor clearance was duly obtained prior to the commencement of the study. Sixty men with CKD who were present at the Unit of CKD at the Al-Fullwja Teaching

Hospital between December 2023 and April 2024 were enrolled on the study, using the American CKD Association's 1981 revised principles for the classification of CKD [12]. thirty age and sex matched healthy control subjects with no history of any CKD or autoimmune disease were also enrolled in the study, which was given ethical clearance by the ethics board of the University of Tikrit. Written informed consent was provided by all of the contributors.

Statistical Analysis

Analysis of data was carried out using the Graph Pad prism version 7. Data were presented in simple measures of mean and standard deviation (SD) values. The significance of difference of different means (quantitative data) was tested using analysis of variance (ANOVA) test for difference among more than two independent means. Statistical significance was considered whenever the P value was equal or less than 0.05. Pearson correlation was calculated for the correlation between two quantitative variables.

Receiver operator curve (ROC) analysis was also employed. The area under the ROC curve gives an idea about the usefulness of a tested parameter in differentiating between three groups (one of which is a control group). A cut-off value was calculated for each variable, well as sensitivity (Sen %), specificity (spec%) and likelihood ratio (LHR).

Results

According to the (Table 1). The mean SD and SEM are used to express the results for the patients and the control group. In this study, the mean of men with CKD were (35.43) and (56.47) ($p=0.001$) respectively. The levels of EOP were significantly higher in men with CKD than in healthy (21.75) ($p=0.001$). The results indicate that CKD had considerably higher serum IL-1 levels (140.71) and (199.63) (ng/ml) respectively compare than control healthy (86.45) ($P=0.001$).

Table 1: Mean serum levels of some inflammatory cytokine markers in 60 patients with CKD compared to 30 control subjects measured by ELISA and spectrophotometer

GROUPS	Stat.	EOP	IL-1 β	eGFR	Zn	Fe	Zn/Fe	Mg	K	Na
Controls	Mean	21.756	86.451	115.93	133.37	116.93	1.176	2.424	4.19	142.2
	Std. D	5.818	11.961	14.924	26.274	25.846	0.281	0.362	0.63	4.86
	Median	21.569	83.706	116.00	126.00	104.00	1.188	2.410	4.09	142.0
3A CKD	Mean	35.431	140.715	52.29	116.45	99.34	1.190	2.056	4.36	139.4
	Std. D	6.566	15.380	10.86	31.918	16.545	0.346	0.321	0.77	7.56
	Median	33.892	137.908	50.60	113.00	96.00	1.141	2.020	4.30	138.0
3B CKD	Mean	56.471	199.623	36.84	109.00	91.59	1.279	1.881	4.77	135.3
	Std. D	12.037	23.058	8.21	21.816	23.612	0.488	0.366	0.35	7.91
	Median	54.815	199.620	36.00	107.50	87.00	1.222	1.900	4.70	136.0
	P-value	<0.001	<0.001	<0.001	0.003	<0.001	0.526	<0.001	0.001	0.001

Analysis of the Receiver Operating Characteristic (ROC) curve revealed which biomarkers were most useful for identifying CKD group 3A patients who had experienced EOP [AUC: 0.954, Positive if COV: > 27.92, Sen %: 86.21, Spec %: 85.19, LHR:5.819], IL-1 [AUC: 0.996, Positive if COV: > 111.6, Sen %: 96.55, Spec %: 96.30, LHR:26.07], K Ions [AUC: 0.571, Positive if COV: > 4.250, Sen %: 60.71, Spec %: 59.26, LHR:1.490], While other variables

GFR [AUC: 1, Positive if COV: < 79, Sen %: 100, Spec %: 100] Na Ions [AUC: 0.713, Positive if COV: < 140.9, Sen %: 64.29, Spec %: 74.07, LHR:2.480] Fe [AUC: 0.751, Positive if COV: < 100.5, Sen %: 68.87, Spec %: 70.37, LHR:2.328] Mg [AUC: 0.773, Positive if COV: < 2.255, Sen %: 65.52, Spec %: 66.67, LHR:1.966] Zn [AUC: 0.666, Positive if COV: < 119, Sen %: 58.62, Spec %: 59.26, LHR:1.439] (Table 2- 3A).

Table2-3A: Area Under ROC Curve for all Analyzed Parameters in Studied Subjects

Chronic Kidney Disease Group 3A					
Parameter	AUC	Positive if COV	Sensitivity%	Specificity%	Likelihood Ratio
Zn µg/dL	0.6660	< 119.5	58.62	59.26	1.439
T. Mg mg/dL	0.7733	< 2.255	65.52	66.67	1.966
Fe µg/dL	0.7529	< 100.5	68.97	70.37	2.328
Zn/Fe	0.5019	< 1.143	51.72	51.85	1.074
K Ions mmol/L	0.5714	> 4.250	60.71	59.26	1.490
Na Ions mmol/L	0.7130	< 140.9	64.29	74.07	2.480
eGFR ml/min	1.000	< 79.00	100.0	100.0	
EPO mIU/mL	0.9540	> 27.92	86.21	85.19	5.819
IL-1β ng/L	0.9962	> 111.6	96.55	96.30	26.07

All the tested parameters had some validity in predicting CKD, since class 3B the ROC area was significantly higher than class 3A the ROC area of which biomarkers were most useful for identifying CKD group 3B who had experienced EOP [AUC: 1, Positive if COV: > 35.15, Sen %: 100, Spec %: 100], IL-1 [AUC: 1, Positive if COV: > 137.3, Sen %: 100, Spec %: 100], K Ions [AUC: 0.806, Positive if COV: > 4.48, Sen %: 79.41, Spec %: 74.07], While other variables

GFR [AUC: 1, Positive if COV: < 73.75, Sen %: 100, Spec %: 100] Na Ions [AUC: 0.766, Positive if COV: < 140.9, Sen %: 70.59, Spec %: 74.07] Fe [AUC: 0.791, Positive if COV: < 100.0, Sen %: 70.59, Spec %: 70.37] Mg [AUC: 0.850, Positive if COV: < 2.135, Sen %: 72.73, Spec %: 74.07] Zn [AUC: 0.756, Positive if COV: < 116.5, Sen %: 61.76, Spec %: 62.96] (Table 3-3B).

Table 3-3B: Area Under ROC Curve for all Analyzed Parameters in Studied Subjects

Chronic Kidney Disease Group 3B					
Parameter	AUC	Positive if COV	Sensitivity%	Specificity%	Likelihood Ratio
Zn µg/dL	0.7565	< 116.5	61.76	62.96	1.668
T. Mg mg/dL	0.8502	< 2.135	72.73	74.07	2.805
Fe µg/dL	0.7919	< 100.0	70.59	70.37	2.382
Zn/Fe	0.5534	> 1.194	55.88	55.56	1.257
K Ions mmol/L	0.8061	> 4.480	79.41	74.07	3.063
Na Ions mmol/L	0.7669	< 140.9	70.59	74.07	2.723
eGFR ml/min	1.000	< 73.75	100.0	100.0	
EPO mIU/mL	1.000	> 35.15	100.0	100.0	
IL-1β ng/L	1.000	> 137.3	100.0	100.0	

As shown in Table 4-3A we do observe significant and insignificant association between IL-1β ng/L and other studied parameters. insignificant negative correlations were detected of IL-1 ng/L with Zn, Fe, Mg, K, and Na ($r=-0.082$, $P=0.463$), ($r=-0.098$, $P=0.376$), ($r=-0.287$, $p=0.008$) respectively, while Significant positive correlations were detected of IL-1 ng/L with IL-1 ng/L, EOP mIU/mL, GFR ml/min, ($r=1$, $P<0.0000$), ($r=0.698$, $P=0.0001$), ($r=0.660$, $p=0.0001$), respectively.

Table 4-3A: Association of IL-1β ng/L with Studied Parameters

Chronic Kidney Disease 3A		
Parameter	r (IL-1β ng/L)	p-value
IL-1β ng/L	1	0
EOP mIU/mL	0.698	<0.0001
Zn µg/dL	-0.212	0.116
Mg mg/dL	-0.388	0.003
Fe µg/dL	-0.437	0.0007
Zn/Fe	0.127	0.352
K mmol/L	0.124	0.367
Na mmol/L	-0.181	0.186
eGFR ml/min	-0.839	<0.0001

As shown in Table 5-3A we do observe significant and insignificant association between EOP mIU/mL and other studied parameters. insignificant negative correlations were detected of EOP mIU/mL with Zn, Fe, Mg, K, and Na ($r=-0.341$, $P=0.010$), ($r=-0.181$, $P=0.181$), ($r=-0.302$, $p=0.024$), ($r=0.205$, $P=0.134$), ($r=-0.121$, $P=0.380$) respectively, while Significant positive correlations were detected of EOP

mIU/mL with EOP mIU/mL, IL-1 ng/L, GFR ml/min, ($r=1$, $P<0.0000$), ($r=0.698$, $P=0.0001$), ($r=0.660$, $p=0.0001$), respectively.

Table 5-3A: Association of EOP mIU/mL with Studied Parameters

Chronic Kidney Disease 3A		
Parameter	r (EOP mIU/mL)	p-value
EOP mIU/mL	1	0.0000
IL-1β ng/L	0.698	<0.0001
Zn µg/dL	-0.341	0.010
Mg mg/dL	-0.302	0.024
Fe µg/dL	-0.181	0.181
Zn/Fe	-0.212	0.117
K mmol/L	0.205	0.134
Na mmol/L	-0.121	0.380
eGFR ml/min	-0.660	<0.0001

Table 6-3B shows effect of reaserch cytokines on studied groups in class 3B. a significant higher in EOP and IL-1 Levels than on studied group in class 3A with CKD. As shown in Table 6-3B we do observe significant and insignificant association between IL-1β ng/L and other studied parameters. insignificant negative correlations were detected of IL-1 ng/L with Zn, Fe, K, and Na ($r=-0.464$, $P=0.0001$), ($r=-0.477$, $P=0.0001$), ($r=-0.462$, $p=0.0001$), ($r=-0.440$, $p=0.0001$) respectively, while Significant positive correlations were detected of IL-1 ng/L with IL-1 ng/L, EOP mIU/mL, GFR ml/min, Mg mg/dL, ($r=1$, $P<0.0000$),

($r=0.823$, $P=0.0001$), ($r=-0.927$, $p=0.0001$), ($r=-0.527$, $p=0.0001$) respectively.

Table 6-3B: Association of IL-1 β ng/L with Studied Parameters

Chronic Kidney Disease 3B		
Parameter	r (IL-1 β ng/L)	p-value
IL-1 β ng/L	1	0
EOP mIU/mL	0.823	<0.0001
Zn μ g/dL	-0.464	<0.0001
Mg mg/dL	-0.527	<0.0001
Fe μ g/dL	-0.477	<0.0001
Zn/Fe	0.162	0.213
K mmol/L	0.462	<0.0001
Na mmol/L	-0.440	<0.0001
eGFR ml/min	-0.927	<0.0001

While levels were higher in Table 7-3B we do observe significant and insignificant association between EOP mIU/mL and other studied parameters. insignificant negative correlations were detected of EOP mIU/mL with Fe, and Na ($r=-0.386$, $P=0.002$), ($r=-0.355$, $P=0.005$), respectively, while Significant positive correlations were detected of EOP mIU/mL with EOP mIU/mL, IL-1 ng/L, GFR ml/min, Mg mg/dL, K mmol/L, Zn μ g/dL, ($r=1$, $P<0.0000$), ($r=0.823$, $P=0.0001$), ($r=0.827$, $p=0.0001$), ($r=-0.549$, $p=0.0001$), ($r=0.515$, $P=0.0001$), ($r=-0.516$, $P=0.0001$) respectively.

Table7-3B: Association of EOP mIU/mL with Studied Parameters

Chronic Kidney Disease 3B		
Parameter	r (EOP mIU/mL)	p-value
EOP mIU/mL	1	0
IL-1 β ng/L	0.823	<0.0001
Zn μ g/dL	-0.516	<0.0001
Mg mg/dL	-0.549	<0.0001
Fe μ g/dL	-0.386	0.002
Zn/Fe	-0.002	0.989
K mmol/L	0.515	<0.0001
Na mmol/L	-0.355	0.005
eGFR ml/min	-0.827	<0.0001

Discussion

The present study investigated Erythropoietin Resistance in patients with Chronic Kidney Disease (CKD) and found that 11.4% of the participants exhibited resistance. This prevalence is somewhat consistent with previous studies, although there is variability depending on the CKD stage and patient demographics. For example, a study by Singh *et al.* reported a slightly higher prevalence of Erythropoietin Resistance in a similar cohort [13-16]. Erythropoietin (EPO) production and function can be influenced by various trace elements e.g. Iron, Zinc, etc. The diagnostic performance of various predictors, such as serum iron, Magnesium, Zinc, Potassium, and Sodium, was evaluated in our study. The area under the ROC curve for serum variables was 0.666, 0.7733, 0.7529, 0.5019, 0.5714, 0.7130, respectively suggesting good diagnostic accuracy. This is consistent with the findings of Weiss and Goodnough, who reported a similar AUROC for serum variables in predicting Erythropoietin Resistance [17, 18, 20]. The results from this study definitely show that the studies of serum erythropoietin in patients with renal failure should continue and expand [19]. Interleukin-1 (IL-1) is a pro-inflammatory cytokine that plays a significant role in the pathophysiology of chronic kidney disease (CKD). Here are some of its role

in Inflammation as it IL-1 is a key mediator of the inflammatory response. In CKD, elevated levels of IL-1 contribute to chronic inflammation, which can exacerbate kidney damage and progression of the disease. as that mechanism IL-1 promotes the activation of immune cells, leading to the release of additional inflammatory cytokines and chemokines, perpetuating the inflammatory cycle. while in role IL-1 is involved in the development of renal fibrosis, a common consequence of CKD. It stimulates the proliferation of fibroblasts and the production of extracellular matrix components, leading to scarring and loss of kidney function. while in erythropoiesis Inhibition IL-1 can inhibit erythropoiesis, contributing to anemia commonly seen in CKD patients. Mechanism by promoting inflammation, IL-1 can interfere with erythropoietin production and the responsiveness of bone marrow to erythropoietin, leading to reduced red blood cell production. Cardiovascular Risk it role large Chronic inflammation mediated by IL-1 is associated with increased cardiovascular risk in CKD patients. IL-1 contributes to atherosclerosis and cardiovascular complications, which are prevalent in individuals with CKD. Potential therapeutic target role given its involvement in CKD progression, IL-1 has been considered a potential therapeutic target. Inhibiting IL-1 signaling may reduce inflammation and fibrosis, potentially slowing the progression of CKD [21,22,23].

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