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Association of *Interleukin-18 (IL-18) gene* expression with some serum parameters in a sample of patients with chronic kidney disease

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Abstract

Background IL-18 is a cytokine, which is a type of protein that helps regulate the immune system and inflammatory responses in the body. a pro-inflammatory cytokine, has garnered attention for its potential role in the progression of CKD. **Aim** the aim of study to evaluated level of Interleukin-18(IL-18), urea, creatinine, sodium, potassium, calcium and phosphate compared with control and study correlation between them. In addition, estimation of Fold of Interleukin-18. **Methods** the research involved 100 individuals from Iraq, divided into two groups. The first group for CKD patients(n:50), The second group (n:50) serving as the control. Participants were recruited from the dialysis center at Madinat Al-Amamin Al-Kadhim in Teaching Hospital in Baghdad between October 2023 and February 2024. **Results** the Kidney function tests showed higher urea and creatinine levels in CKD patients (76.58±45.90, 5.07±5.11) compared to controls (26.04±5.12, 0.33±0.12). In CKD patients, mean ± SD of K, Cl, and PO4 rose (4.62±0.81, 106.90 ± 5.63, and 4.91 ± 1.14) at p-value <0.001. Na levels was similar between CKD patients (137.70±3.71) and controls (138.4±3.02) at p-value >0.05. Increased mean ± SD of Interleukin-18 Conc. (112.22 ± 100.96) at p-value <0.05. The calculated fold expression ratios for the gene were 1.48 in CKD patients and 1.00 in control. The expression of the IL-18 gene showed highly significant differences (p<0.001) in CKD patient group when compared to control group. **Conclusion**: IL-18 demonstrates high sensitivity and specificity. Because it is not dependent on several non-renal parameters.

Key words: CKD, Interleukin-18 gene, Interleukin-18 level, Creatinine, Urea, electrolyte.

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Introduction.

Chronic Kidney Disease (CKD) is a progressive disorder characterized by a gradual decline in kidney function that can persist over months or even years. When symptoms of renal impairment persist for more than three months, it is often identified as chronic kidney disease (1, 2). An essential aspect of CKD classification is that it can usually be determined through non-invasive tests. This classification is significant because it is associated with various outcomes, including the progression of kidney disease, cardiovascular disease, and overall mortality. Additionally, it allows for the implementation of treatment measures at earlier stages, which can slow disease progression, lower cardiovascular disease (CVD) risk, and improve both quality of life and survival (3, 4). Conventional biomarkers, such as blood creatinine, may

not be effective in detecting early signs because of their susceptibility to factors such as age, gender, muscle mass, and diet (5) .IL-and is driven by the activation of fibroblasts and the deposition of extracellular matrix proteins. IL-18 is a member of the interleukin-1 family of cytokines and is primarily produced by macrophages, dendritic cells, and other innate immune cells. It is synthesized as a precursor protein (pro-IL-18) and is activated by caspase-1, a process that converts it into its mature, biologically active form. IL-18 exerts its effects by binding to its receptor, IL-18, which leads to the activation of nuclear factor kappa B (NF-κB) and the subsequent release of pro-inflammatory cytokines (6). Elevated IL-18 levels have been associated with increased fibrosis in the kidneys. Fibrosis is a key feature of CKD and is driven by the activation of fibroblasts and the deposition of extracellular matrix proteins (7). This discovery indicates that IL-18 has the potential to detect high-risk groups at an earlier stage and with greater accuracy (8). This enables prompt interventions that can potentially slow down the evolution of the disease and minimize complications (9). This study aims to examine the relationship between levels of Interleukin-18 in patients with CKD in a representative sample of Iraqi adults.

Material and methods Samples collection

The research involved 100 individuals from Iraq, divided into two groups. The first group comprised 50 CKD patients, including females and males, The second group consisted of 50 serving as the control, including females and males, Participants were recruited from the dialysis center at Madinat Al-Amamin Al-Kadhimin Teaching Hospital in Baghdad between October 2023 to February 2024. Diagnosis was conducted through clinical examination by a

18 levels have been associated with increased fibrosis in the kidneys. Fibrosis is a key feature of CKD

Nephrologist. All participants provided written and informed consent. The control group was sourced from the National Blood Transfusion Center, selected based on specific criteria, such as no pregnancy, no familial history of renal failure, immunodeficiency, malignancy, or defects in the parathyroid gland or osteoporosis.

Sample Collection

Ten ml of venous blood was carefully drawn into appropriate sample tubes. Samples were allowed to clot for 30 minutes, and then centrifuged at 4000 rpm for 10 minutes. The yielded serum was divided into aliquots and stored at -70 ºC until the time of analysis. Serum was analyzed for urea, creatinine, potassium, sodium, albumin, calcium, and phosphorus [10].

Measurement of electrolyte and biochemical parameters

In this study, analysis Japanese company (FUJIFILM, Code No. 293-77601) was used to measure [Na], [Cl], [K], urea, creatine, calicum and phosphate level.

Determination of serum *Interleukin-18*.

The level of Interleukin-18 was measured using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Cat. No. In-Hu2146).

Gene expression of IL-18 by Quantitative Real Time PCR (qRT–PCR)

The total RNA underwent reverse transcription into complementary DNA (cDNA) using the EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit, as provided by TransGen Biotech Co in China, within a reaction

volume of 20 µl, following the manufacturer's guidelines. The Quantitative PCR System (Germany). Each qRT-PCR reaction involved 2 µl of cDNA, 1 µl for both the forward and reverse primers (with a concentration of 10 µM) as listed in Table-2, and 10 µl of the PerfectStartTM Green qPCR SuperMix kit from TransGen Biotech Co, China. The thermal profile consisted of an initial step at 94 °C for 5 minutes (one cycle), followed by 40 cycles involving denaturation at 94 °C for 5minutes, annealing at 58 °C for *IL-18* and GAPDH for 15 seconds, and extension at 72 °C for 20 seconds. The final dissociation stage spanned from 55 to 95 °C, with each degree lasting 5 seconds. The specificity of the amplified product was confirmed through melting curve analyses. Real Time PCR (qRT–PCR) was carried out using the QIAGEN Rotor gene Q Real-time
To evaluate the relative expression of the *IL-18* gene in the samples from the study groups, the expressions were normalized to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the 2-Ct technique [10]. Compared to the healthy controls, the data were presented as the fold change in *IL-18* gene expression within the study groups. This allowed for a normalization of the expression levels against the reference gene (*GAPDH*). The median fold expression levels of *IL-18* in the study groups were then utilized to assess whether there were statistically significant differences in *IL-18* gene mRNA expression levels.

Table 1: The study's designed primers

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta (°C)
	IL-18 (Gene Expression)			
Forward	GGAATTGTCTCCCAGTGCAT	20	126	60
Reverse	CAGACCTTCCAGATCGCTTC	20		
	GAPDH- Glyceraldehyde3-phosphatedehydrogenase			
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	72
Reverse	GAGCCCCAGCCTTCTCCATG	20		

IL-18 genes expression Calculation

The fold variations of the quantitative expression of the mature RNAs were determined using the relative cycle threshold ($2^{-\Delta\Delta Ct}$) approach, which was first described by Livak and Schmittgen in 2001. It is the ratio of the relative gene expression between the control group and the test group. The double delta (threshold cycle) analysis was used to assess the expression of *IL-18* genes, in which the housekeeping reference genes. The calculations were as the following: By using the real-time cycler software, the (CT) was calculated for each sample. The samples were duplicated and the average results were computed. The Ct values for the target genes *IL-18*, which were being evaluated in both patients and controls, were reported. The ΔCt, or difference in CT

values, which is also referred to as the "normalized raw data," was determined by subtracting the specified normalization factor from the Ct value of each target gene and the housekeeping gene.

ΔCt (control)=CT (gene)-CT(HKG) ΔCt (patient)=CT (gene)-CT(HKG) ΔΔCt=ΔCt(patient)- ΔCt (control)

Ethical approval

The present study was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki. The technique was performed following the acquisition of both verbal and written consent from the patients before collecting the samples, in compliance with document number (4782 in November 25, 2023).

Statistical analysis

The results of the present study were analyzed related to the objectives and presented according to the general description of the sample. Microsoft Excel 2010 and SPSS (version 25) software was used for statistics analysis. Microsoft package (Excel and Word). The data are expressed as mean ± SD, Differences were considered significant when p values was $P < 0.05$.

RESULTS

The results in Table 2, shows mean ± SD in different parameter in patients with CKD. Kidney function test as urea and creatinine increased in CKD patients that were (76.58±45.90 ,5.07±5.11) mg/dl respectively as compared with control (26.04±5.12, 0.33±0.12) mg/dL. As well as mean ± SD of K, Cl, and PO4 increased in CKD patients (4.62±0.81, 106.90 ± 5.63, and 4.91 ± 1.14) respectively at p-value <0.001. While no differences in Na level between CKD patients (137.70±3.71) respectively and control (138.4±3.02) respectively at p-value >0.05. Furthermore, increase mean ± SD of Interleukin-18 Conc. (112.22 ± 100.96) respectively at p-value <0.01.

Table(2): the descriptive statistics for all the study variable that collected from CKD patients and controls

	Patients	Controls	
Parameter	Mean±SD		P-value
Urea mg/dl	76.58±45.90	26.04±5.12	0.0001***
Creatinine mg/dl	5.07±5.11	0.33±0.12	0.0000001***
Na millimole/ L	137.70±3.71	138.4±3.02	0.1
K millimole/ L	4.62±0.81	4.13±0.55	0.00000001***
Cl millimole/ L	106.90±5.63	102.9±3.69	0.00000001***
Ca	8.47±0.96	8.88±0.96	0.0000001***
PO_4	4.91±1.14	4.35±0.71	0.0000001***
Interleukin-18 Conc. pg/mol	112.22 ± 100.96	2.35±0.97	0.0000000001***

Correlation between the parameters

In Table (3), showed correlation between Log2 Fold Change (LFC) of Interleukin-18 Gene Expression and level of Phosphate, Calcium, Sodium, potassium, Chloride, creatinine, and urea. The result shows no correlation between LFC of Interleukin-18 gene expression with Calcium (r=-0.09) and Sodium (-0.08). While moderate negative correlation between LFC of Interleukin-18 and Phosphate (r=0.2) Potassium(r=.0.2), but moderate positive between LFC of Intrleukin-18 and Chloride (0.3). As well as positive correlation between LFC of Interleukin-18 Potassium(r=0.4), Urea(r=0.4), Creatinine(r=0.5).

Table 3: Pearon's Product-Moment Correlation of Log2 Fold Change (LFC) of *Interleukin-18* Gene Expression with Serum Parameters in Patients and Control Samples

	t test value	df	p-value	95 percent confidence interval	Correlation(r)
Phosphate	2.8178	98	0.00585**	0.08174012, 0.44618858	0.273763
Calcium	0.92954	98	0.3549	-0.2846770, 0.1048573	-0.09349
Potassium	2.5662	98	0.0118*	0.05733843, 0.42633568	0.250931
Sodium	-0.8346	98	0.406	-0.2758749, 0.1142952	-0.08401
Chloride	3.9643	98	0.00014***	0.1891445 0.5295058	0.371752
Urea	5.698	98	1.28E-07***	0.3352848 0.6332234	0.498851
Creatinine	6.2717	98	9.94E-09***	0.3800151 0.6641224	0.537132

IL-18 gene expression

The results demonstrated that using GAPDH for normalization in qRT-PCR is a highly reliable approach, particularly in clinical studies. Furthermore, the 2-Ct value and the ratio of 2-Ct for various study groups compared to the control group were employed to assess changes in GAPDH expression across different study groups, as presented in Table-4. The 2 - ΔΔCt value for CKD patients was 0.000331049, and for control it was 0.000223413. The calculated fold expression ratios for the gene were 1.48 and 1.00, respectively. These variations in gene fold expression among the study groups underscore the utility of the GAPDH gene as a reliable control. The expression of the *IL-18* gene showed highly significant differences ($p<0.001$) in CKD patient group when compared to control group.

DISCUSSION

IL-18 known as interferon-gamma inducing factor is a protein which in humans is encoded by the *IL18* gene, the protein encoded by this gene is a pro-inflammatory cytokine (11). This study focused on several markers, such as serum IL18, creatinine, urea, fold of interleukin-18, and some electrolytes, to be examined as early indicators for patients with CKD. This study shows the highest mean of Interleukin-18 in CKD.

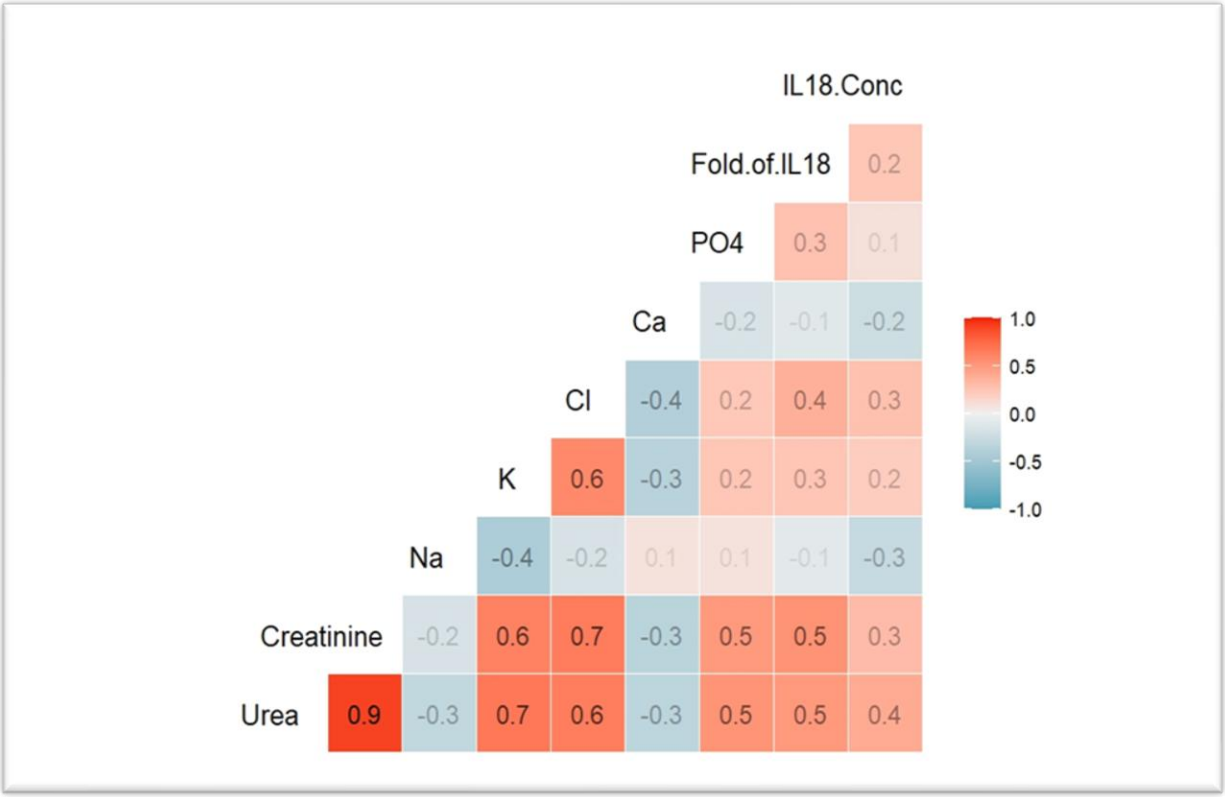

Figure (1): summary the correlation between serum variable with IL18 concentration and the log fold change of IL18 gene expression tested against each other using Person method, r=0; there is no relation between the variable, r= 0 - 0.30; negligible correlation, r=0.30 - 0.50; moderate correlation, r=0.50 - 1 highly correlated, r=+: perfectly positively correlate and r= -: perfectly negatively correlated.

patients, as compared with control. on the other hand up regulation Fold of IL-18 in CKD patients, this result may be due to Interleukin-18 has different genetic patterns, mutations, or re-organization in gene expression. Notably, this study is the first to reveal potential *Interleukin-18* gene expression level ranges in CKD patients and healthy controls in the Iraqi population. We found, IL-18 mRNA levels in Iraqi CKD patients were significantly higher than those in the healthy control group. Specifically, the gene expression was 1.48 times higher in CKD patients than healthy controls. In molecular research, the use of housekeeping genes relies on

the assumption that their expression remains consistent in the cells or tissues being studied (12). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the most commonly employed housekeeping genes for assessing gene expression data (13). In a study conducted by Robert and colleagues (14), the expression of 1,718 genes across 72 different types of normal human tissues was investigated using quantitative real-time polymerase chain reaction (qRT-PCR), with GAPDH serving as a reference gene. The IL-18 gene, which encodes Interleukin-18, is located on chromosome 11 in humans. Specifically, it is situated on the long arm of chromosome 11 at position 11q13.1.(15). Another study discussed the potential mechanisms by which IL-18 contributes to kidney damage, including its role in inflammation and fibrosis. Elevated IL-18 levels could serve as an indicator of disease severity and progression. Consequently, serum IL-18 levels can be considered as an early indicator of renal disorders. Additionally, identifying chronic kidney disease (CKD) in its first phase is crucial for prompt management to effectively mitigate the decline in kidney function, hence enhancing both survival rates and quality of life (16). As compared to the control group, CKD patients had the highest mean levels of urea and creatinine, according to the study (17). Most medical experts agree that elevated blood urea and serum creatinine levels are important indicators of decreased kidney function in patients with chronic kidney disease (CKD). The current study recorded an increase in K, Cl, and PO4 levels in the patient group compared to the healthy group. The cause of this is kidney disease, as the fundamental role of the kidney is to regulate the equilibrium of bodily fluids, electrolytes, and acid-base balances. Chronic renal illness frequently results in a range of abnormalities, including elevated levels of phosphate and potassium, as well as metabolic acidosis. These irregularities can result in serious problems such as vascular calcification, muscular atrophy, and mineral bone diseases. (18). The current study also demonstrated no substantial reduction in Na levels. Patients with CKD are at an increased risk of developing hyponatremia due to their impaired ability to concentrate or dilute urine (19).

CONCLUSION

The results of this study indicated higher serum levels of Interleukin-18 in patients with chronic kidney disease compared to the control group. This indicates a direct association between IL-18 levels with kidney function. An increase in the level of some salts was observed. The level is inconclusive, especially in some individuals with diabetes for a longer period and other comorbidities.

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