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# Impact of IL-10, and IL-33, Polymorphisms on Atherosclerosis Risk: Insights from a Jordanian Cohort

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**Abstract**

Atherosclerosis, a complex and chronic inflammatory disease, remains a major cause of cardiovascular morbidity and mortality worldwide. Growing evidence suggests that genetic variations in inflammatory cytokines, such as interleukin-10 (IL-10) and interleukin-33 (IL-33), may influence individual susceptibility to the disease. This study explored the relationship between polymorphisms in the IL-10 and IL-33 genes and the risk of developing atherosclerosis in a Jordanian population.

We analyzed blood samples from 100 patients with clinically confirmed atherosclerosis and 110 healthy, unrelated controls. DNA was extracted and genotyped using established PCR (PCR-SSP) technique of IL-10 and PCR-MS for IL-33 polymorphism, serum cytokine levels were measured using ELISA. Our results revealed that the T allele and T/T genotype of the IL-33 rs7044343 variant were significantly more common in healthy controls than in patients, suggesting a potential protective role against coronary artery disease. While overall IL-33 genotype frequencies did not differ significantly between groups, the data did show deviations from Hardy-Weinberg equilibrium in the patient group, which may reflect underlying genetic differences related to disease presence.

In addition, patients with atherosclerosis had notably lower serum levels of IL-33, supporting its proposed anti-inflammatory and cardioprotective effects. For IL-10, the -1082 A>G (rs1800896) polymorphism showed borderline significance in genotype distribution, hinting at a possible but less clear role in disease development.

These findings underscore the importance of genetic background in shaping individual responses to inflammatory processes in atherosclerosis. While IL-33 appears to play a more prominent role, both cytokines merit further investigation. Understanding how these gene variants interact with lifestyle and environmental factors could eventually lead to more personalized strategies for the prevention and management of atherosclerosis.

**Keywords**: Atherosclerosis, Cytokine Gene Polymorphisms, IL-10, IL-33, Coronary Artery, Disease (CAD), Genetic Diversity, Jordanian Population

## **1.** Introduction:

Atherosclerosis is a chronic inflammatory condition affecting the walls of medium and large arteries. The progression of atherosclerosis is regulated by both innate and adaptive immune responses, which are coordinated through a complex network of cytokines that influence all stages of the disease [1]. Cytokines play a crucial role in the maturation, differentiation, and functional activation of immune cells. They regulate various cellular processes, including proliferation and differentiation, and maintain a balance between pro-inflammatory and anti-inflammatory pathways, which can impact the progression of atherosclerosis [2].

Anti-inflammatory interleukins are recognized for suppressing pro-inflammatory signaling pathways and modulating immune responses. These interleukins employ various mechanisms to reduce inflammation by producing pro-inflammatory cytokines and regulating immune cell activation and function. Key anti-inflammatory cytokines of interest, interleukin-33 (IL-33), and interleukin-10 (IL-10) [3].

Interleukin-10 (IL-10) is a prototypical anti-inflammatory cytokine that plays a pivotal role in regulating the Th1/Th2 balance, shifting the response toward Th2. It has been extensively studied in the context of atherosclerosis and is considered an anti-atherosclerotic cytokine due to its diverse mechanisms, including Th2 polarization. IL-10 inhibits the expression of inflammatory genes across various cell types and suppresses antigen presentation and T-cell proliferation [4]. The IL-10 gene is located on chromosome 1, spanning the 1q31 to 1q32 regions [5]. Its promoter region is highly polymorphic, with biallelic variants such as -1082 A > G (rs1800896), −819 T > C (rs1800871), and −592 A > C (rs1800872). These variants are known to affect the promoter region of the IL-10 gene, thereby influencing its transcriptional activity and resulting in altered plasma levels of IL-10. Specifically, certain polymorphisms—such as the -1082

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A>G (rs1800896)—are associated with increased or decreased binding affinity of transcription factors, which modulates the rate of gene transcription. This regulatory effect has been observed in various inflammatory conditions, including acute coronary syndromes (ACS), where altered IL-10 expression may impact the balance between pro- and anti-inflammatory responses. [5].

Interleukin-33 (IL-33) is a recently characterized member of the IL-1 cytokine family. Stromal cells express epithelial and endothelial cells. Its expression increases in response to pro-inflammatory stimuli. IL-33 functions both as a conventional cytokine and as a nuclear factor that regulates gene transcription, serving as an "alarmin" that signals immune activation in response to cellular stress or damage. IL-33 is thought to play a protective role in the development of atherosclerosis by inducing the production of interleukin-5 (IL-5) and promoting the generation of antibodies against oxidized low-density lipoprotein (ox-LDL), a modified form of LDL that contributes to plaque formation.[6]. The IL-33 gene is located on chromosome 9 (9p24.1)[7].

Studies have shown that administering interleukin-33 (IL-33) to apolipoprotein E-deficient (ApoE$^{-/-}$) mice, a widely used animal model for atherosclerosis characterized by impaired lipid metabolism and spontaneous plaque development, leads to the induction of protective antibodies against oxidized low-density lipoprotein (ox-LDL) and increased production of Th2 cytokines. This immune response results in a significant reduction of atherosclerotic plaque formation in the aortic sinus [8]. Furthermore, research on tag single nucleotide polymorphisms (tag SNPs)—genetic variants selected to represent regions of the IL-33 gene (rs7025417, rs10975514, and rs10975519)—in coronary artery disease (CAD) patients from the Chinese Han population has highlighted the contribution of IL-33 polymorphisms to disease susceptibility [9,10].

A range of single nucleotide polymorphisms (SNPs) with functional relevance has been identified in cytokine genes, influencing interleukin production and contributing to the pathogenesis of atherosclerosis [11]. In this study, we specifically focus on two anti-inflammatory cytokines—interleukin-10 (IL-10) and interleukin-33 (IL-33)—and examine the association between their gene polymorphisms and clinically confirmed atherosclerosis in a Jordanian population. Rather than assessing genetic predisposition in healthy individuals, this study investigates patients already diagnosed with atherosclerosis. Our objectives are to determine the frequency of IL-10 and IL-33 polymorphisms in these patients compared to healthy controls and to explore how these genetic variations correlate with circulating levels of IL-10 and IL-33, thereby shedding light on their potential role in disease progression and inflammatory regulation.

## 2. Patients and Methods

### *2.1. Study Population*

This study included 100 consecutive Jordanian patients (87 males and 13 females) with clinically confirmed atherosclerosis, recruited from Prince Hamza Hospital, a public healthcare facility in Amman, Jordan. Additionally, 110 unrelated healthy blood donors (60 males and 50 females) were included as the control group. All participants underwent a comprehensive clinical evaluation.

Laboratory test results, including complete blood count (CBC), cardiac enzyme levels, renal function tests, lipid profile, and C-reactive protein (CRP) levels, were retrieved from the patients' medical records. These analyses were conducted by the clinical laboratories of Prince Hamza Hospital as part of routine diagnostic workup. Participants also completed a brief standardized questionnaire designed to collect demographic and clinical information, including age, sex, smoking status, family history, and comorbidities.

Blood samples were collected by trained hospital phlebotomists under sterile conditions. Approximately 10 mL of venous blood was drawn from each participant into ethylenediaminetetraacetic acid (EDTA) tubes. The samples were transported on ice to the research laboratory. Plasma was separated by centrifugation and stored at –80°C for subsequent analysis of cytokines using enzyme-linked immunosorbent assay (ELISA). The remaining cellular fraction was used for DNA extraction and genotyping analysis.

The study protocol received ethical approval from the Ethical Committee of Prince Hamza Hospital 7/37/2/1426 and from the Ethics committee for scientific research (1/8/2021) of Zarqa University. Written informed consent was obtained from all participants prior to their inclusion in the study.

### *2.2. DNA Extraction*

Blood samples were collected in sterile EDTA tubes. Genomic DNA was extracted from whole blood using a standard commercial kit (Qiagen Ltd., UK) according to the manufacturer's instructions.

### *2.3. SNP Typing*

The IL-10 single nucleotide polymorphism (SNP) at position −1082 (G/A) was genotyped using the polymerase chain reaction sequence-specific primer (PCR-SSP) method, as previously described by Talaat et al. (2018). To amplify the IL-10 promoter region containing the −1082 SNP, DNA samples were subjected to PCR using allele-specific primers listed in Table 1. Each DNA sample was run in two separate reactions—one specific for the G allele and the other for the A allele—using a final reaction volume of 25 µL. The PCR mixture contained DreamTaq Green PCR Master Mix (2×, Fermentas), 10 pmol of the allele-specific forward primer, 10 pmol of the reverse primer, 3.5 pmol of each internal control primer, and approximately 100 ng of genomic DNA. The thermal cycling protocol began with an initial denaturation at 94°C for 2 minutes, followed by 5 cycles at 96°C for 25 seconds, 70°C for 45 seconds, and 72°C for 20 seconds. This was followed by 11 cycles at 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds, and concluded with 15 cycles at 96°C for 25 seconds, 55°C for 60 seconds, and 72°C for 2 minutes. After amplification, PCR products were separated on a 2% agarose gel and visualized under UV light. The size of each product was determined relative to a 100 bp DNA ladder (Fermentas). The expected product sizes were 258 bp for the −1082 region, 233 bp for the −819 region, and 429 bp for the internal control fragment. **[18]**

The single nucleotide polymorphism (SNP) rs7044343 in the IL-33 gene was genotyped using a conventional polymerase chain reaction (PCR) method. Genomic DNA was amplified using a specific primer pair targeting the region containing the rs7044343 polymorphism. Table (1), producing a 547 base pair (bp) amplicon. Each PCR reaction was performed in a final volume of 25 µL using DreamTaq Green PCR Master Mix (Fermentas), 10 pmol of each primer, and approximately 100 ng of genomic DNA. The thermal cycling conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, with a final extension step at 72°C for 7 minutes. PCR products were resolved on a 2% agarose gel and visualized under UV illumination to confirm the expected 547 bp band.

**Table 1.** Primers used for the detection of IL-10, IL-33, and IFN-γ polymorphisms in atherosclerosis patients and controls.

| Gene | Primer | Sequence | Product Size |
|---|---|---|---|
| IL-10 | Forword 1 (G) | 5' - CTACTAAGGCTTCTTT GGGAG-3' | 258 bp |
| rs1800896 | Forword 2 (A) | 5' - ACTACTAAGGCTTCTTTGGGAA-3' | 258 bp |
| | Reverse | 5' - CAGTGCCAACTGAGAATTTGG- 3' | |
| IL-33 | Forword | 5' - CAGCAGGACACAGAAAAACAGATGTATGC- 3' | 547 bp |
| rs7044343 | Reverse | 5' - GCAACCAGAAGTCTTTTGTAGGACTCAG- 3' | |

*2.4. Measurement of Serum IL-10 and IL-33 by enzyme-linked immunosorbent assay (ELISA)*

Measurement of Serum Anti-inflammatory Cytokines (IL-10, and IL-33) were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Inc., USA), following the manufacturer's instructions. The intensity of the resulting color was measured at a wavelength of 450 nm using an ELISA microplate reader (DiaSourceImmunoAssays®, Belgium). Raw absorbance readings were converted into concentrations using a standard curve, and the cytokine levels in each sample were reported in pg/ml.

*2.5. Statistical Analysis*

Data analysis was performed using IBM SPSS software, version 20.0 (Armonk, NY: IBM Corp). Categorical variables were presented as numbers and percentages, and comparisons between groups were made using the chi-square test or the Monte Carlo correction test when more than 20% of cells had expected counts below 5. Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Quantitative data were described using the range (minimum-maximum), mean, standard deviation, and median. Student's t-test was applied for comparisons between two groups with normally distributed data, while one-way ANOVA was used for comparisons across multiple groups.

For non-normally distributed data, the Mann-Whitney U test was applied for comparisons between two groups, and the Kruskal-Wallis test was used for multiple group comparisons. Spearman's rank correlation coefficient was used to assess associations between non-normally distributed variables. The study of population's genetic distribution was assessed for Hardy-Weinberg equilibrium. A p-value of less than 0.05 was considered statistically significant.

## 3. Results

*3.1. Patient Characteristics*

Table 2 presents the demographic data and results of various biochemical parameters for the study participants. Among the atherosclerosis group, there were 87 (87%) male and 13 (13%) female patients, while the control group included 60 (54.5%) males and 50 (45.5%) females. The median age of the atherosclerosis patients was 56.5 years (range: 27.0–80.0 years), compared to a median age of 35.5 years (range: 18.0–71.0 years) in the control group. Atherosclerosis patients showed significantly higher levels of white blood cells (WBC) compared to controls ($p < 0.01$). However, there was no significant difference in hemoglobin levels between the two groups.

*3.2. Serum Levels of Anti-inflammatory Cytokines*

As shown in Table 2, there was a statistically significant increase in IL-33 levels among healthy controls compared to atherosclerosis patients ($p \leq 0.001$), with median values of 22.4 (4.48–84.1) in controls versus 5.46 (0–42.6) in patients. No statistically significant differences were observed in the levels of IL-10 between the two groups.

**Table 2**: Comparison of Demographic and Biochemical Parameters between Atherosclerosis Patients and Controls

| Parameter | Patients (n = 100) | Controls (n = 110) | Test Statistic | p-value |
|---|---|---|---|---|
| Age (years) | | | | |
| Mean ± SD | 55.9 ± 10.3 | 35.6 ± 12.9 | t = 12.647* | <0.001* |
| Median (Min – Max) | 56.5 (27 – 80) | 35.5 (18 – 71) | | |
| Gender | | | | <0.001* |
| Female | 13 (13.0%) | 50 (45.5%) | $\chi^2$ = 26.273* | |
| Male | 87 (87.0%) | 60 (54.5%) | | |
| *WBC (10^9) / L | | | | <0.001* |
| Mean ± SD | 10.3 ± 3.19 | 6.58 ± 1.56 | t = 10.581* | |
| Median (Min – Max) | 10.25 (2.9 – 20.4) | 6.20 (4 – 11) | | |
| Hemoglobin g/dl | | | t = 0.151 | 0.880 |
| Mean ± SD | 13.52 ± 2.11 | 13.47 ± 2.20 | | |
| Median (Min – Max) | 13.8 (8.3 – 18.3) | 13.1 (8.9 – 17.8) | | |
| IL-33-P (pg/ml) | | | U = 1135.0* | <0.001* |
| Mean ± SD | 7.04 ± 8.39 | 22.4 ± 9.96 | | |
| Median (Min – Max) | 5.46 (0 – 42.6) | 22.4 (4.48 – 84.1) | | |
| IL-10 (pg/ml) | | | U = 4722.0 | 0.075 |
| Mean ± SD | 3.56 ± 2.54 | 4.48 ± 3.43 | | |
| Median (Min – Max) | 3 (0 – 12) | 5 (0.10 – 15) | | |

*SD: Standard deviation; t: Student's t-test; U: Mann-Whitney test; $\chi^2$: Chi-square test; p: p-value for comparison between groups. A p-value ≤ 0.05 indicates statistical significance.

### *3.3. IL-10,and IL-33 Polymorphism Analysis*

Table 3 presents the genotype and allele frequencies of IL-10 (-1082 A>G, rs1800896) and IL-33 (rs7044343) polymorphisms in atherosclerosis patients and healthy controls.

- IL-33 Gene Polymorphism:
  - *Genotype Frequencies*: The CC genotype was absent in both groups. The CT genotype was more prevalent among patients (82%) compared to controls (73.6%). The TT genotype was found in 18% of patients and 26.4% of controls. The difference in genotype distribution was not statistically significant ($\chi^2 = 2.109$, $p = 0.146$).
  - *Allele Frequencies*: Regarding IL-33 polymorphism, the CC genotype was absent in both atherosclerosis patients and healthy controls. The CT genotype was more prevalent among patients (82%) compared to controls (73.6%), while the TT genotype appeared in 18% of patients and 26.4% of controls. Despite these variations, the overall genotype distribution between groups was not statistically significant ($\chi^2 = 2.109$, $p = 0.146$). Similar to IL-10, allele frequency analysis revealed significant deviations from Hardy-Weinberg equilibrium in both groups (patients: $HW\chi^2 = 48.291$, $p < 0.001$; controls: $HW\chi^2 = 37.354$, $p < 0.001$), warranting further exploration of genetic or methodological factors that could explain this deviation.
- IL10-1082 Gene Polymorphism:
  - *Genotype Frequencies*: The GG genotype was rare, observed in 6% of patients and 0.9% of controls. The GA genotype was the most common in both groups (80% in patients and 80.9% in controls). The AA genotype was observed in 14% of patients and 18.2% of controls. The genotype distribution showed borderline significance ($\chi^2 = 4.471$, MCp = 0.099).
  - *Allele Frequencies*: The distribution of IL-10 genotypes among atherosclerosis patients and healthy controls showed a predominance of the heterozygous **GA** genotype in both groups (80% in patients and 80.9% in controls). The GG genotype was relatively rare, observed in 6% of patients and only 0.9% of controls. The AA genotype appeared in 14% of patients and 18.2% of controls. Although these differences in genotype frequencies approached statistical significance, they did not reach conventional significance levels ($\chi^2 = 4.471$, MCp = 0.099). Analysis of allele frequencies showed a slight predominance of the A allele over the G allele in both groups. Importantly, significant deviations from Hardy-Weinberg equilibrium (HWE) were observed in both patients ($HW\chi^2 = 37.247$, $p < 0.001$) and controls ($HW\chi^2 = 49.076$, $p < 0.001$), which may suggest underlying population structure, genotyping error, or selective pressure on this locus.

Overall, the analysis of polymorphisms did not reveal significant differences between atherosclerosis patients and controls, except for certain deviations from Hardy-Weinberg equilibrium.

### *3.4. Correlation between IL-33 and IL-10 in the Patients Group:*

A positive correlation was observed between IL-33 and IL-10 levels in the patient group (Table 4), with a Spearman correlation coefficient of rs = 0.190 and a p-value of 0.059, indicating a trend toward statistical significance. This may suggest a potential relationship between these two cytokines, though it did not reach the conventional threshold for significance.

### *3.5. Correlation between IL-33 and Different Parameters in the Patients Group:*

Table 5 presents the relationship between IL-33 levels and various parameters in the patient group. There was no statistically significant difference in IL-33 levels between males and females (p = 0.845), although females had a slightly higher mean IL-33 level (8.15 ± 11.1) compared to males (6.88 ± 7.97). Similarly, no significant association was found between IL-33 levels and troponin I status (p = 0.910), with comparable mean values in the negative (6.37 ± 6.79) and positive (7.11 ± 8.84) groups. Analysis of IL-

33 gene polymorphism revealed that individuals with the TT genotype had higher IL-33 levels (9.90 ± 11.0) than those with the CT genotype (6.41 ± 7.63), but the difference did not reach statistical significance (p = 0.252). Overall, IL-33 levels did not show significant variation with respect to gender, troponin I status, or genotype in this cohort.

### *3.6. Correlation between IL-10 and Different Parameters in the Patients Group:*

Table 6 summarizes the relationship between IL-10 levels and various clinical parameters in the patient group. No significant difference in IL-10 levels was observed between females and males (p = 0.211), with females showing a slightly higher mean IL-10 level (4.26 ± 2.20) compared to males (3.45 ± 2.59). However, IL-10 levels were significantly higher in patients with negative troponin I results (4.85 ± 2.84) compared to those with positive troponin I (3.25 ± 2.43), indicating a possible association between lower IL-10 levels and cardiac injury (p = 0.024). Regarding IL-10 gene polymorphisms, patients carrying the GG genotype exhibited the highest IL-10 levels (6.17 ± 2.99), followed by the AA genotype (4.23 ± 3.12) and GA genotype (3.24 ± 2.29). Although this difference did not reach statistical significance (p = 0.057), it suggests a trend toward genotype-dependent variation in IL-10 expression

**Table 3**: Comparison of Gene Polymorphism between Atherosclerosis Patients and Controls

| Parameter | Patients (n = 100) | Controls (n = 110) | $\chi^2$ | p-value |
|---|---|---|---|---|
| IL-33 | | | 2.109 | 0.146 |
| CC | 0 (0%) | 0 (0%) | | |
| CT | 82 (82%) | 81 (73.6%) | | |
| TT | 18 (18%) | 29 (26.4%) | | |
| Allele | | | | |
| C | 82 (41%) | 81 (37%) | | |
| T | 118 (59%) | 139 (63%) | | |
| HW $\chi^2$ | 48.291 | 37.354 | | <0.001* |
| IL10-1082 | | | 4.471 | MCp=0.099 |
| GG | 6 (6%) | 1 (0.9%) | | |
| GA | 80 (80%) | 89 (80.9%) | | |
| AA | 14 (14%) | 20 (18.2%) | | |
| Allele | | | | |
| G | 92 (46%) | 91 (41%) | | |
| A | 108 (54%) | 129 (59%) | | |
| HW $\chi^2$ | 37.247 | 49.076 | | <0.001* |

*HW$\chi^2$: Chi-square for Hardy-Weinberg equilibrium; $\chi^2$: Chi-square test; MC: Monte Carlo; p-value ≤ 0.05 indicates statistical significance.

**Table (4):** Correlation between IL-33 with IL-10 with in patients group (n = 100)

| | IL-33-P | |
|---|---|---|
| | rs | p |
| IL-10 | 0.190 | 0.059 |

rs: Spearman coefficient

**Table (5):** Relation between IL-33 and different parameters in patients group (n = 100)

| | N | IL-33 Mean ± SD. | IL-33 Median (Min. – Max.) | U | P |
|---|---|---|---|---|---|
| Gender | | | | | |
| Female | 13 | 8.15 ± 11.1 | 6.53 (0 – 31.3) | 546.5 | 0.845 |
| Male | 87 | 6.88 ± 7.97 | 5.25 (0 – 42.6) | | |
| TROPONINE I | | | | | |
| Negative | 23 | 6.37 ± 6.79 | 4.45 (0 – 20.7) | 679.0 | 0.910 |
| Positive | 60 | 7.11 ± 8.84 | 4.75 (0 – 42.6) | | |
| IL-33 | | | | | |
| CT | 82 | 6.41 ± 7.63 | 4.41 (0 – 31.3) | 611.0 | 0.252 |
| TT | 18 | 9.90 ± 11.0 | 8.99 (0 – 42.6) | | |

SD: Standard deviation U: Mann Whitney test

p: p value for comparing between relation between IL-33 and different parameters

**Table (6):** Relation between IL-10 and different parameters in patients group (n = 100)

| | N | IL-10 Mean ± SD. | IL-10 Median (Min. – Max.) | Test of Sig. | P |
|---|---|---|---|---|---|
| Gender | | | | | |
| Female | 13 | 4.26 ± 2.20 | 5 (1 – 7) | U=444.5 | 0.211 |
| Male | 87 | 3.45 ± 2.59 | 3 (0 – 12) | | |
| TROPONINE I | | | | | |
| Negative | 23 | 4.85 ± 2.84 | 5 (1 – 12) | U=469.0* | 0.024* |
| Positive | 60 | 3.25 ± 2.43 | 3 (0 – 8) | | |
| IL-10 | | | | | |
| GG | 6 | 6.17 ± 2.99 | 5.5 (4 – 12) | | |
| GA | 80 | 3.24 ± 2.29 | 3.0 (0 – 8) | H=5.715 | 0.057 |
| AA | 14 | 4.23 ± 3.12 | 5.0 (0.1 – 9) | | |

SD: Standard deviation ,U: Mann Whitney test, H: H for Kruskal Wallis test

p: p value for comparing between relation between IL-10 and different parameters

*: Statistically significant at $p \leq 0.05$

## 4. Discussion

Understanding how genetic variations affect the development of atherosclerosis has become increasingly important, especially when it comes to cytokine gene polymorphisms. Research has shown that certain genetic variants—particularly in genes like *IL-10* and *IL-33*—can significantly influence cytokine production and may play a role in driving disease progression [12,13].

In Jordan, atherosclerosis is a major public health concern, contributing heavily to the rising rates of illness and death from cardiovascular disease. Recent studies have revealed alarmingly high rates of risk factors among Jordanians, including dyslipidemia (around 75%), obesity (about 32%), hypertension (37%), smoking (31%), and diabetes (21%). Many individuals carry multiple of these risk factors at once [19], highlighting the urgent need to address both lifestyle-related and genetic contributors to disease.

While behavioral and environmental risk factors have been widely explored in Jordan, research into genetic predispositions—especially involving cytokine genes like *IL-10* and *IL-33*—remains limited. In light of this gap, our

study set out to investigate how specific polymorphisms in *IL-10* (-1082 A>G, rs1800896) and *IL-33* (rs7044343) are associated with plasma cytokine levels and atherosclerosis status in a Jordanian population.

The IL-33 gene, located on chromosome 9 (9p24.1), encodes a cytokine that serves both as a nuclear factor for gene transcription regulation and as a traditional signaling cytokine. IL-33 acts as an "alarmin," alerting the immune system to stress or tissue damage. In our study, we observed that the T allele and T/T genotype at the IL33 - rs7044343 position were more prevalent among the Jordanian control group (96.6% and 26.4%, respectively) than in the patient group (86.4% and 18%, respectively) (Table 3). This SNP analysis suggests that rs7044343 (T) may be linked to a protective effect against coronary artery disease (CAD). Although our results did not reveal significant differences in IL-33 genotype frequencies between patients and controls, the Hardy-Weinberg equilibrium (HW$\chi^2$) indicated significant deviations in both groups, implying potential selection pressures or population stratification effects.

Research has highlighted IL-33's role in promoting protective Th2 cytokines and ox-LDL antibodies, which may help mitigate atherosclerotic plaque formation [8]. The differences between our findings and those of previous studies may be attributed to variations in population-specific genetic backgrounds, as well as differences in study design, methodology, or sample sizes. Notably, despite the recognized role of IL-33 in atherosclerosis development, there remains a scarcity of research exploring its genetic susceptibility to CAD.

In agreement with these findingsTu, et al [9], identified an association between the IL-33 rs7025417 polymorphism and an increased risk of CAD within the Chinese Han population, suggesting that variations in IL-33 gene expression and plasma levels could influence disease susceptibility. Interestingly, we found that the rs7044343 variant was associated with a reduced risk of coronary artery disease (CAD), suggesting a potential protective role of this polymorphism in the studied population.

However, previous studies have presented conflicting associations. For instance,[14] demonstrated that the rs7044343 CC genotype was linked to a decreased risk of rheumatoid arthritis (RA) and lower serum IL-33 levels. Conversely, this CC genotype was associated with an increased risk of systemic sclerosis. These findings align with the association reported by [15,16] in systemic sclerosis, as we observed a significant relationship between the rs7044343 T allele and a decreased risk of premature CAD. Furthermore, while Li et, al. [14] reported lower serum IL-33 levels in RA patients with the CC genotype, in our study we found that individuals carrying the CC genotype of the rs7044343 variant produced higher levels of IL-33 in monocytes compared to those with the CT or TT genotypes. IL-33 plays a complex role in the immune system—on one hand, it can drive inflammation in autoimmune diseases, but on the other, it can also help regulate immune responses and promote tissue protection. In the context of atherosclerosis, IL-33 has been shown to reduce plaque development by encouraging anti-inflammatory immune pathways. This dual nature may explain our finding that the C allele was associated with a lower risk of premature coronary artery disease (CAD). Our results suggest that the protective effect may be partly due to increased IL-33 production. It is worth noting that differences in how IL-33 was measured—such as the type of cells studied or how they were stimulated—might explain why our findings differ from previous reports.

IL-10 is a well-established anti-inflammatory cytokine known for its regulatory role in immune responses, particularly concerning atherosclerosis progression [4,17]. The IL10 gene promoter region is highly polymorphic, and the -1082 A>G (rs1800896) variant has been associated with variations in cytokine expression levels. Our results showed that the GG genotype was infrequent among both patients and controls, while the GA genotype was predominant. Although the genotype distribution approached borderline significance (MCp=0.099), significant deviations from Hardy-Weinberg equilibrium were noted in both groups. These findings indicate that IL10-1082 polymorphisms may influence atherosclerosis by modulating cytokine expression and immune regulation, although further research, including functional studies and larger sample sizes, is necessary to clarify their specific impacts.

Our findings revealed a positive, though non-significant, correlation between IL-33 and IL-10 levels ($rs = 0.190$, $p = 0.059$), hinting at potential coordinated regulation of these cytokines. This aligns with Jiang et al. experimental data suggesting IL-33 can drive IL-10 production by promoting M2 macrophage polarization and regulatory T-cell expansion, thus dampening arterial inflammation and plaque formation [20].

In our study, IL-33 levels did not vary significantly across gender, troponin I status, or genotype, though the TT genotype trended toward higher IL-33 expression. This lack of statistical significance may stem from our limited sample size or population-specific genetic backgrounds; similarly, clinical studies have reported inconsistent associations between IL-33 concentration and cardiovascular markers [21].

Regarding IL-10, the absence of gender-related differences aligns with prior research indicating that basal IL-10 production is relatively stable across sexes [22,23].

However, we observed significantly higher IL-10 levels in patients with negative troponin I, suggesting a protective, anti-inflammatory role during cardiac injury—a finding supported by multiple studies demonstrating IL-10’s ability to suppress pro-inflammatory cytokines, reduce ventricular remodeling, and improve myocardial outcomes [24,25].

Although the relationship between IL-10 genotypes and cytokine levels (highest in GG carriers) was not statistically significant ($p = 0.057$), this trend resonates with literature linking IL-10 promoter variants (e.g., -1082G) to increased expression and protective effects in acute coronary syndrome [22]. The borderline p-value indicates that a larger sample might confirm this genetic association.

Our findings suggest that IL-33 may play a supportive, upstream role in enhancing IL-10's anti-inflammatory and potentially cardioprotective effects. In this study, we measured serum levels of both cytokines to better understand their involvement in atherosclerosis. Interestingly, IL-33 levels were significantly lower in patients compared to healthy controls ($p < 0.001$), reinforcing its potential protective role. In contrast, IL-10

levels did not show a significant difference between groups, highlighting the complexity of cytokine regulation in cardiovascular disease. These variations, whether driven by genetic differences, demographic factors, or environmental influences, underscore the need for further research in larger and more diverse populations to fully unravel the interplay between these immune mediators and atherosclerosis risk.

## 5. Conclusion

Our findings suggest that certain genetic differences—like variations in the IL-33 gene—may play a role in how atherosclerosis develops. This highlights the importance of looking at genetic diversity and population-specific traits when studying complex conditions like heart disease. Understanding how these gene variants work, especially in combination with environmental factors, could help guide more personalized approaches to preventing and treating atherosclerosis in the future.

## 6. Declarations:

*6.1. Funding:*

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*6.2. Data availability:*

Derived data supporting the results of this study are available from the corresponding author on request.

*6.3. Authors' contributions:*

All authors made a substantial contribution to all processes.

*6.4. Ethics approval statement:*

The study was examined and given approval by Zarqa University's Ethics Committee for Scientific Research (ECSR), with approval number 1/8/2021, in compliance with the regulations for the protection of human beings and the ethical principles governing research projects.

*6.5. Consent to participate:*

Informed consent was taken from all participants.

*6.6. Consent for publication:*

Informed consent was provided by all participants.

*6.7. Competing interest:*

The authors declare that they have no competing interests.

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