Interleukin-17A (IL-17A) gene polymorphisms and susceptibility to chronic kidney failure in adolescents

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Abstract

Background: Chronic kidney failure in adolescents is a serious condition that can progress to end-stage renal disease (ESRD) and require long-term hemodialysis. Immune dysregulation and persistent inflammation contribute to disease progression, and interleukin-17A (IL-17A) is a key pro-inflammatory cytokine implicated in chronic inflammatory pathways. **Objective:** To assess whether IL-17A gene polymorphisms are associated with susceptibility to chronic kidney failure and to explore their relationship with inflammatory and immunological markers among adolescent patients receiving hemodialysis.**Methods:** A hospital-based cross-sectional study was conducted at Tikrit Teaching Hospital, enrolling 100 adolescent patients with clinically diagnosed ESRD on hemodialysis and 100 age-matched healthy controls. Genomic DNA was extracted from peripheral blood, and IL-17A polymorphisms were genotyped using the ARMS-PCR technique. Serum inflammatory/immunological markers (C3, C4, NF-κB, COX-2, IL-6) were compared across genotypes, and hemodialysis duration was analyzed as a clinical correlate. **Results:** The GG genotype was more frequent among hemodialysis patients (42%) than controls (28%), although this difference was not statistically significant ($p = 0.144$). The AG genotype was less common in patients (34%) than in controls (58%) and was associated with a reduced likelihood of chronic kidney failure ($p = 0.017$). The AA genotype occurred in 24% of patients and 14% of controls ($p = 0.207$). Among patients, the GG genotype was associated with significantly higher levels of C3, C4, NF-κB, COX-2, and IL-6 compared with AG and AA genotypes ($p = 0.001$). The GG genotype also corresponded to the longest hemodialysis duration (9.77 ± 2.17 years), while AA was associated with the shortest duration (5.37 ± 1.17 years; $p = 0.011$). **Conclusion:** In adolescents, IL-17A polymorphisms may influence susceptibility to chronic kidney failure and are associated with inflammatory activity in those with ESRD. The GG genotype appears linked to heightened inflammatory responses and longer hemodialysis duration, whereas the AG genotype may have a protective association. Further adolescent-focused studies are recommended to confirm these findings and clarify clinical implications.

Keywords: adolescents, chronic kidney failure, end-stage renal disease, Hemodialysis, IL-17A, gene polymorphism, inflammation, NF-κB, IL-6

1 Introduction

Kidney is important organ, its function is regulation of the blood composition and volume, filtration the urine by eliminating the metabolic wastes, secretion of the end metabolism products and maintenance the acid or balance in the body via regulate fluids and electrolytes [1]. Renal failure (RF) is common disease, usually is defined as the decrease of kidney function, glomerular filtration rate (GFR) this use to estimate the kidney function reduction usually is less than 60 mL / min . Resulting in uremia, metabolic acidosis, anemia, electrolyte imbalances and endocrine disorders. Its main causes are diabetes, hypertension, glomerulonephritis, and polycystic kidney disease. Haemodialysis is the most frequent treatment method for CRF. However, it has been argued that a number of restrictions and modifications accompany this treatment, which have a detrimental impact on the quality of patient's life and affect individuals' physical and psychological well-being [2]. Chronic Renal failure is a continuous psychological process for patients and their families in order to accept their new image and to be adjusted to the new condition of haemodialysis. The quality of life of patients requiring dialysis is affected significantly, since it is associated with changes in their daily habits and in their lifestyle for both themselves and their families. At the same time, their physical

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health, their functional status, their personal relationships and their social and economic status are greatly affected. Chronic kidney disease is an emergent worldwide public health problem. In the United States, the prevalence of end-stage renal disease (ESRD) is increasing [3].

Interleukin-17A (IL-17A) is a cytokine, produced by multiple cell types, such as CD4 +ab T cells, gamma-delta T cells, natural killer cells, neutrophils, macrophages, dendritic cells, lymphoid tissue inducer cells, mast cells and plasma cells. IL-17A participates in tissue inflammation by inducing the expression of chemokines, pro-inflammatory cytokines and matrix metalloproteases. Besides its role in host defense against infectious diseases, IL-17A is involved in different autoimmune and inflammatory diseases. interleukin-17A (IL-17A) member of the IL-17 family of cytokines, which also includes IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25) and IL-17F. IL-17A is a pleiotropic cytokine that participates in tissue inflammation by inducing the expression of chemokines, pro-inflammatory cytokines and matrix metalloproteases [4].

Based on the emerging evidence from experimental animal models and human studies, IL-17A has been put forward as a central player in the mammalian immune system. As the kidney can be affected by systemic, organ-specific and dysregulated immunity, as an innocent bystander in host defense and through targeted allogeneic responses in transplantation [5]. Being the major cause of acute renal failure in native kidneys and in kidney allografts, ischemia-reperfusion injury is characterized by an inflammatory process, mediated by innate and adoptive immune responses. Acute kidney injury (AKI) is enhanced by the production of pro-inflammatory cytokines and leukocytes, whereas regulatory cells and immunomodulatory cytokines attenuate injury. In a mouse model of cisplatin nephrotoxicity, which mimics many features of human AKI, the role of IL-17A has been investigated. Activation of Toll-like receptor-2 (TLR2) and the apoptosis-associated speck-like protein containing a carboxy-terminal (ASC-CARD) inflammasome resulted in the generation of IL-17A, which is a significant contributor to cisplatin-induced AKI by recruitment of innate effector cells [6]. A significant immune dysregulation has been reported in patients with end-stage renal disease. One of the immunological characteristics in patients with terminal renal insufficiency is the impaired T lymphocyte-associated immunological profile. End-stage renal disease is characterized by a significant immune activation, as represented by the increase of IL-17A producing effector memory T cells. In addition, a decreased number of T naive cells is found in this group, which is an illustration of an immune impairment [7]. In a study of 105 chronic hemodialysis patients, a Th17/Treg cell imbalance was observed with an increased number of Th17 cells and a decreased number of Treg cells [8].

Polymorphisms in the IL-17 pathway genes might contribute to the risk of chronic kidney disease. In the Spanish RENASTUR cohort study with 650 healthy elderly individuals, a significantly higher frequency of SNP rs4819554 AA homozygotes was observed among individuals with an estimated glomerular filtration rate <60 mL/ min/1.73 m2. An association was found between the allele rs4819554 A and the risk of developing end stage renal disease, as well as with an increased IL-17RA expression and higher concentrations of Th17 cell subsets. The rs4819554 is part of the IL17RA promoter and the nucleotide change could affect the binding of transcription factors by creating a site for the TF AP-4 (activating enhancer binding protein 4, core binding sequence CAGCTG). The association between kidney failure and SNPs linked to differences in IL-17RA protein expression and/or function might be explained by the role of Th17 cells and components of the IL17 pathway in kidney damage [9]. The aim of this study is to investigate the role of interleukin-17A gene polymorphisms in the pathogenesis of kidney failure.

2 Materials and methods

This cross-sectional hospital-based study was done in Tikrit Teaching Hospital, the work started in April 2023 after taking the approval from Ministry of Health/Saladin Health Directorate. The study included 100 patients with CKD who underwent regular hemodialysis were included in this study at Tikrit Teaching Hospital. They were clinically diagnosed by nephrologists as ESRD patients (on hemodialysis), based on their history, clinical examination, renal function tests and other laboratory tests, undergoing hemodialysis.

The study also included 100 adult persons looks healthy with no prior medical or family history of CKD as control participants in this study.

2.1 Exclusion criteria

Patients suffering from kidney failure but without renal dialysis, patients with other diseases such as chronic and autoimmune diseases, as well as chronic infections.

2.1.1. Ethical approval. Approval of the research committee /Saladin Health Directorate/ Ministry of Health/Republic of Iraq was obtained for the proposal of the study, and the practical study to be done in Tikrit Teaching Hospital (160, 08/03/2023).

2.1.2. Clinical and laboratory information. Full clinical examination by a physician to exclude the other causes included in the exclusion criteria such as kidney failure but without renal dialysis, patients with other diseases such as chronic and autoimmune diseases, as well as chronic infections, also assessing the biochemical parameters, imaging studies and renal biopsy when required.

An interview was carried out with these patients using questionnaire form designed by the investigator including age, sex ,duration of CKD, duration of hemodialysis sessions, and frequency of hemodialysis sessions per week.

2.2 Methods

Five ml of blood was collected by vein puncture 5 ml syringes from each patient (before hemodialysis (pre —HD) and control enrolled in this study. Blood samples were placed into two sterile test tubes, in one of them 1.5 ml of blood was put in test tube containing anticoagulant EDTA and used for detection of IL-17A gene polymophism.

2.2.1. Interleukin-17A gene polymorphism.

a. DNA extraction. In EDTA tubes, blood samples are obtained from patients and healthy controls.then DNA was extracted from whole blood by using (Geneaid kit) according to the kit instruction.

b. Principle. The magnetic beads in the kit have specific polymeric groups of adsorbed nucleic acid (DNA/RNA) on the surface.

c. Kit contents.

Constituents of kit	B-200	Main components
	32 T /Kit	
Proteinase K	480 μL x1 tube	<5% Proteinase K
Nucleic acid extraction reagent prepackaged in 96-well plates	16 T x2 Plates	Extraction Reagent I, Extraction Reagent II , Elution Buffer, Magnetic beads solution

d. Procedure.

1. After taking all the components out of the kit, invert the 96-well plate to mix the liquid in each well, then let the wells stand vertically for 3 to 5 minutes.

2. Carefully open the aluminum packaging of the 96-well plate. Add 15 µL of proteinase K to positions A1-H1 and A7-H7. Then, add 200 µL of the sample to each well and mix using a Vortex for 5 seconds. Place the 96-well plate in the correct position on the instrument and insert the magnetic rod sleeve.

3. Start the nucleic acid isolation process by running the system and selecting either the ZYBIO-B-200 or ZYBIO-B-200Q program.

4. Begin the extraction process by pressing the start button on the toolbar. The machine lowers magnetic rods into each comb, inserts the comb into the designated wells, and establishes a magnetic field.

5. Each sample is attributed to its own well in the sample area.

6. After removing the iron comb, only the plastic comb remains, which is used to mix the samples with the iron atoms while analyzing the sample.

7. Once mixing is complete, the liquid is moved to the third column where lysis occurs.

8. At 65°C, a magnetic field is applied for 40 seconds. The liquid is then transferred to the fourth column containing a washing solution to remove impurities by using the iron comb to create a magnetic field that attracts iron atoms, which are associated with RNA or DNA, including any present virus.

9. After transferring the liquid to the elution solution at 85°C for 15 seconds and mixing thoroughly, the RNA or DNA is separated from the iron atoms.

10. The samples are then stored at very cold temperatures after extraction, ready for master mix preparation and amplification for each sample. This process takes nine minutes.

11. Finally, the 96-well plates are removed, and the nucleic acid solution from positions A6-H6 and A12-H12 is pipetted into a 1.5 mL centrifuge tube for the next step.

2.2.2. Primers used in the study. PCR primers were designed by web-based allele specific primer designing tool based on primer 3. In order to access the success of PCR an internal control, human growth hormone (HGH) was amplified using a pair of primers designed from the nucleotide sequence of HGH. The primer sequences used for amplification of *IL-17A* were as follows (Table 1):

Table 1: The primer sequences used for the amplification of IL-17A

Primer Type	Sequence
Allele-Specific Sense Primer C	5'-GAT ATG CAC CTC TTA CTG CAC TC-3'
Allele-Specific Sense Primer T	5'-GAT ATG CAC CTC TTA CTG CAC TT-3'
Antisense Common Primer	5'-AGTTGTACAGGCCCAGTGTA-3'
Control Primer (HGH) Sense	5'-CCT TCC AAC CAT TCC CTT A-3'
Control Primer (HGH) Antisense	5'-TCA CGG ATT TCT GTT GTG TTTC-3'

2.3 Amplification refractory mutation system – ARMS-PCR

The allele-specific PCR also called as an ARMS- PCR (amplification refractory mutation system) or PASA (PCR amplification of specific alleles) or AS-PCR used to detect the SNPs, ARMs – PCR protocol was used for SNPs detection of IL-17A gene.

2.3.1. Principles. Amplification Refractory Mutation System PCR (ARMS-PCR) provides an exciting methodology to simultaneously detect and type haplotype/single nucleotide polymorphism based on differential size of PCR products for a particular allele when resolved on agarose gel electrophoresis(Chander et al., 2016). Template DNA, primers, nucleotides, and DNA polymerase are all required for each PCR assay. The DNA polymerase is the enzyme responsible for joining individual nucleotides to generate the PCR product. The nucleotides include the four bases – adenine, thymine, cytosine, and guanine (A, T, C, G) – that are found in DNA. These serve as the building blocks for the DNA polymerase to utilize in producing the PCR product. the primers in the reaction specify the exact DNA product to be amplified. The primers are short DNA fragments having a specific sequence that complements the target DNA to be identified and amplified. These serve as a base for the DNA polymerase to construct upon.

The basic ARMS-PCR steps are:- Initiation step, Denaturation step, Annealing step, Extension / Elongation step, Final Extension

a. Primers reconstitution and dilution. The primers are lyophilized before use. A mass in Picomoles is the unit of measurement for a lyophilized primer. The subsequent steps are done for the reconstitution and dilution of the primers:

1. The tube is centrifuged at 10000 xg for 5-10 min before decapping.
2. The chosen volume from nuclease free water are added according to the manufacturer to obtain a 100 pmoles /μL (master stock).
3. The Primers are re- mixed by suitable vortexing.
4. To obtain a 10 pmoles/L concentration, ten microliters of the master stock are transferred to a 0.5 mL eppendorf tube containing 90 mL of nuclease-free water (working stock). Both the master and working stocks are held at -20 degrees Celsius.

The working stock is warmed up and kept on ice for use in PCR and then stored at -20 °C after each use.

b. ARMS-PCR master mix. PCR Master Mix includes Nuclease-Free Water and PCR Master Mix, 2X. PCR Master Mix is a premixed, ready-to-use solution that contains appropriate quantities of Taq DNA polymerase, dNTPs, MgCl2, and reaction buffers for effective PCR amplification of DNA templates.

c. Optimization of ARMS-PCR conditions. The following methods are used to optimize PCR conditions:

PCR Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95°C	1 min	1 cycle
Denaturation	95°C	15 sec	10 cycles
Annealing	55°C	50 sec	10 cycles
Extension	72°C	40 sec	10 cycles
Denaturation	95°C	20 sec	20 cycles
Annealing	56°C	50 sec	20 cycles
Extension	72°C	50 sec	20 cycles

d. Thermocycler program for DNA amplification. To obtain the best results of amplification of alleles of IL-17A gene, The two alleles put in one program of the PCR thermocycler is shown in above table and the melting temperature for primer 50 – 65 C.

2.3.2. Agarose gel electrophoresis. Agarose gel electrophoresis is a widely used procedure in various areas of biotechnology. This simple, but precise, analytical procedure is used in research, biomedical and forensic laboratories. Of the various types of electrophoresis, It is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for determining the size of DNA molecules in the range of 500 to 30,000 base pairs.

a. Procedure. A 1.5 g agarose in 100 ml of a solution was prepared by the following steps

a1. Preparation of solution. 10X TBE buffer (tris borate EDTA) was dilute with deionized water to make 1X TBE buffer (tris borate EDTA). (One volume of 10X TBE buffer with 9 volume of deionized water: 1:10 dilution).

a2. Preparation of agarose gel.

1. Firstly 1. 5 g of agarose were weighted and placed into a conical flask, and then added 90 ml of distilled water and 1o ml of 1X TBE buffer. This gel was utilized to identify the PCR product's band.
2. While waiting for the gel solution to appear clear and pure, the solution was agitated over a hot plate, then allowed to warm before being poured into the tray.
3. At 60-65°C, five liters of stock ethidium bromide (10 mg/mL) were added to the gel, resulting in a fluid content of 0.5 Lml.
4. Adhesive tape was used to seal the ends of the gel chambers
5. About 1 inch from one end of the tray, a comb was placed into the gel chamber
6. A gel solution was put into the chamber and allowed to solidify at room temperature for around 30 minutes.
7. The comb was removed, and the chamber was placed in a horizontal electrophoresis tank filled with TBE buffer, which was utilized for the gel agarose preparation
8. Approximately 4 ml of sample was placed into each well with extreme caution to avoid well damage and cross contamination of nearby wells.
9. The negative pole was connected to the unit's negative side, while the positive pole was connected to another.
10. According to the size of the DNA fragment, electrophoresis was performed at (70 volts for 1.5 hours) and (80 volts for 60 minutes) or while waiting for dye indicators to travel to the necessary distance.

b. Loading of samples. The comb was removed after the gel had become clear and solid, and the glass plates were placed in a submarine horizontal gel electrophoresis chamber. 1 X TBE buffer was added to the chamber until it reached above the level of the wells, and the sample was loaded into the gel wells using a 4l micropipette.

c. Electrophoresis conditions. An electric field (70 volts for 1.5 hours) and (80 V for 1 hour) were applied to the system after the sample was applied, allowing the negatively charged nucleic acids to migrate across the gel to the positive electrode (anode).

d. DNA ladder. In current study, 4 μL of DNA ladder was used [Sentol (Russia)] and band size ladder was 100- 1000 bp. Without the need for sample loading dye, the ladder type employed in this investigation can be given directly.

e. Gel- band visualization. The agarose gel was placed in a UV transilluminator device and exposed to UV light to view the DNA bands, and images were taken with a digitcaera connected to a PC.

2.4 Statistical analysis

Computerized statistically analysis was performed using Minitab version 23 statistic program. Comparison was carried out using, Chi-square, T-Test correlation and for determination of probability value (P-value). The P value> 0.05 was considered statistically significant, while for those which its P value was greater than 0.05 considered non-significant statistically.

3 Results

The comparison of demographic characteristics between Hemodialysis patients and the Control Group revealed that the distribution of males and females did not significantly differ, with 54% males in the Hemodialysis group and 63% in the Control Group (Chi-square test, p = 0.19). In terms of age distribution, the Chi-square test indicated no significant differences across age groups (p = 0.14). The mean age for Hemodialysis patients was 58.81±12.8, slightly higher than that of the Control Group (57.56±11.6), although the t-test did not show a statistically significant difference (p = 0.23), see Table 2. Example of imaging DNA fragments by agarose gel electrophoresis after restriction enzyme digestion is presented in Figure 1.

Table 2: Demographic characteristics of the studied groups

Demographic characteristics	Hemodialysis patients	Control group	Statistics
Sex			
Male	54 (54%)	63 (63%)	Chi-square: 1.66P-value: 0.19
Female	46 (46%)	37 (37%)	
Total	100 (100%)	100 (100%)	
Age years			
28-37	10 (10%)	16 (16%)	Chi-square: 6.82P-value: 0.14
38-47	14 (14%)	16 (16%)	
48-57	25 (25%)	30 (30%)	
58-67	27 (27%)	27 (27%)	
68-77	24 (24%)	11 (11%)	
Mean±SD	58.81±12.8	57.56±11.6	T-test: 1.66P-value: 0.23

For patients, the PCR-RFLP product for the IL-17A gene after digestion with the XagI restriction enzyme is shown in the 4.1% agarose gel image in Figure 3. The gel was visualized using the gel documentation system under the UV transilluminator after staining with ethidium bromide. The L-strand contains a 25/100 bp mixed DNA marker. The 3rd, 4th, 6th, 9th, and 11th strips exhibit a single fragment band (102 bp)

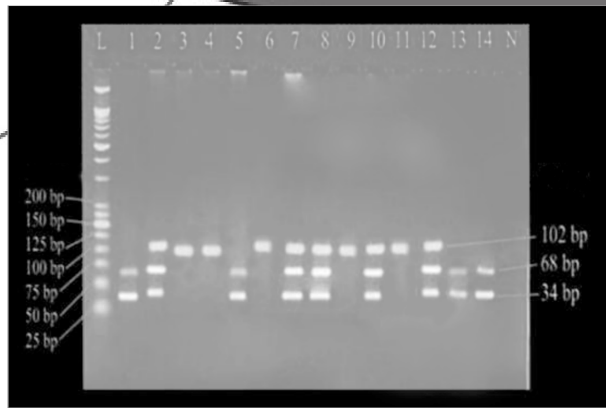

Figure 1: Example of imaging DNA fragments by agarose gel electrophoresis after restriction enzyme digestion

indicating the AA genotype. The 1st, 5th, 13th, and 14th lanes show two fragment bands (68 bp and 34 bp) corresponding to the GG genotype. However, the 2nd, 7th, 8th, and 10th strands exhibit three fragment bands (102 bp, 68 bp, and 34 bp) that represent the AG genotype.

The study showed that the GG (homozygous wild type) genotype of IL-17A is more prevalent in hemodialysis patients (42%) compared to the control group (28%), with an odds ratio of 1.86 (95% CI: 0.81 - 4.29) and a p-value of 0.144, indicating no significant difference. The AG (heterozygous mutant type) genotype is less common in hemodialysis patients (34%) than in controls (58%), with an odds ratio of 0.37 (95% CI: 0.17 - 0.84) and a significant p-value of 0.017, suggesting a protective effect. The AA (homozygous mutant type) genotype is observed in 24% of patients and 14% of controls, with an odds ratio of 1.94 (95% CI: 0.69 - 5.43) and a p-value of 0.207, indicating no significant difference. The allele frequencies of G (57%) and A (43%) are identical between patients and controls, both with an odds ratio of 1 (95% CI: 0.57 - 1.75) and a p-value of 1, showing no difference in allele distribution between the groups, see Table 3.

Table 3: Comparison of IL-17A genotypes between hemodialysis patients and a control group

IL-17A Genotypes	Hemodialysis patients		Control group		Odd Ratio	95% C.I.	p-value
	No.	%	No.	%			
GG (homozygous wild type)	42	42%	28	28%	1.86	0.81 - 4.29	0.144
AG (heterozygous mutant type)	34	34%	58	58%	0.37	0.17 - 0.84	0.017
AA (homozygous mutant type)	24	24%	14	14%	1.94	0.69 - 5.43	0.207
Total	100	100%	100	100%	-	-	-
Allele frequency							
G	57	57%	57	57%	1	0.57 - 1.75	1
A	43	43%	43	43%	1	0.57 - 1.75	1

The distribution of IL-17A genotypes among hemodialysis patients indicated a similar pattern between male and female patients, with no significant gender-based differences (P-value: 0.91). Among the 100 patients, the GG (homozygous wild type) genotype was observed in 42 patients, including 18 males (24%) and 18 females (18%). The AG (heterozygous mutant type) genotype was present in 34 patients, with 18 males (18%) and 16 females (16%). The AA (homozygous mutant type) genotype was found in 12 patients, equally distributed between males and females, each comprising 12% of the total. Overall, the patient cohort consisted of 54% males (27 patients) and 46% females (43 patients). The nearly equal distribution of genotypes across genders and the high P-value suggest no significant association between IL-17A genotypes and gender in this group of hemodialysis patients, see Table 4.

Table 4: Distribution of IL-17A genotypes among hemodialysis patients according to gender

IL-17A Genotypes	Hemodialysis patients n:50				
	Total No.	Male		Female	
		No.	%	No.	%
GG (homozygous wild type)	42	24	24%	18	18%
AG (heterozygous mutant type)	34	18	18%	16	16%
AA (homozygous mutant type)	24	12	12%	12	12%
Total	100	54	54%	46	46%
P-value: 0.011					

The distribution of IL-17A genotypes among hemodialysis patients and their respective mean durations of dialysis, combined with standard deviation (SD), indicates Patients with the GG (homozygous wild type) genotype had a mean dialysis duration of 9.77 ± 2.17 years. Those with the AG (heterozygous mutant type) genotype had a mean duration of 7.11 ± 2.01 years. Patients with the AA (homozygous mutant type) genotype had the shortest mean dialysis duration at 5.37 ± 1.17 years (P-value: 0.011). These results suggest a significant variation in the duration of dialysis among different IL-17A genotypes, with the GG genotype associated with the longest dialysis duration, followed by the AG genotype, and the AA genotype with the shortest duration, see Table 5.

Table 5: Relation of of IL-17A genotypes with the duration of hemodialysis

IL-17A Genotypes	Total No.	duration of dialysis (year)	
		Mean	SD
GG (homozygous wild type)	42	9.77	2.17
AG (heterozygous mutant type)	34	7.11	2.01
AA (homozygous mutant type)	24	5.37	1.17
P-value: 0.011			

The study showed that patients with the GG genotype exhibited higher mean levels of C3 (318.32 ± 36.79 pg/ml), C4 (133.97 ± 14.15 pg/ml), NF-κB (15.06 ± 1.50 pg/ml), COX-2 (9.21 ± 2.53 pg/ml), and IL-6 (225.90 ± 46.39 pg/ml) compared to those with AG and AA genotypes. Patients with the AG genotype had intermediate mean levels of these parameters: C3 (246.52 ± 28.49 pg/ml), C4 (103.75 ± 10.96 pg/ml), NF-κB (11.66 ± 1.16 pg/ml), COX-2 (7.14 ± 1.96 pg/ml), and IL-6 (174.95 ± 35.93 pg/ml). The AA genotype group (n=12) had the lowest mean levels: C3 (229.77 ± 26.55 pg/ml), C4 (96.70 ± 10.21 pg/ml), NF-κB (10.87 ± 1.08 pg/ml), COX-2 (6.65 ± 1.82 pg/ml), and IL-6 (163.06 ± 33.48 pg/ml). These results indicated that the GG genotype is associated with higher levels of these immunological markers, followed by the AG and AA genotypes, respectively (P-value: 0.001), see Table 6.

4 Discussion

The study's findings on the distribution of IL-17A genotypes among hemodialysis patients compared to a control group reveal interesting trends, although most differences are not statistically significant. The GG (homozygous wild type) genotype, while more prevalent in hemodialysis patients (42% compared to 28% in controls), does not show a significant difference. This was in agreement with Mohsen et al. [10] study,

Table 6: Comparison of immunological parameters among different IL-17A genotypes in hemodialysis patients

Immunological parameters		IL-17A Genotypes			P-value
		GG (homozygous wild type) n:21	AG (heterozygous mutant type) n:17	AA (homozygous mutant type) n:12	
C3 (pg/ml)	Mean	318.32	246.52	229.77	0.001
	SD	36.79	28.49	26.55	
C4 (pg/ml)	Mean	133.97	103.75	96.70	0.001
	SD	14.15	10.96	10.21	
NF-κB (pg/ml)	Mean	15.06	11.66	10.87	0.001
	SD	1.50	1.16	1.08	
COX-2 (pg/ml)	Mean	9.21	7.14	6.65	0.001
	SD	2.53	1.96	1.82	
IL-6 (pg/ml)	Mean	225.90	174.95	163.06	0.001
	SD	46.39	35.93	33.48	

who show a non-marked difference between patients and controls when genotype T/T and G/G of IL-17A genotypes. Moreover, Romanowski et al [11]. showed an association of the *IL-17A* *GG* genotype with with ESRD. Moreover, research conducted by Domanski et al. [12] suggests a possible association between the *IL-17A* gene polymorphisms and the chronic histopathological changes detected in kidney biopsies after transplantation. It appeared that tubular atrophy and interstitial fibrosis were more severe among individuals with the *IL-17A* rs2275913 polymorphism was found to be associated with a higher grade of tubulitis among patients with the *A* allele, and with a higher grade of arteriolar hyaline thickening and a mesangial matrix increase among patients carrying the *G* allele. Different studies have investigated the rs2275913 polymorphism in the context of various diseases. Previous research has demonstrated a positive correlation between the rs2275913 polymorphism of the IL-17A gene and ulcerative colitis in Korean and Japanese populations [13, 14]. Domanski et al. [12] showed that the rs2275913 polymorphism of the IL-17A gene is significantly associated with tubulitis, thickening of the arterial hyaline, and increased mesenchymal matrix in kidney transplants. Additionally, studies have explored IL-17-related polymorphisms in renal diseases, including diabetic nephropathy (DN). Coto et al. [15] found an association between the rs4819554 polymorphism in the IL-17 receptor A (IL17RA) promoter region and renal dysfunction, suggesting that this polymorphism could serve as an early marker of kidney disease.

Furthermore, Kim et al. [7] suggested that genetic alterations in IL-17E and IL-17R are associated with an increased risk of end-stage renal disease (ESRD), highlighting the role of the inflammatory cascade in the pathogenesis of ESRD. However, they also noted that the rs4819554 polymorphism of the IL17RA gene did not significantly increase the risk of ESRD. The distribution of IL-17A genotypes among hemodialysis patients indicated a similar pattern between male and female patients, with no significant gender-based differences (P-value: 0.91). Among the 100 patients, the GG (homozygous wild type) genotype was observed in 42 patients, including 18 males (24%) and 18 females (18%). The AG (heterozygous mutant type) genotype was present in 34 patients, with 18 males (18%) and 16 females (16%). The AA (homozygous mutant type) genotype was found in 12 patients, equally distributed between males and females, each comprising 12% of the total. Overall, the patient cohort consisted of 54% males (27 patients) and 46% females (43 patients). The nearly equal distribution of genotypes across genders and the high P-value suggest no significant association between IL-17A genotypes and gender in this group of hemodialysis patients.. The lack of significant gender differences in IL-17A genotype distribution suggests that the genetic factors contributing to renal failure in these patients are not influenced by gender. This finding is important because it indicates that the potential role of IL-17A in the pathogenesis of renal disease is consistent across genders, reinforcing the idea that other factors—such as environmental influences, comorbid conditions, and overall genetic background—are likely more critical in determining susceptibility to severe kidney disease requiring hemodialysis [7]. In line with our findings, Mohsen et al. [10] reported that the most frequent IL-17A genotype detected among both males and females was the heterozygote allele (AG), with a prevalence of 60.6% in males and 53.6% in females. This supports the observation that the AG genotype is common across genders in different patient populations. While our study found a lower prevalence of the AG genotype in hemodialysis patients (34% overall, with 18% in males and 16% in females).

Moreover, the nearly equal distribution of genotypes across genders in this cohort highlights that IL-17A may not be a gender-specific marker of risk for renal failure. This could imply that therapeutic approaches targeting IL-17A-related pathways in hemodialysis patients can be applied similarly to both male and female patients without the need for gender-specific adjustments. The findings also underscore the importance of considering a broad range of genetic and non-genetic factors when evaluating the risk of renal disease progression in diverse patient populations. The distribution of IL-17A genotypes among hemodialysis patients and their respective mean durations of dialysis, combined with standard deviation (SD), indicates Patients with the GG (homozygous wild type) genotype had a mean dialysis duration of 9.77 $\pm$ 2.17 years. Those with the AG (heterozygous mutant type) genotype had a mean duration of 7.11 $\pm$ 2.01 years. Patients with the AA (homozygous mutant type) genotype had the shortest mean dialysis duration at 5.37 $\pm$ 1.17 years (P-value: 0.011). These results suggest a significant variation in the duration of dialysis among different IL-17A genotypes, with the GG genotype associated with the longest dialysis duration, followed by the AG genotype, and the AA genotype with the shortest duration.

The study showed that patients with the GG genotype exhibited higher mean levels of C3 (318.32 $\pm$ 36.79 pg/ml), C4 (133.97 $\pm$ 14.15 pg/ml), NF-κB (15.06 $\pm$ 1.50 pg/ml), COX-2 (9.21 $\pm$ 2.53 pg/ml), and IL-6 (225.90 $\pm$ 46.39 pg/ml) compared to those with AG and AA genotypes. Patients with the AG genotype had intermediate mean levels of these parameters: C3 (246.52 $\pm$ 28.49 pg/ml), C4 (103.75 $\pm$ 10.96 pg/ml), NF-κB (11.66 $\pm$ 1.16 pg/ml), COX-2 (7.14 $\pm$ 1.96 pg/ml), and IL-6 (174.95 $\pm$ 35.93 pg/ml). The AA genotype group (n=12) had the lowest mean levels: C3 (229.77 $\pm$ 26.55 pg/ml), C4 (96.70 $\pm$ 10.21 pg/ml), NF-κB (10.87 $\pm$ 1.08 pg/ml), COX-2 (6.65 $\pm$ 1.82 pg/ml), and IL-6 (163.06 $\pm$ 33.48 pg/ml). These results indicated that the GG genotype is associated with higher levels of these immunological markers, followed by the AG and AA genotypes, respectively (P-value: 0.001)..The elevated levels of C3, C4, NF-κB, COX-2, and IL-6 observed in patients with the GG genotype may be attributed to several factors. The high levels of C3, C4, NF-B, COX-2 and IL-6 observed in patients with the GG genotype can be caused by various factors. This genotype probably leads to increased activation of inflammatory pathways and elevated gene expression of pro-inflammatory markers. Some important signaling pathways, such as NF-κB, may be activated differently in people with the GG genotype. This genotype may also interact with environmental, epigenetic, or other genetic factors to enhance the immune response. In addition, individuals with the GG genotype may have a genetic predisposition that increases their susceptibility to chronic inflammatory conditions, resulting in higher observed levels of these biomarkers. These findings suggest that the GG genotype is associated with a stronger inflammatory phenotype, potentially influencing disease progression.

5 Conclusions

The study suggests a possible association between IL-17A gene polymorphisms and CKD progression. The GG genotype is linked to increased inflammatory responses and prolonged hemodialysis duration, while the AG genotype may confer a protective effect. These findings highlight the potential role of IL-17A polymorphisms as genetic markers for CKD risk and progression, warranting further investigation into targeted therapeutic approaches.

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