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Research Article

The impact of serum interleukin-18 and its gene polymorphism in coronary atherosclerosis

Radhwan Shukur Uso* **and Ruqaya Muammed Gharib AL-Barzinji**

Department of Basic Sciences, College of Medicine, Hawler Medical University - Erbil, Kurdistan Region, Iraq

Correspondence: radhwanshukur.24@gmail.com

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ABSTRACT: Introduction: Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality worldwide and is mainly driven by chronic inflammation and endothelial dysfunction. Interleukin-18 (IL-18) is among the inflammatory mediators involved, and IL-18 gene polymorphisms may also influence disease pathogenesis. This study aimed to evaluate the diagnostic and prognostic significance of biomarkers and IL-18 variants in CAD in order to improve non-invasive early detection and risk stratification.
Methods: This prospective case-control study included 200 Kurdish subjects recruited at the Erbil Cardiac Centre, comprising 100 patients with CAD confirmed by angiography and 100 controls. Clinical evaluation was supplemented with biochemical and hematological tests. Serum IL-18 and hsCRP levels were measured using ELISA and turbidimetry, respectively, while IL-18 SNPs (-607 T/G; rs1946518) were genotyped using ARMS-PCR.
Results: The findings showed a significant increase in IL-18 and hsCRP levels in CAD patients compared with controls. Notably, the TT and GG genotypes of IL-18 rs1946518 were observed only in patients, and these genotypes were associated with increased biomarker levels. No significant differences were detected in the genotype distribution, allele frequencies, or haplotype frequencies of IL-18 gene polymorphisms between the CAD and control groups. Positive correlations were observed between IL-18 expression and hsCRP, indicating a shared inflammatory process.
Conclusion: Elevated IL-18 levels, together with IL-18 gene polymorphisms, suggest a synergistic role of genetic and inflammatory factors in CAD progression among patients with coronary atherosclerosis. Integrated assessment enhances diagnostic accuracy, supporting its potential as a non-invasive approach for early CAD detection and risk stratification.

Keywords: coronary artery disease, interleukin-18, (–607 T/G), polymorphism

I. INTRODUCTION

Atherosclerosis is a chronic inflammatory condition that progresses to cardiovascular diseases such as coronary artery disease (CAD). It is strongly influenced by inflammation, making it a major global health concern because of its adverse effects on the coronary arteries [1].

Coronary artery disease (CAD) is a leading cause of mortality worldwide, resulting from atherosclerotic blockage of the coronary arteries [2]. Atherosclerotic plaques consist of smooth muscle cells, inflammatory cells, lipids, and other components, and are formed in response to oxidative stress and inflammatory signals [3]. A key event in the development of atherosclerosis is the migration and proliferation of smooth muscle cells [4]. Various risk factors for CAD include age, sex, lifestyle, family history, genetic variations, ethnicity, dyslipidaemia, hypertension, and obesity [5].

Interleukin-18 (IL-18), a pro-inflammatory cytokine, is part of the IL-1 superfamily and is recognized for inducing interferon-gamma in natural killer cells and T lymphocytes, which play vital roles in atherosclerotic plaque formation. Elevated levels of IL-18 are associated with inflammatory diseases, underscoring its significant role in the inflammatory cascade [6]. The IL-18 gene, located on chromosome 11q22.2–q22.3, consists of five introns and six exons [7]. Research indicates a correlation between IL-18 polymorphisms, specifically rs1946518 (C > A) at position -607 and rs187238 (G > C) at position -137, and the risk of coronary artery disease [8, 9].

The liver and mature adipocytes are the main producers of high-sensitivity C-reactive protein (hs-CRP), an acute-phase protein that functions as a non-specific marker of inflammation, infection, and tissue damage and is crucial for evaluating the risk of various diseases, including cardiovascular disease [10, 11]. Hypertension related to coronary artery disease (CAD) is a significant global health issue, with its prevalence rising rapidly, and hypertensive individuals accounting for approximately one-quarter of the global adult population [12, 13]. Metabolic syndrome is characterized by traits such as insulin resistance, obesity, hypertension, and elevated LDL levels, all of which contribute independently to atherosclerosis and are commonly observed in individuals with diabetes [14].

Coronary atherosclerosis is a major health concern and a leading cause of illness and death worldwide. This study investigates the role of IL-18 and its gene polymorphisms in the progression of coronary atherosclerosis, given the lack of research in this area, by examining the impact of serum levels of specific cytokines.

II. MATERIALS AND METHODS

A. Research methodology and participant selection

This prospective case-control study was conducted at the Cardiac Centre of the Surgical Specialty Hospital, Erbil, from June to December 2024. A total of 200 Kurdish subjects were recruited and

divided into two groups: coronary atherosclerosis patients (CAPs) and controls [15].

- *CAP group:* 100 patients (67 men, 33 women; aged 37–75 years) with ≥ 45% stenosis in one of the major coronary arteries, as confirmed by coronary angiography. All patients presented with angina and underwent PCI for revascularization in accordance with the 2024 ESC guidelines on chronic coronary syndromes.
- *Control group:* 100 participants (68 men, 32 women; aged 30–72 years) were enrolled from blood donors and cardiac centers. According to the 2019 ESC Guidelines, they had no clinical evidence of CAD or other high-risk diseases, apart from manageable conditions such as hypertension or diabetes.

Strict exclusion criteria were applied to exclude individuals with stroke, heart failure, recent surgeries, or other confounding factors. Ethical approval for the study was obtained from the Research Ethics Committee of Hawler Medical University (Meeting Code: 7, Paper Code: 18, May 27, 2024). Verbal informed consent was obtained from all participants, who were assured of confidentiality and their right to withdraw from the study at any time.

B. Clinical Assessment

Medical history and sociodemographic data were collected using structured questionnaires and interviews. Information was obtained on smoking status, hypertension, diabetes mellitus (DM), obesity, and family history of coronary artery disease (CAD). The classification criteria were as follows:

- *Hypertension:* systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, or current use of antihypertensive medication.
- *Diabetes mellitus:* fasting blood glucose > 126 mg/dL, HbA1c > 6.5%, or a previous clinical diagnosis of diabetes.
- *Obesity:* body mass index (BMI) ≥ 30 kg/m^2.
- *Smoking:* active smoking history of ≥ 10 pack-years.

All patient groups received standard medical treatment, including beta-blockers, statins, angiotensin-converting enzyme (ACE) inhibitors, and metformin, as clinically indicated.

C. Body Mass Index

Per WHO recommendations, BMI is calculated as weight (kg) divided by height (m^2) and classified as underweight (< 18.5), normal (18.5–24.9), overweight (25–29.9), and obese (≥ 30).

D. Sample Collection

A 10 mL venous blood sample was collected before angiography, centrifuged at 2500 rpm for 15 min, and stored at −80°C for serum analysis. For molecular analysis, DNA was extracted from 3 mL of whole blood collected in EDTA tubes during angiography.

E. Coronary Angiography

This procedure was performed through the right femoral artery. Affected vessels were identified, and PCI was performed for newly detected arterial stenoses.

F. Laboratory Investigations

The enzyme-linked immunosorbent assay (ELISA) was used to measure proteins. The binding of antigens to antibodies produced a colorimetric reaction proportional to the concentration, which was measured spectrophotometrically [16].

Serum interleukin-18 (IL-18) concentrations were determined using ELISA kits (SUNLONG BIOTECH, China). An immunoassay was performed on a Cobas C311 analyzer to measure C-reactive protein (CRP) (Roche Diagnostics GmbH).

- *Lipid profile:* triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol were measured using a Cobas C311 analyzer.
- *HbA1c:* glucose monitoring over a period of 3 months was performed using a Cobas Integra 400 analyzer.

The Medonic M-series M32 analyzer (Sweden) was used to determine RBC, Hb, Hct, WBC, and platelet counts.

G. DNA extraction and quality control

Whole blood was used to obtain genomic DNA. The quality and quantity of DNA were evaluated using [17]:

- *Nanodrop spectrophotometer:* for DNA concentration and purity.
- *Agarose gel electrophoresis:* for inspection and contamination testing.

H. SNP selection and primer design

The IL-18 gene polymorphism -607T/G (rs1946518) on chromosome 11q22.3 was selected based on its clinical significance. NCBI Primer-BLAST was used to design the primers, and they were validated using previous research (Tables 1 and 2).

Table 1. SNPs, chromosomal location, functional sites, and substitutions (NCBI)

Gene	Chromosome	dbSNP	Position	SNP
IL-18	11q22.3	rs1946518	-607	T/G

I. PCR amplification

The PCR reaction was performed in a 50 μl mixture containing 2x Taq DNA Polymerase Master Mix, 10 pmol primers, DNase-free water, and template DNA, using a Bioresearch PTC-200 Gradient thermocycler. The amplification protocol is outlined in Table 3. One of the two allele-specific forward primers and a general antisense primer were used to detect the IL-18 polymorphism.

The PCR reaction conditions were optimized by adjusting the annealing temperature for each primer. The PCR protocol for gene polymorphism detection was then performed as detailed in Table 4.

J. Agarose gel electrophoresis

DNA fragments were separated in 1X TBE on 1.0–1.5% agarose gels. Samples were loaded with loading buffer and electrophoresed at 75 V/cm^2 and then at 85 V/cm^2 for 20 and 30 minutes, respectively. Bands were visualized under UV light (312 nm) after ethidium bromide staining.

Table 2. Primer sequences were specifically designed for use in ARMS-PCR genotyping detection

Gene polymorphisms	Minor allele	Primer name	Primer sequence (5′-3′)	allele	Amplicons size (bp)	Source of primers
IL-18 (–607 T/G)	G	F1:	GTTGCAGAAAGTGTAAAAATTATTAC	T	196	[18]
		F2:	GTTGCAGAAAGTGTAAAAATTATTAA	T	196	
		R:	TAACCTCATTCAGGACTTCC	G	301	
		IPC:	CTTTGCTATCATTCCAGGAA		301	

Table 3. DNA Amplification components of IL-18

No.	PCR components	Concentration	Volume (μl)
1	Master Mix	2x	12.5
2	Forward Primer	10 Pmol	1
3	Reverse Primer	10 Pmol	1
4	DNase free Water	-	8.5
5	Template DNA	50ng/μl	2
		Total	25

Table 4. PCR cycling for gene polymorphism detection

Gene polymorphism	PCR cycles	Temperature	Time	Source of PCR Cycling
IL-18 (–607 T/G)	Initial denaturation	95 °C	7 min.	[18]
	PCR cycling (35 cycles) Denaturation	95 °C	30 sec.	
	Annealing	50 °C	45 sec.	
	Extension	72 °C	50 sec.	
	Final extension	72 °C	7 min.	
	Hold	20 °C	∞	

K. Statistical Analysis

SPSS v26.0 was used to analyze the data. IL-18 levels (pg/mL) were standardized through ELISA quantification. Continuous variables were reported as mean ± SE and compared using one-way ANOVA or Student's t-test. The chi-squared test was used to analyze categorical variables. Pearson correlation coefficients were used to assess linear relationships [19]. Online calculators were used to determine genotype and allele frequencies and to test Hardy–Weinberg equilibrium.

III. RESULTS

In this study, there were 100 CAD patients and 100 controls. The patients were older and more often diabetic and hypertensive, with higher systolic blood pressure, BMI, and HbA1c levels (all $p< 0.001$). LDL-C and HDL-C levels were lower, and a significant increase in inflammatory biomarkers (IL-18 and hsCRP) was observed in the patient group. WBC counts were also higher. Angiography revealed single-vessel disease in 44% of patients, most commonly involving the LAD (83%). All patients were treated with dual antiplatelet therapy, and 44% received either metformin or insulin (Table 5).

DM refers to diabetes mellitus, HTN to hypertension, BMI to body mass index, HbA1c to hemoglobin A1c, IL-18 to interleukin-18, hsCRP to high-sensitivity C-reactive protein, HDL-C to high-density lipoprotein cholesterol, LDL-C to low-density lipoprotein cholesterol, and WBC to white blood cell count. For the single nucleotide polymorphism analysis of IL-18 (rs1946518), 25 patients and 25 control participants were randomly selected from the 100 patients and 100 controls. The primers yielded IL-18 products of 196 bp and 301 bp. The PCR product was electrophoresed and visualized on a 1.5% agarose gel. Important variables included patient/control status, IL-18 polymorphism, and amplicon size (Figures 1 and 2).

Table 5. Some data on sociodemographic, clinical features, and baseline information of study groups

Variable	Patients	Controls	P Value
Age (years)	58.6	48.8	–
Diabetes (%)	48	7	0.001**
Hypertension (%)	64	26	0.001**
BMI (kg/m^2)	30.5	28.4	0.001**
HbA1c (%)	6.93	5.62	0.001**
IL-18 (pg/ml)	63.9	8.1	0.001**
hsCRP (mg/L)	6.07	2.75	0.028*
HDL-C (mg/dL)	34.8	37.3	0.032*
LDL-C (mg/dL)	74.9	84.4	0.027*
WBC (10^3/µL)	8.35	7.32	0.001**

DM refers to Diabetes Mellitus, HTN to Hypertension, BMI -body mass index: HbA1c to hemoglobin A1c- IL-18 to interleukin-18; hsCRP to high-sensitivity C-reactive protein; HDL-C to high density lipoprotein cholesterol: LDL-C - low-density lipoprotein cholesterol: and WBC to white blood cell count.

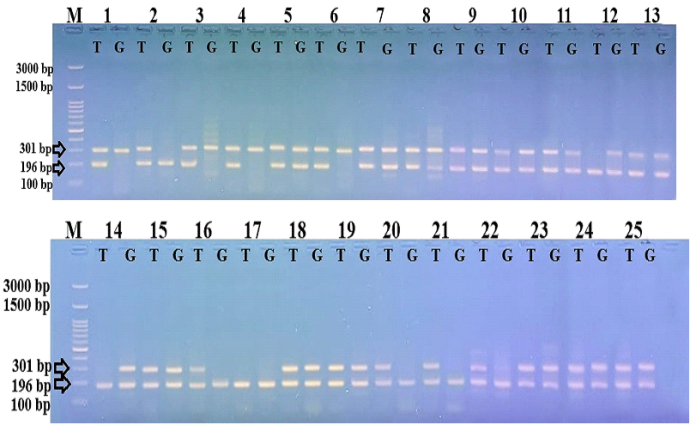

Figure 1. Control samples were analyzed by gel electrophoresis of IL-18 SNP (rs1946518) on a 2% agarose gel with ethidium bromide staining. The PCR product size was 196 bp, and the reference gene size was 301 bp. The DNA ladder ranged from 3000 to 100 bp. Lanes 1–5 and 25 represent the tested samples, with homozygous genotypes (TT or GG) displaying a single band and heterozygous genotypes (TG) showing bands in two separate lanes.

A. IL-18 gene T>G gene polymorphism genotypes and allele frequencies among study groups

Three genotypes (TT, TG, and GG), corresponding to the T and G alleles, were analyzed. The frequencies of these genotypes and their alleles were recorded in the patient and control groups. The patient group was in Hardy–Weinberg equilibrium (HWE) ($p> 0.05$), while the control group was not ($p< 0.05$). The wild

genotype (TT) was found at a higher rate in patients (25%) than in controls (16%), but this difference was not statistically significant (p> 0.05).

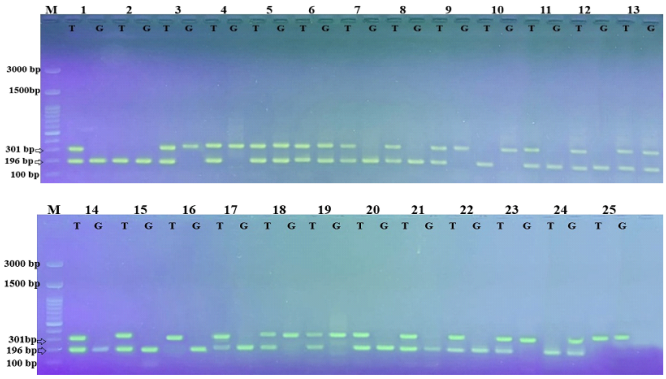

Figure 2. Gel electrophoresis was performed on patient samples to analyze the IL-18 SNP (rs1946518) using a 2% agarose gel stained with a safety stain. The amplified PCR product had a molecular size of 196 bp, while the reference gene measured 301 bp. A DNA ladder of 3000–100 bp was used, with lanes 1 to 5–25 representing the tested samples. Homozygous genotypes (TT or GG) showed a single band for each sample, while heterozygous genotypes (TG) revealed bands in two lanes, reflecting both T and G alleles.

The frequency of allele T was 0.604 and 0.580 in the patient and control groups, respectively. The control group had a higher percentage of heterozygous (TG) genotypes, whereas the patient group had a lower percentage (84% and 70.83%, respectively). The differences were not significant (p> 0.05). Moreover, a higher proportion of the homozygous (GG) genotype was present in the patient group than in the control group (4.16% and 0.0%, respectively), with non-significant differences (p> 0.05). The frequency of allele G was higher in the control group than in the patient group, as illustrated in Table 6.

Table 6. IL-18 gene T>G gene polymorphism genotypes and allele frequency among study groups

IL-18 gene T > G	Patient (Numbers = 24)	Control (Numbers = 25)	X^2	P value
Genotype Frequency No (%)				
Wild Type (TT)	6 (25.0)	4 (16.0)	0.611	0.435
Heterozygote (TG)	17 (70.83)	21 (84.0)	1.219	0.269
Homozygous mutant (GG)	1 (4.16)	0 (0.0)	1.020	0.312
Allele Frequency				
T	0.604	0.580		
G	0.396	0.420		
HWE X2	5.550	13.109		
HWE P*	0.062	0.002**		
HWE: Hardy–Weinberg equilibrium; X2: chi-square. *Significant deviation was observed from HWE (P<0.05).				

B. Chi-square analysis of genotype and allele distribution among study groups

Although the TT genotype was more prevalent in the patient group than in the control group, the difference was not statistically significant (p> 0.05). Conversely, the TG genotype showed a higher distribution in the control group (p> 0.05). Notably, the patient group had a higher proportion of the T allele (60.48%), whereas the control group had a higher proportion of the G allele (42%). Chi-square analysis revealed no significant differences in allele frequency between patients and controls for either allele (p> 0.05) as detailed in Table 7.

Table 7. Chi-square investigation of genotype and allele distribution among study groups

IL-18 gene T > G	Patient (Numbers = 24)		Control (Numbers = 25)		X^2	P value
	No.	(%)	No.	(%)		
Genotype						
TT	6	(25.0)	4	(16.0)	0.611	0.435
TG	17	(70.83)	21	(84.0)	1.219	0.269
GG	1	(4.16)	0	(0.0)	1.020	0.312
TG+GG	18	(75.0)	21	(84.0)	1.231	0.267
Total	24	100	25	100		
Alleles						
T	29	(60.48)	29	(58.0)	0.059	0.808
G	19	(39.58)	21	(42.0)	0.049	0.825
IL-18: Interleukin-18, was analyzed for significant differences using chi-square (X^2) and Fisher's exact test at a p-value level of <0.05.						

C. Comparison of IL-18 Level Within the Patient Group With Regard to IL-18 Gene Polymorphism

Patients in the heterozygous TG subgroup showed higher concentrations than those with other IL-18 gene polymorphisms, although the differences were not significant (p> 0.05). A similar lack of significant difference was observed when comparing the combined TG+GG group with the TT genotype, as detailed in Table 8.

Table 8. Comparison of IL-18 level among the patient group with regard to IL-18 gene polymorphism

IL-18 gene polymorphism	Patients No. 24	P value
TT	19.04±1.36 a	0.390*
TG	33.35±6.12 a	
GG	21.47±0.51 a	
TG+GG	32.72±5.81	0.196**
TT	19.04±3.35	
IL-18: Mean ± SE was used to report interleukin-18 results. *ANOVA and t-tests** were used for statistical comparisons, with significance thresholds set at p < 0.05 for significance and p > 0.05 for non-significance. Significant differences are shown by groups with distinct letters, and non-significant differences are indicated by similar letters.		

D. Comparison of IL-18 Level Within Study Groups With Regard to IL-18 Gene Polymorphism

The TG and combined TG+GG genotypes exhibited significantly higher concentrations in the patient group than in the control group (p< 0.05). The TT genotype had lower IL-18 concentrations than the other genotypes, with patients showing elevated levels compared to controls, which was also significant (p< 0.05). The GG genotype could not be evaluated because of the lack of control values, suggesting a potential disease-specific link. The presence of the G allele in either TG or GG form was associated with increased levels in patients, indicating its potential role as a risk allele (Table 9).

E. Comparison of Some Inflammatory Markers, Lipid Profile, and Hematological Indices Among Study Groups With Regard to Different IL-18 Genotype Distributions

Comparison of variables among CAD patients with different IL-18 genotypes (TT vs. TG+GG) indicated that the TG+GG group was older. Significant differences were noted in inflammatory biomarkers, with the TG+GG group showing the highest levels of IL-18 and hsCRP, while the control group had the lowest levels.

No significant differences in lipid profiles were found among the groups, although HDL cholesterol was lower in TT patients than in controls. Hematological indices showed reduced WBC levels in the TT wild-type control group, with no significant intergroup differences. More details are presented in Table 10.

Table 9. Comparison of IL-18 Level Within Study Groups With Regard to IL-18 Gene Polymorphism

IL-18 gene polymorphism	Patients No. 24	Control No. 25	P value
TT	19.04±1.36	9.35±0.37	0.001**
TG	33.35±6.12	8.78±0.42	0.001**
GG	21.47±0.51	-	-
TG+GG	32.72±5.81	8.78±0.42	0.001**
Values are expressed as mean ± SE, with significance determined by t-tests, where p > 0.05 denotes non-significance and p < 0.05 denotes significance.			

Table 10. Comparison of some inflammatory markers, lipid profile, and hematological indices among study groups with regard to different IL-18 genotype distributions

Variables	Study groups			
	Patient No. 24		Control No. 4	P value
	TT No. 6	TG+GG No. 18	TT No. 4	
Mean ± SE				
Age in Years	51.33±4.45a	54.33±2.13a	36.50±2.10b	0.06
BMI (Kg/m^2)	31.60±2.48a	32.34±0.86a	28.10±0.78a	0.196
Inflammatory Biomarkers				
IL-18 (pg/ml)	19.04±1.36ab	32.72±5.81a	9.35±0.37b	0.090
hsCRP (mg/L)	4.44±1.83a	4.52±1.17a	1.35±0.30a	0.456
Lipid profile and lipid ratio				
HDL-C (mg/dL)	34.14±3.10 a	34.26±1.98 a	37.22±3.39 a	0.793
LDL-C (mg/dL)	67.95±10.30 a	84.63±5.83 ab	101.45±9.47 b	0.116
Hematological indices				
WBC (10^3 /µL)	8.26±1.14 ab	8.26±0.41b	5.77±1.06 a	0.102
Values are expressed as mean ± SE, with comparisons made using ANOVA. Different letters indicate significant group differences, with significance defined as *P < 0.05 (significant) and **P < 0.01 (highly significant).				

IV. DISCUSSION

The mean age of patients in our study is aligned with the previous literature [20, 21]. Obstructive CAD was more common among men and was dominated by males, which is congruent with previous results [22]. The risks of coronary atherosclerosis and cardiovascular events increased in cases of hypertension and diabetes, as reported in previous studies [23].

In the study, IL-18 concentration and hs-CRP were highly significantly elevated in patients with coronary atherosclerosis compared to controls, indicating a strong positive correlation between these biomarkers. This aligns with prior findings regarding inflammatory responses and endothelial dysfunction in atherosclerosis [24].

IL-18, a pro-inflammatory cytokine in the IL-1 superfamily, is mainly secreted by macrophages and activates TNF-α, IL-6, and CRP. It has been linked to atherosclerosis, as reported by [25], and to CAD. Circulating IL-18 has been proven to be an independent biomarker of CAD in a meta-analysis by [26]. Hepatocytes produce C-reactive protein (CRP), a member of the pentraxin family, in reaction to IL-6. It suggests plaque instability and cardiovascular risk and is a hallmark of the acute-phase response [27].

The correlation between environmental risk factors and genetic variations is significant, supporting innovations in the treatment and prevention of coronary artery disease [28]. In particular, the IL-18 rs1946518 gene, especially the -607 T/G variant in its promoter region, is under intensive research for its involvement in various human diseases.

The crucial involvement of IL-18 in the inflammation cascade is demonstrated by its elevated levels in inflammatory diseases [6]. The IL-18 gene, situated on chromosome 11q22.2–q22.3, consists of five introns and six exons. Studies indicate a correlation between IL-18 polymorphisms, specifically rs1946518 (T > G) at position -607 and rs187238 (T > G) at position -137, and the risk of coronary artery disease (CAD) [8, 9].

In the existing study, we designed to investigate the relationship of IL-18 -607 and rs1946518 (T > G) and IL-18 level with coronary atherosclerosis disease susceptibility and severity among Kurdish people in Erbil governorate. The patient group was in Hardy–Weinberg equilibrium (HWE) regarding IL-18 -607 T/G genotype polymorphism ($p > 0.05$). In comparison, the control group showed a significant difference ($p < 0.05$).

This study examined the abnormal deviations from Hardy–Weinberg equilibrium (HWE) in the control group, relating these unusual results to factors such as insufficient genotyping in heterozygous individuals and the limited sample size, along with the collection of control samples from individuals across multiple hospitals, which impacts the accuracy of genotype frequency estimation, Table 6. The distribution of the -607 T/G genotype in the control group conformed to Hardy–Weinberg equilibrium but showed a significant difference between expected and observed frequencies ($\chi^2 = 13.109$), Table 6. This SNP is common in the Kurdish community, where the wild-type T allele offers protection against susceptibility, while the mutant G allele is influenced by environmental factors.

A higher proportion of the T allele was observed in the patient group (60.48%), while a higher proportion of the G allele was observed in the control group (42%). The statistical analysis for allelic frequency showed no significant differences between patients and controls ($p > 0.05$), Table 7. This finding has been further supported by determining the level of IL-18 protein in all patient groups. When IL-18 protein was compared within the same patient group, it showed that carriers of TT had a higher genotype distribution in the patient group compared to the control group, but the difference was statistically insignificant ($p > 0.05$), Table 8. Moreover, the present study revealed insignificant differences ($p > 0.05$) in IL-18 protein level between patients and the control group for genotype TG, and illustrated its higher genotype distribution in the control group compared to the patient group. Since we could not detect the GG genotype in the control group, we could not make a comparison regarding this genotype, Table 9. This finding demonstrated that the G allele may be associated with atherosclerosis susceptibility and severity and with its complicated events.

The results of the current study seem to be supported by previously published studies in which a higher frequency of the TT genotype was found in the patient group, whereas the TG genotype showed a higher frequency in the control group [29, 30]. IL-18 is a pro-inflammatory cytokine formed by immune cells such as dendritic cells and macrophages. It affects neutrophils, T cells, and natural killer cells, enhancing the production of tumor necrosis factor-alpha and interferon-gamma. IL-18 plays a crucial role in inflammatory conditions, including autoimmune disorders, atherosclerosis, and even infectious diseases [31, 32]. Variations of

the -607 T/G IL-18 gene influence serum IL-18 concentrations and polymorphisms, with varying results across countries. Research has linked IL-18 polymorphism risk to breast cancer [33, 34]. The IL-18 -607 polymorphism may be linked with a higher risk of breast cancer, especially in Asian and diverse populations [35, 36]. Additional research demonstrated that allele C of the IL-18 gene SNPs and the G/C genotype at the -137 position are protective factors against coronary artery disease (CAD) (Lu et al., 2013b), while no link was found for the -607 (C/A) polymorphism [29]. These results conflicted with previous findings. Additionally, they confirmed our findings by demonstrating a high concentration of IL-18 in the atherosclerotic tissues of individuals with CAD.

Regarding the distribution of risk factors in patients based on IL-18 polymorphism, no significant differences were observed among the three genotypes, indicating no association between this variant and CAD risk factors in our study. Lu et al. [37] found an association between the IL-18 (-607) allele C and CAD susceptibility, while Opstad et al. (2011) found no significant relationships between the studied polymorphisms and CAD. Also, another previous study performed on IL-18 -607 C/A polymorphisms and polycystic ovary syndrome (PCOS) revealed conflicting results. A study by Ibrahim and Al-Saffar (2018) found no significant association, while [38] reported a significant correlation with increased PCOS risk. Additionally, a study on Greek patients indicated that IL-18 levels are elevated in patients and rise further with obesity and insulin resistance [39]. Moreover, Umare et al. [40] indicated that IL-18 -607 polymorphisms are linked to disease susceptibility and are associated with renal and neurologic involvement in Indian patients with systemic lupus erythematosus. In contrast, [41] found no relation between IL-18 gene variations and rheumatoid arthritis or cardiovascular disease risk factors, differing from the findings of [42].

The study also identified a significant association between the TT, TG, and GG genotypes, and T and G distribution alleles at the rs1946519, 656 T/G IL-18 gene position, with cardiovascular disease linked to hypertension, dyslipidemia, and diabetes, compared to the healthy group, indicating that these SNPs may affect both disease risk and phenotype severity [43]. A study conducted within an Asian population observed a significant relationship between the IL-18 (-137 G/C) gene variation and the risk of diabetic nephropathy, suggesting that alterations in the IL-18 gene promoter region may disrupt its normal function and lead to immune dysfunction and increased disease susceptibility [44]. Additionally, research among the Japanese population indicates that genetic variations in the interleukin gene are linked with kidney disorders in chronic kidney disease [45]. Also, Kariž and Petrovič [46] reported that IL-18 variations at -607 C/A and -137 G/C do not correlate with myocardial infarction (MI) risk in diabetics, suggesting that they are insufficient as risk markers. A study in Saudi Arabia similarly found no significant associations at three SNP sites with CAD [47]. Moreover, numerous studies indicate that genetic variations in the IL-18 pathway are linked to circulating IL-18 levels in coronary artery disease (CAD), with variants in the IL18R1 and IL18RAP genes associated with elevated IL-18 levels and a heightened risk of MI [48, 49]. Receptor polymorphisms may affect IL-18 signaling control, increasing systemic levels. [50] indicated that genetic variations influencing IL-18 production or signaling can contribute to chronic inflammation and promote plaque formation in atherosclerosis.

Recent research reported a significant correlation between IL-18 and hypertriglyceridemia, cardiometabolic risk, insulin resistance, and even inherent genetic protection [51]. The TG+GG genotype subgroup demonstrated insignificant correlations between IL-18 and both TC and non-HDL cholesterol. These findings may be explained by increased lipid consumption through systemic inflammation or by more aggressive cholesterol-lowering medication usually prescribed to higher-risk genotypes. [52] supported this explanation and observed that CAD patients bearing IL-18 risk alleles frequently exhibit elevated cytokine levels while simultaneously maintaining reduced cholesterol levels, attributable to intensive statin therapy or lipid depletion driven by inflammation. The absence of significant correlations with additional lipid markers shows the specific metabolic role of IL-18, which seems to be significantly influenced by genotype, treatment status, and inflammatory state [53]. These results confirm that the IL-18 polymorphism has a complex, non-linear effect on lipid metabolism and cardiovascular risk [54].

The current study revealed a correlation between TG+GG genotypes and elevated IL-18 levels, corroborated by [55], which indicated that specific IL-18 promoter genotypes, such as CC at -137, result in enhanced IL-18 expression. Some research categorizes TG+GG as a risk genotype linked to elevated inflammation and older age. However, certain meta-analyses indicated that specific minor alleles of IL-18 polymorphisms, such as the C-allele in rs187238 or the A-allele in rs1946518, could provide protective effects against CAD risk. The observed variation could originate from differences in population genetics or in the definitions of the TG+GG genotype. Moreover, increased IL-18 levels may not reliably signify long-term risk owing to compensatory mechanisms including IL-18BP and other regulatory factors [56]. hsCRP, which comes from the liver, is sensitive to metabolic syndrome, while IL-18, which comes from macrophages, is more associated with plaque variability [57]. Therefore, weak correlations between IL-18 polymorphisms and hsCRP are expected, as they represent separate inflammatory pathways, analogous to different instruments within the same group. Minor or non-significant correlations between IL-18 and HbA1c in CAD samples agree with previous research specifically designed to examine this relationship [58].

Moreover, although IL-18 levels may rise in the presence of insulin resistance or diabetes, its genotype variations generally do not correlate with HbA1c, demonstrating that IL-18 genotypes reflect inflammatory susceptibility rather than chronic glycemic status. Recent studies demonstrated a link between IL-18 and kidney injury, specifically emphasizing the association of its promoter variants with post-transplant kidney function and the risk of nephropathy. IL-18 levels may indicate renal inflammation and injury; however, their relationship with conventional renal markers such as urea and creatinine is context-dependent, influenced by medical conditions (chronic CKD versus AKI) and genetic factors. The concentration of IL-18 is elevated during acute kidney injury; however, its genetic polymorphisms do not correlate with baseline kidney function [59]. This observation supports the idea that renal markers vary with physiological and pathological changes, independent of IL-18 genotype [59].

IL-18 concentrations are predominantly affected by inflammation rather than age, and although BMI influences low-grade inflammation, IL-18 gene variations do not predict BMI-related alterations. Relationships between age and BMI have been observed

only slightly, indicating that genotype affects inflammatory signaling. In the population, IL-18 levels and genotype exhibited modest correlations with age, hsCRP, and BMI markers, indicating minimal connections with inflammatory and metabolic processes in the CAD study [57, 60].

The present cross-sectional study might not consider dynamic relationships affecting IL-18 activity, as factors like genetic variation and unknown regulators (e.g., IL-18 binding protein) play a role [61]. Despite no substantial differences in IL-18 genotype or allele frequencies in patients with CAD versus controls, inflammation and environmental factors significantly influence IL-18 levels, indicating that inflammation is critical in coronary artery disease (CAD).

V. CONCLUSION

The present study confirms that IL-18 serum concentration is significantly elevated in coronary artery disease (CAD), which contributes to disease progression. Additionally, the IL-18 gene rs1946518 polymorphism is related to increased IL-18 concentration and a higher risk of CAD, suggesting a genetic influence on vascular inflammation. Combined measurement of these biomarkers improves CAD detection, suggesting a valuable combined assessment approach for early diagnosis and risk assessment. These findings highlight the complex interplay of genetic and inflammatory factors in CAD and support further research to validate their clinical utility and guide personalized treatment strategies.

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INTEREST CONFLICT

The authors declare no conflict of interest.

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