

Association analysis of IL-6 polymorphisms and relevant biomarkers in patients with renal failure

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Received October 7, 2025; Accepted February 20, 2026

DOI: 10.3892/br.2026.2213

Abstract. The present study aimed to clarify the relationship between polymorphisms present in the interleukin-6 (IL-6) gene (rs1800795 and rs1800797) and the susceptibility to chronic kidney disease (CKD), while concurrently examining critical biochemical markers (urea, creatinine, albumin, calcium, phosphate and glucose) alongside the levels of immunological factors [IL-6, tumor necrosis factor- α (TNF- α) and antinuclear antibodies (ANAs)]. A detailed investigation was carried out on a group of 51 subjects (22 male and 29 female subjects) who were 40–60 years old. The serum concentrations of IL-6, TNF- α and ANA were quantitatively evaluated using enzyme-linked immunosorbent assays, whereas the levels of various biochemical markers were determined by the Cobas 6000 analytical system. Genomic DNA was isolated and the IL-6 single nucleotide polymorphisms were genotyped by employing the PCR-restriction fragment length polymorphism methodology. The analysis of body weight showed notable differences between the male and female participants; however, other chemical markers did not indicate any sex-related variations. The correlation analyses illustrated moderate statistically significant relationships between albumin and calcium, together with an association between urea and phosphate, while the levels of IL-6, TNF- α and ANA portrayed a non-significant fluctuation. Analysis of the IL-6 gene showed that carriage of the rs1800795 C allele was associated with increased susceptibility to CKD, whereas rs1800797 was not significantly associated with CKD. Integrating genetic, immunological and biochemical data may improve understanding of CKD susceptibility, although validation in larger cohorts is required.

Introduction

Immunomodulators encompass elements that can influence the immune response and are derived from both exogenous sources and endogenous production (1,2). Within the field of immunology, the mode of action of the agent is essential for its classification and can be distinctly classified into immunostimulants, immunoadjuvants or immunosuppressants. Research indicates that immunostimulants have the potential to enhance the protective functions of the immune system against pathogenic microorganisms, typically operating through mechanisms that demonstrate notable efficacy. When the innate and adaptive immune systems commence their activities, these agents may yield enhanced outcomes (3). The biological framework of humans is made up of two primary parts that bolster their defense systems: Innate immunity and adaptive immunity. Furthermore, both innate and adaptive immune systems can be categorized into cellular and humoral immune responses (4). Acute renal failure is marked by a rapid deterioration of kidney function, evidenced through a decrease in the glomerular filtration rate (GFR) (5).

Cytokines are small, secreted proteins with a molecular weight of <40 kDa that are produced by a diverse spectrum of cells to regulate and modulate the immunological response (6). The release of pro-inflammatory cytokines initiates the activation of immune cells and subsequently stimulates the production and secretion of additional cytokines (7,8). However, a previous investigation has indicated that the simultaneous release of both pro-inflammatory and anti-inflammatory cytokines is crucial for any cellular and humoral immune response (9). The pleiotropic cytokine interleukin-6 (IL-6) plays a notable regulatory role in hematopoietic homeostasis and immunological signaling cascades, metabolic pathways and organogenesis. The significance of IL-6 in a variety of renal pathologies, including lupus nephritis, IgA nephropathy, diabetic nephropathy, acute kidney injury (AKI) and chronic kidney disease (CKD), is increasingly recognized (10). Specifically, IL-6 is integral to the pathophysiology of renal impairment and it possesses significant prognostic implications regarding the extent of such an impairment (11). The human IL-6 gene is located on chromosome 7p21 and consists of five exons with four introns

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Key words: immunomodulatory, gene polymorphisms, renal failure, biochemical markers

in between. Several single nucleotide polymorphisms (SNPs) in its promoter region, including -174 G>C, -634 C>G, -572 G>C and -597 G>A, have been linked to the control of IL-6 transcriptional activity (12). Amongst the identified variants, the -174 G>C SNP within the promoter region has garnered the greatest research focus, owing to its documented impact on modulating IL-6 gene expression (13,14).

Antinuclear antibodies (ANAs) comprise a diverse array of autoantibodies that exhibit reactivity towards structural components of the cell nucleus. The ANA assay serves as a preliminary diagnostic tool for the identification of autoimmune disorders (15). ANAs have also been detected in cases of acute and chronic infections, malignancies, adverse reactions to pharmacological agents and even among individuals with no discernible health issues (16). ANAs can directly target self-derived proteins, leading to disruptions in DNA synthesis and protein biosynthesis, which may result in immune dysregulation, metabolic dysfunctions of organs and further complications. Thus, under certain conditions, elevated levels of ANAs may represent a threat to the physiological integrity of the organism (17).

The kidneys are essential in regulating glucose homeostasis via mechanisms of glucose filtration, reabsorption, utilization and endogenous synthesis. The development of hyperphosphatemia and hypocalcemia in patients with CKD will cause an alteration in the negative feedback systems responsible for modulating parathyroid hormone production, thereby inducing secondary hyperparathyroidism. Thus, the presence of hyperparathyroidism is a notable marker of mineral dysregulation in patients who are affected by CKD (18).

Tumor necrosis factor- α (TNF- α) is a cytokine that exhibits pleiotropic effects across diverse cell types; it has been recognized as a principal modulator of inflammatory responses and participates in the pathogenesis of certain inflammatory and autoimmune disorders. This cytokine is produced by activated macrophages, natural killer cells and T lymphocytes and serves as an initiator for a variety of inflammatory mediators, including additional cytokines and chemokines (19-21). Albumin represents the predominant protein found within human plasma, constituting ~50% of the overall protein composition of serum; it serves as a frequently employed clinical biomarker (22).

While a considerable amount of research has explored the effects of IL-6 gene polymorphisms in relation to CKD, the results have illustrated variability, particularly regarding the relationships between the rs1800795 and rs1800797 variants and the concentrations of circulating IL-6 in individuals with end-stage renal disease (ESRD) receiving hemodialysis. Furthermore, a significant deficiency in current research is apparent, which integrates genetic, immunological and biochemical dimensions through a unified analytical perspective, concentrating on populations originating from the Middle East, particularly those with Kurdish ethnic identities. The effects of sexual disparities associated with these gender distinctions persist to demand comprehensive scrutiny in the scholarly discourse. As a result, the present study aims to resolve this research gap by conducting a detailed investigation into the genetic polymorphisms of IL-6 and their associations with different biomarkers in patients with CKD.

Materials and methods

Patients. A wide-ranging analysis was executed with a cohort of 51 individuals (22 male and 29 female). The individuals were 40-60 years old and all were diagnosed with ESRD (stage V), receiving hemodialysis 3 times a week. In the present study, individuals diagnosed with ESRD were stratified as exhibiting stage V renal failure in accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (23). A GFR <15 ml/min/1.73 m² characterizes ESRD, leading to the requirement for initiating dialysis. Anyone outside the outlined age group, as well as children and teenagers and those with early disease manifestation were excluded from the present study. Samples were collected from dialysis centers located in the Sulaymaniyah Governorate, Kurdistan Region of Iraq in the municipalities of Rania and Qaladeze (Rania Dialysis Center and Qaladeze Dialysis Center), from September 2024 to January 2025.

Controls. In total, 51 unrelated healthy volunteers (20 men and 31 women, age range: 25-60 years) were used for the molecular genetic analysis. These healthy individuals were used only as controls for comparing IL-6 genotype and allele frequencies, they were not included in the clinical or biochemical analyses. Healthy volunteers with a history of chronic kidney disease or other major chronic illnesses were excluded from the present study.

Sample analysis. Specimens were obtained under standard conditions and then separated into two aliquots. The first aliquot was designated for the assessment of biochemical markers and inflammatory cytokines, whereas the second aliquot was reserved for molecular investigations. Serum extraction from whole blood was executed through centrifugation at 1,000 x g for 30 min at room temperature. Prior to the initiation of any analytical procedures, aliquots of the samples were meticulously prepared and stored at -20°C. The enzyme-linked immunosorbent assay (ELISA) methodology was employed to quantify the concentrations of human IL-6, TNF- α and ANAs. The investigation was performed in accordance with the defined principles, methodologies and result analyses as outlined in the kits used. The determination of serum levels of IL-6, TNF- α and ANA was performed with the human IL-6 ELISA (cat. no. QT-EH0201; Wuhan Fine Biotech Co., Ltd.), human TNF- α ELISA (cat. no. QT-EH0302; Wuhan Fine Biotech Co., Ltd.) and ANA (ORGENTEC Diagnostika GmbH; product no. ORG 200; 24 Alegria) kits. All samples were evaluated thrice and the conclusive concentrations were defined through standard calibration curves established using successive dilutions of the indicated standards.

The biochemical parameters (urea, creatinine, albumin, calcium, phosphate and glucose) were systematically analyzed employing the Cobas 6000 analytical system. This system uses enzymatic reaction methods to analyze urea (urease GLDH method), creatinine (enzymatic conversion in conjunction with colorimetric detection) and glucose (hexokinase approach), culminating in marked optical variations through substrate conversion. Colorimetric assessment was also used to ascertain albumin concentration utilizing bromocresol green dye-binding. Calcium concentrations were quantified through

the formation of arsenazo III complexes and phosphate levels were assessed via molybdate ultraviolet complex formation. The apparatus incorporates autonomous calibration with stringent quality management systems, ensuring analytical exactness and consistency.

Genomic DNA isolation and genotyping. After obtaining informed consent, EDTA-anticoagulated peripheral whole blood was drawn from each patient. The extraction of genomic DNA was carried out utilizing the AddPrep Genomic DNA Extraction Kit (AddBio, Inc.; cat. no. 10023). PCR amplification was performed using Add Taq Master Mix, 2X concentration (AddBio, Inc.) in accordance with the manufacturer's guidelines. Genotyping of IL-6 polymorphisms was performed using the PCR-restriction fragment length polymorphism (RFLP) method (Table I). In total, two independent PCR assays were carried out to detect distinct polymorphic loci within the IL-6 gene: *HinIII* (rs1800795, G>C) and *FokI* (rs1800797, G>A). Appropriate primers were used to amplify the corresponding gene regions from the subjects via PCR and the reaction products were digested using the appropriate restriction enzymes (Table I). The digested products were analyzed on 2% agarose gels, stained with Safe Gel Stain Dye and examined under UV transillumination (Fig. 1). Each gel was examined by two observers unaware of the status of the subject. If there was any conflict, the analysis was repeated.

Ethical considerations. The research protocol was approved by the Biology Department, the Scientific Committee and the Directorate of the Research Center at the University of Raparin (Rania, Sulaymaniyah, Iraq; approval no. 71; reference no. 2866; May 28, 2023). Written informed consent to participate was obtained from all participants before enrolment and sample collection.

Statistical analysis. The biostatistical evaluation was conducted employing the SPSS software suite (version 22; IBM Corp.). A one-way analysis of variance was utilized to assess the data (immunological and biochemical parameters). Sex-based comparisons between male and female patients with ESRD were performed using one-way ANOVA. Since only two groups were compared, no post hoc test was required. Pearson's correlation test was used to assess associations among inflammatory, demographic and biochemical parameters. Genotype frequencies in patients and controls were evaluated using the Hardy-Weinberg equilibrium (HWE) test by χ^2 test of each SNP. To analyze IL6 SNP genetic models, the SNPStats tool (<https://www.snpstats.net/>) was used (24). Genotype frequencies, proportions, odds ratios (ORs) and 95% confidence intervals (CIs) are used to summarize logistic regression analysis. The Kruskal-Wallis test was used to analyze serum IL-6 concentrations across rs1800795 and rs1800797 genotypes in patients with CKD. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Demographic characteristics of the investigated cohort. The cohort included 22 male (43.1%) and 29 female (56.9%) patients; this distribution was descriptive, and no statistical

Table I. PCR and restriction fragment length polymorphism procedures and products of the IL-6 gene variants (rs1800795 and rs1800797).

Gene variant	Primers (5'-3')	Thermocycler conditions	PCR product (bp)	Restriction enzyme	Restriction products (bp)
rs1800795 (-174C/G)	F: TGACTTCAGCTTTACTCTTTGT R: CTGATTGGAAACCTTATTAAAG	95°C 4 min, (95°C 30 sec; 58°C 35 sec; 72°C 60 sec) x 35, 72°C 5 min	198	<i>HinIII</i>	C allele: 122, 45 and 31; G allele: 167 and 31
rs1800797 (-597G/A)	F: GGAGTCACACACTCCACCT R: CTGATTGGAAACCTTATTAAAG	95°C 4 min, (95°C 30 sec; 57°C 30 sec; 72°C 30 sec) x 35, 72°C 5 min	527	<i>FokI</i>	G allele: 527; A allele: 461 and 66
F, forward; R, reverse.					

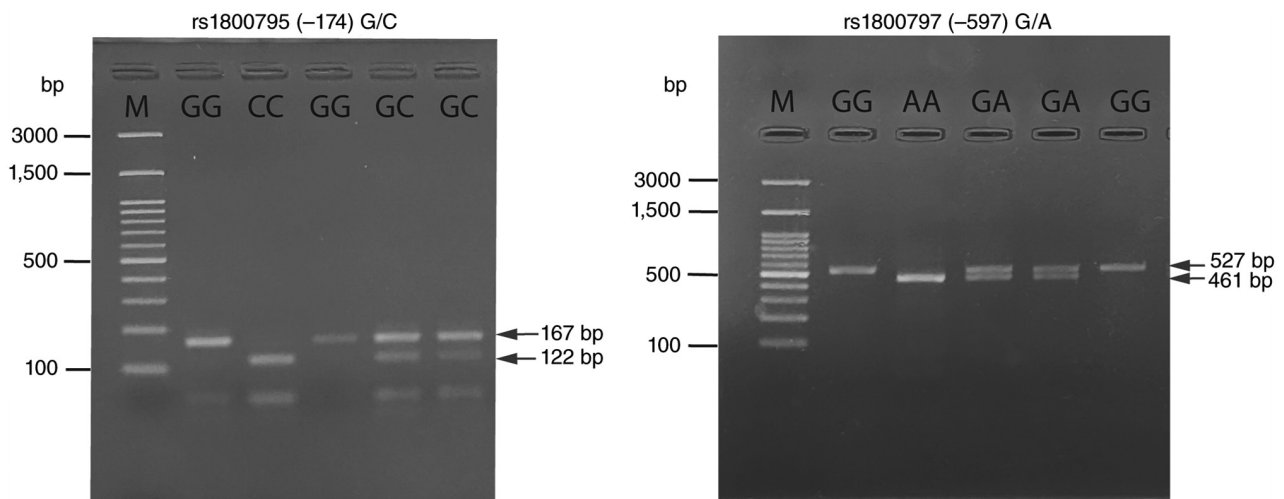


Figure 1. Discriminating between the interleukin-6 alleles of rs1800795 (-174G/C) and rs1800797 (-597 G/A) single nucleotide polymorphisms by PCR and restriction enzyme digestion. Samples from each of the two positions were amplified using specific primers and analyzed before and after digestion with *Hin*III and *Fok*I by agarose gel electrophoresis. The ladder is the 100 bp DNA ladder from Yorkshire Bioscience. Sizes of the largest restriction fragments in each of the two positions are indicated with arrows.

comparison of sex proportions was performed. Among the examined demographic, biochemical and inflammatory variables, body weight was significantly higher in male than female patients ($P=0.007$; Tables II and III), whereas age and the other measured parameters did not differ significantly between sexes. However, the greater number of female participants in this convenience sample should not be interpreted as evidence of a sex difference in CKD prevalence or incidence.

Age showed small, non-significant correlations with IL-6 ($r=0.166$), TNF- α ($r=0.217$), creatinine ($r=-0.185$), urea ($r=-0.062$), albumin ($r=-0.081$), calcium ($r=0.021$), phosphate ($r=-0.265$), ANA ($r=0.110$) and glucose ($r=0.153$) (Table IV). These findings indicate that age was not significantly correlated with the measured inflammatory or biochemical variables in this cohort. Furthermore, creatinine, urea, albumin, calcium, phosphate, TNF- α , ANA and glucose did not differ significantly between male and female patients ($P>0.05$; Table III).

Most inflammatory, demographic and biochemical variables did not show statistically significant correlations. However, significant correlations were observed between albumin and calcium ($r=0.509$, $P<0.01$), urea and phosphate ($r=0.305$, $P<0.05$) and TNF- α and urea ($r=-0.302$, $P<0.05$) (Table IV).

Distributions of IL-6 gene polymorphisms between groups. Table V presents the genotype distributions for the IL-6 promoter variants rs1800795 and rs1800797, together with the HWE statistics for cases and controls (controls were included only for molecular studies for the comparison of genotype and allele frequencies). For rs1800795, 51.0% of patients and 78.4% of controls carried the GG genotype, 43.1 and 19.6% carried the GC genotype and 5.9 and 2.0% carried the CC genotype, respectively. HWE testing showed that rs1800795 genotype frequencies were in equilibrium in both patients ($\chi^2=0.35$, $P=0.55$) and controls ($\chi^2=0.16$, $P=0.69$).

For rs1800797, 58.8% of patients and 68.6% of controls had the GG genotype, 33.3 and 29.4% the GA genotype, and 7.8 and 2.0% the AA genotype, respectively. Genotype distributions

for rs1800797 were also consistent with HWE in both groups (patients: $\chi^2=0.50$, $P=0.47$; controls: $\chi^2=0.17$, $P=0.67$).

Association of rs1800795 and rs1800797 with CKD in different genetic models. The association between genetic models of the IL-6 rs1800795 and rs1800797 genotypes and susceptibility to CKD was performed using SNPStats software (Table VI). For IL6 rs1800795, a statistically significant association with CKD was found under the codominant model ($P=0.013$), with higher frequencies of the GC and CC genotypes observed in patients with CKD compared with the control group. Significant associations were also detected using the dominant model (GC+CC vs. GG, $P=0.0034$) and the overdominant model (GC vs. GG+CC, $P=0.0098$). However, the recessive model did not show significant results ($P=0.30$), which may be due to the lower frequency of CC homozygotes. Although the odds ratios for rs1800795 were <1 across several models, this is likely a result of the reference coding utilized in SNPStats. The GC and CC genotypes were more frequent among patients with CKD than in the control group, indicating an increased susceptibility to the disease for carriers of the C allele.

By contrast, IL6 rs1800797 (*Fok*I) showed no significant association with CKD under the codominant, dominant, recessive and overdominant models (all $P>0.05$). Genotype and allele distributions were comparable between patients with CKD and the controls across all inheritance models.

Serum IL-6 concentrations across rs1800795 and rs1800797 genotypes in patients with CKD. Serum IL-6 levels in patients with CKD were measured using ELISA. The IL-6 levels did not differ significantly among the rs1800795 genotypes (Kruskal-Wallis test, $P=0.069$). The median (min-max) IL-6 concentrations were 1.10 (0.0-53.2), 0.65 (0.0-49.9) and 0.80 (0.7-1.7) pg/ml for the GG, GC and CC genotypes, respectively. Similarly, analysis of the rs1800797 genotypes showed no significant differences in IL-6 levels in patients with CKD (Kruskal-Wallis test $P=0.66$). The median (min-max) IL-6 concentrations were 0.80 (0.0-53.2), 0.80 (0.0-7.7) and 0.75

Table II. Sex-based comparison of biochemical and inflammatory parameters.

Biochemical parameters	Mean $\pm$ standard deviation	Standard error of the mean
Weight (kg)		
Male	74.0909 $\pm$ 10.46950	2.23210
Female	63.2759 $\pm$ 15.51013	2.88016
Creatinine (mg/dl)		
Male	8.3436 $\pm$ 2.30019	0.49040
Female	8.9566 $\pm$ 12.26722	2.27797
Urea (mg/dl)		
Male	135.8773 $\pm$ 27.14893	5.78817
Female	124.6421 $\pm$ 40.35681	7.49407
Albumin (g/dl)		
Male	3.5823 $\pm$ 0.64323	0.13714
Female	3.7803 $\pm$ 0.36188	0.06720
Calcium (mg/dl)		
Male	8.8855 $\pm$ 2.19645	0.46828
Female	8.9748 $\pm$ 1.21011	0.22471
Phosphate (mg/dl)		
Male	5.3368 $\pm$ 1.92865	0.41119
Female	5.1762 $\pm$ 1.60423	0.29790
ANA (IU/dl)		
Male	0.7682 $\pm$ 0.65928	0.14056
Female	0.7586 $\pm$ 0.60975	0.11323
Glucose (mg/dl)		
Male	125.0727 $\pm$ 41.18986	8.78171
Female	146.1862 $\pm$ 79.55239	14.77251
IL6 (pg/ml)		
Male	1.518182 $\pm$ 1.945602	0.414804
Female	6.703448 $\pm$ 15.494849	2.877321
TNF- α (pg/ml)		
Male	7.677273 $\pm$ 11.384071	2.427092
Female	32.282759 $\pm$ 107.180396	19.902900

IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; ANA, anti-nuclear antibody.

(0.2-0.8) pg/ml for the GG, GA and AA genotypes, respectively. These results are summarized in Table VII.

Discussion

Recent findings indicate that biological sex affects the metabolic response to lifestyle interventions. In particular, men and women respond differently to changes in diet and to physical activity, which leads to variations in metabolic outcomes (25). Sex inequities notably impact the global prevalence of CKD and the availability of healthcare services, thereby highlighting the necessity for sex-sensitive healthcare approaches. In addition, it has also been demonstrated that the differences between the sexes are evident in the management of CKD and hypertension, demanding customized therapeutic plans for each sex due to

Table III. Statistical differences in patients: Implications for clinical monitoring.

Biochemical parameters	F-value	P-value ^a	Mean difference
Weight (kg)	6.396	0.007	10.81505
Creatinine (mg/dl)	1.155	0.818	-0.61292
Urea (mg/dl)	2.108	0.266	11.23520
Albumin (g/dl)	5.032	0.169	-0.19807
Calcium (mg/dl)	1.524	0.854	-0.08937
Phosphate (mg/dl)	0.615	0.747	0.16061
ANA (IU/dl)	0.030	0.958	0.00956
Glucose (mg/dl)	5.103	0.263	-21.11348
IL6 concentration (pg/ml)	8.866	0.126	-5.185266
TNF- α (pg./ml)	3.200	0.290	-24.605486

^aTwo-tailed P-values were reported. Male and female patients with end-stage renal disease were compared using one-way ANOVA. IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; ANA, antinuclear antibody.

their biological variations and health risk disparities (26,27). Additionally, it has been asserted that women appear to have a lower risk of developing acute kidney injuries that happen within healthcare facilities, indicating that estrogen has a clear effect in diminishing the related threats (26,27).

The interplay of pro-inflammatory vs. anti-inflammatory cytokine gene alterations is pivotal in understanding CKD emergence; it provides a cross-sectional analysis that scrutinizes the extent to which genetic variations in cytokine genes may affect individual susceptibility to CKD. The present study revealed the importance of genetic traits in modulating inflammation among those with CKD, arguing that variations in cytokine genes linked to both inflammation types might have a role in how the disease develops (28). The KDIGO clinical practice guidelines provide a framework for the classification and management of kidney disease, emphasizing early detection, monitoring of renal function and management of underlying causes. In patients receiving hemodialysis, fluid status is managed through dialysis prescription and clinical monitoring (23). The KDIGO guidelines are systematically applied to establish uniform criteria to ensure consistency in the management of patients suffering from AKI and to enhance clinical methodologies within the discipline of nephrology (23,29).

Empirical research reveals that, due to the protective characteristics of estrogen, women are less susceptible to AKI and recover more swiftly, while men are more likely to suffer from intensified damage related to androgens (30-34). In alignment with the expected anthropometric variations associated with biological sex, in the present study, male participants exhibited a markedly increased body mass compared with female participants. However, no notable differences were identified between the sexes concerning biochemical or renal metrics, suggesting that their metabolic profiles were similar. This finding corresponds with the characteristics of the intended population, which comprised

Table IV. Pearson correlation coefficients among the inflammatory, demographic and biochemical parameters.

Variable	IL-6 (pg/ml)	TNF- α (pg/ml)	Age (years)	Weight (kg)	Creatinine (mg/dl)	Urea (mg/dl)	Albumin (g/dl)	Calcium (mg/dl)	Phosphate (mg/dl)	ANA (IU/dl)	Glucose (mg/dl)
IL-6 (pg/ml)	1	-0.025	0.166	-0.055	-0.024	-0.009	0.048	0.173	0.032	-0.075	0.029
TNF (pg/ml)	-0.025	1	0.217	-0.162	-0.054	-0.302 ^a	-0.222	-0.041	-0.104	0.191	0.011
Age (years)	0.166	0.217	1	-0.086	-0.185	-0.062	-0.081	0.021	-0.265	0.11	0.153
Weight (kg)	-0.055	-0.162	-0.086	1	0.132	0.042	-0.116	-0.136	-0.124	-0.151	-0.036
Creatinine (mg/dl)	-0.024	-0.054	-0.185	0.132	1	0.047	-0.016	-0.165	0.248	0.07	0.005
Urea (mg/dl)	-0.009	-0.302 ^a	-0.062	0.042	0.047	1	0.224	0.019	0.305 ^a	0.001	0.212
Albumin (g/dl)	0.048	-0.222	-0.081	-0.116	-0.016	0.224	1	0.509 ^b	0.086	0.033	-0.065
Calcium (mg/dl)	0.173	-0.041	0.021	-0.136	-0.165	0.019	0.509 ^b	1	0.164	-0.066	0.023
Phosphate (mg/dl)	0.032	-0.104	-0.265	-0.124	0.248	0.305 ^a	0.086	0.164	1	0.171	0.161
ANA (IU/dl)	-0.075	0.191	0.11	-0.151	0.07	0.001	0.033	-0.066	0.171	1	0.258
Glucose (mg/dl)	0.029	0.011	0.153	-0.036	0.005	0.212	-0.065	0.023	0.161	0.258	1

^aP<0.05 (two-tailed). ^bP<0.01. Albumin and calcium showed a positive correlation ($r=0.509$, $P<0.01$). IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; ANA, antinuclear antibody.

Table V. Distribution of genotype data for rs1800795 and rs1800797 for the cases (n=51) and controls (n=51).

SNP	Case, n (%)	χ^2	P-value	Controls, n (%)	χ^2	P-value
rs1800795		0.35	0.55		0.16	0.69
GG	26 (51.0)			40 (78.4)		
GC	22 (43.1)			10 (19.6)		
CC	3 (5.9)			1 (2.0)		
rs1800797		0.50	0.47		0.17	0.67
GG	30 (58.8)			35 (68.6)		
GA	17 (33.3)			15 (29.4)		
AA	4 (7.8)			1 (2.0)		

SNP, single nucleotide polymorphism.

individuals in stage V renal failure receiving hemodialysis three times a week. Overall, the results underscore the necessity for a nuanced understanding of sex-related differences in anthropometry while acknowledging the metabolic congruence among the studied groups. The phenomenon of increased body mass, commonly referred to as obesity, has been empirically linked to renal impairment. Obesity exerts a direct deleterious effect on renal function by elevating glomerular pressure and facilitating the accumulation of adipose tissue in the perirenal region, which in turn leads to the generation of oxidative stress and inflammatory responses (35,36). Moreover, excess body weight is often correlated with the onset of diabetes mellitus and hypertension, both of which are recognized as conditions that exacerbate the progression of CKD (37,38). Research indicates that individuals presenting with a body mass index (BMI) of ≥ 30 are at an augmented risk for the development of CKD, while individuals classified as moderately overweight (with a BMI ranging from 25 to

29.9) may experience a gradual decline in renal function over time (25,39-41).

In the present study, most biochemical and demographic factors did not exhibit significant relationships, according to the correlation analysis. Notably, albumin showed a moderate correlation with calcium and urea had a positive correlation with phosphate. There were no other noteworthy relationships found. These results implied that urea-phosphate and albumin-calcium correlations might represent underlying renal and metabolic linkages, even if most parameters vary independently. Higher urea removal efficiency is linked to higher phosphate removal efficiency, according to a study that demonstrated a positive correlation between urea and phosphate reduction ratios during hemodialysis (23,41). The results of the present study demonstrate the usefulness of albumin-adjusted calcium in clinical settings, as demonstrated by its reasonable correlation with ionized calcium levels (42). In a cohort of patients receiving maintenance hemodialysis in

Table VI. Genetic model analysis of IL6 rs1800795 and rs1800797 in cases (n=51) and controls (n=51).

A, rs1800795					
Model	Genotype	Cases, n (%)	Controls, n (%)	OR ^a (95% CI)	P-value
Codominant	G/G	26 (51%)	40 (78.4%)	1.00	0.0013
	G/C	22 (43.1%)	10 (19.6%)	0.30 (0.12-0.72)	
	C/C	3 (5.9%)	1 (2%)	0.22 (0.02-2.20)	
Dominant	G/G	26 (51%)	40 (78.4%)	1.00	0.003
	G/C-C/C	25 (49%)	11 (21.6%)	0.29 (0.12-0.68)	
Recessive	G/G-G/C	49 (94.1%)	50 (98 %)	1.00	0.3
	C/C	3 (5.9%)	1 (2.0%)	0.32 (0.03-3.18)	
Overdominant	G/G-C/C	29 (56.9%)	41 (80.4%)	1.00	0.0098
	G/C	22 (43.1%)	10 (19.6%)	0.32 (0.13-0.78)	
B, rs1800797					
Model	Genotype	Cases, n (%)	Controls, n (%)	OR ^a (95% CI)	P-value
Codominant	G/G	30 (58.8%)	35 (68.6%)	1.00	0.3
	G/A	17 (33.3%)	15 (29.4%)	0.76 (0.32-1.77)	
	A/A	4 (7.8%)	1 (2.0%)	0.21 (0.02-2.02)	
Dominant	G/G	30 (58.8%)	35 (68.6%)	1.00	0.3
	G/A-A/A	21 (41.2%)	16 (31.4%)	0.65 (0.29-1.47)	
Recessive	G/G-G/A	47 (92.2%)	50 (98%)	1.00	0.16
	A/A	4 (7.8%)	1 (2.0%)	0.24 (0.03-2.18)	
Overdominant	G/G-A/A	34 (66.7%)	36 (70.6%)	1.00	0.67
	G/A	17 (33.3%)	15 (29.4%)	0.83 (0.36-1.93)	

^aORs were calculated using SNPStats with the control as the reference outcome; OR <1 indicates a higher likelihood of chronic kidney disease among genotype carriers. OR, odds ratio; CI, confidence interval.

Table VII. Serum IL-6 concentrations across rs1800795 and rs1800797 genotypes in patients with chronic kidney disease.

SNP and genotype	Median IL-6, pg/ml (min-max)	P-value ^a
rs1800795		0.069
GG	1.10 (0.0-53.2)	
GC	0.65 (0.0-49.9)	
CC	0.80 (0.7-1.7)	
rs1800797		0.66
GG	0.80 (0.0-53.2)	
GA	0.80 (0.0-7.7)	
AA	0.75 (0.2-0.8)	

^aKruskal-Wallis test. IL-6, interleukin-6.

an intensive care unit, albumin-corrected calcium levels were found to be highly associated with 28-day in-hospital mortality, highlighting the clinical relevance of this correlation (43).

Environmental factors and genetics promote the advancement of numerous diseases, including cancer, diabetes and

inflammatory diseases, particularly CKD (44,45). Notably, genetic variation is a basic cause of a number of disorders that have been studied across different populations (46). Given the inflammatory characteristics of CKD, the association with the IL-6 gene seems to exert a considerable influence within the etiopathological context of this condition (47). In terms of CKD, an abundance of studies has yielded inconsistent findings across different demographic groups. Some investigations support the results of the present study, whereas others offer conflicting interpretations. The variability in research outcomes highlights the complexity of the relationship between IL-6 and CKD. The present study investigated the association of IL-6 -174 G/C (rs1800795) and IL-6 -597 G/A (rs1800797) with CKD. The genotype distributions of both SNPs were in HWE for cases and controls. The findings indicated that the GC and CC genotypes were more frequent among patients with CKD compared with healthy controls (49 vs. 21.6%). The dominant model showed the strongest statistical significance. This suggests that the presence of the C allele may be associated with CKD in the present cohort. Similar results were reported in South Indian patients with CKD and Sudanese hemodialysis cohorts, where the C allele was associated with advanced kidney disease (48,49) By contrast, studies in Iraqi and European dialysis populations reported no

significant differences in genotype distribution (50-53). Ethnic diversity, geographic variation and the multifactorial nature of the disease may influence these differences, and gene-environment interactions such as diet or lifestyle may modify genetic susceptibility (29,50-53). In the present study, analysis of inheritance patterns further supported the association of rs1800795 with CKD. Significant associations were observed under codominant, dominant and over-dominant models. By contrast, the recessive model did not show any significant correlation, most likely due to the low frequency of the CC homozygous genotype, a common limitation in genetic studies of dialysis patients (14,49). Conversely, rs1800797 showed no association with CKD across any inheritance model, consistent with prior reports indicating minimal impact on disease susceptibility (48).

In the present study, although rs1800795 showed an association with CKD risk, the genotypes of this SNP did not show significant relationships with serum IL-6 levels. This finding aligns with various dialysis studies where IL-6 promoter variants were linked with clinical features, including cardiovascular complications, hypertension and erythropoietin responsiveness, rather than to the baseline serum IL-6 concentration (53,54). In hemodialysis patients, serum levels of IL-6 are influenced by reduced renal clearance, oxidative stress and inflammatory stimuli associated with the dialysis procedure. These pathological factors may mask small genotype-specific differences in IL-6 gene production (55). Likewise, the rs1800797 genotype did not show a correlation with the circulating IL-6 level. Although some studies in other inflammatory conditions have suggested that rs1800797 may influence IL-6 production, evidence of a stable or predictable role in CKD or in patients undergoing dialysis remains limited (49,55). Overall, the findings of the present study suggested that the rs1800795IL-6 gene variant, unlike rs1800797, is associated with CKD susceptibility in patients on hemodialysis. However, neither variant showed a clear influence on the levels of IL-6 found in the blood. This dissociation between genetic risk and measured cytokine levels suggests that these IL-6 variants might contribute to disease susceptibility through biological pathways that are not adequately reflected by a single IL-6 measurement in dialysis patients.

Regardless of the common understanding that IL-6 serves as a significant mediator in the inflammatory pathways of CKD, the present study did find any notable sex differences in IL-6 concentrations or relevant links between circulating IL-6 levels and the biochemical markers explored. This noticeable disjunction emphasizes a vital methodological and biological factor: Circulating IL-6 concentrations among patients with ESRD during hemodialysis might not consistently represent the underlying inflammatory processes or genetic susceptibility at a singular measurement time. Variables such as decreased kidney capability, inflammation initiated by dialysis and marked discrepancies among individuals are likely to add complexity to subtle links. As a result, references to IL-6-associated inflammatory pathways within this discourse aim to elucidate the biological significance of IL-6 in the pathophysiology of CKD, rather than to propose statistically significant correlations observed within the present cohort.

The unavailability of a control group for immunological and biochemical tests acts as a notable hurdle in the present

evaluation, complicating efforts to establish meaningful comparisons between those with renal issues and their healthy counterparts. The presence of a control group would have supported a more extensive evaluation of biomarkers in individuals experiencing CKD and furthered the comprehension of the impact of sex on the differences in these biomarkers. The sample population was characterized by an opportunistic approach, wherein subjects were listed based on their accessibility and clinical diagnosis of ESRD within the predetermined age bracket of 40 to 60 years

In conclusion, the observations of the present study show that, in conjunction with body weight, the primary biochemical and immunological parameters displayed no statistically significant alterations associated with sex among individuals diagnosed with ESRD who were undergoing hemodialysis. Significant correlations were identified between albumin and calcium, along with associations between urea and phosphate, elucidating the complex metabolic issues typically linked to CKD. The IL-6 rs1800795 polymorphism exhibited a statistically significant correlation with the predisposition to CKD, with the C allele present at a higher frequency among affected individuals, while rs1800797 did not demonstrate any such correlation. Additionally, neither type of polymorphism was linked with the levels of IL-6 circulating in the blood, suggesting that the genetic risk could act through pathways not directly identified by standalone serum cytokine assessments (47). These conclusions clarify the diverse attributes of CKD and accentuate the fundamental need for the combination of genetic components with biochemical and immunological studies to enrich our knowledge of the illness and evaluate associated risks.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AFS, KSMI, ASA and AAMA confirm the authenticity of all the raw data. AFS contributed to the investigation by conducting experiments, collecting data and analyzing the results, and contributed to methodology, supervision, original draft preparation and reviewing and editing the manuscript. KSMI contributed to the statistical analysis using SNPStats software analysis, molecular analysis, data visualization and reviewing and editing the manuscript. ASA contributed to formal analysis, interpretation of results and reviewing and editing the manuscript. AAMA contributed to the acquisition, organization and curation of biochemical data and assisted in analysis/interpretation of the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The research protocol was approved by the Biology Department, the Scientific Committee and the Directorate of the Research Center at the University of Raparin (Rania, Sulaymaniyah, Iraq; approval no. 71; reference no. 2866; May 28, 2023). Written informed consent to participate was obtained from all participants before enrolment and sample collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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