

# Association of interleukin-6 174 G>C (rs1800795) gene polymorphisms in a patient with arteriosclerosis in Najaf

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## Abstract

Atherosclerosis is a common chronic disease that mainly affects medium-sized arteries. Clinically, it may present manifest as peripheral arterial disease, ischemic heart disease, or cerebrovascular disease. The aim of this study was to investigate the association of *IL-6* gene polymorphism (rs1800795) with Arteriosclerosis in the AL-Najaf population. Blood samples were taken from 40 arteriosclerosis patients and 20 as a control. Tetra amplification thermal mutagenesis system (T-ARMS-PCR) technique was used to detect the Interleukin-6 (*IL-6*-174 G/C) (rs1800795) gene polymorphism. The results of the genotype apportionment of the *IL-6* gene revealed a significant increase ( $p = 0.016$ ) increase in the GG genotype and the G allele in the -174 G/C single nucleotide polymorphism (SPN) in atherosclerotic patients compared to controls, and the results were significantly more significant. You are more likely to be a mutant allele than controls (OR = 2.231 CI = 1.160–4.291,  $p = 0.01$ ). These results indicate an association of polymorphisms of *IL-6* -174G/C in a patient with atherosclerosis from Iraq.

**Keywords:** Atherosclerosis, Interleukin 6, *IL-6* gene polymorphism, Tetra ARMS – PCR.

## 1. Introduction

Atherosclerosis is described as a persistent inflammatory response of the vascular wall to dyslipidemia, endothelial distress, inflammatory recruitment of leukocytes, and local vascular cell activation. (1). Atherosclerosis is the main pathogenic mechanism associated with Coronary artery disease (CAD). It starts with a subclinical stage that manifests early in life (2) and the clinical symptom mark the final stage of this chronic process. As a result, it is possible to track the development of subclinical atherosclerosis and forecast the likelihood of future cardiovascular or coronary events by measuring its early and intermediate stages. Carotid artery stiffness, ankle-brachial index (ABI), and inner-medial thickness (IMT) are reliable, quantitative, repeatable, well-established indicators of future cardiovascular events including myocardial infarction and stroke and noninvasive signs of atherosclerosis. cardiovascular events in the future, including myocardial infarction and stroke (3)

*IL-6* is a cytokine that regulates cell growth and differentiation as well as the immune response. The human *IL-6* gene is lying on chromosome 7p21 and encodes a protein of 212 amino acids and a 28 amino acid signal peptide. [6]. B cells, lymphocytes, and white blood cells, such as neutrophils, eosinophils, and basophils, are the targets of *IL-6*. B cell differentiation and plasma cell synthesis of IgA, IgM, and IgE are two of *IL-6*'s effects [5].

A key modulator of the inflammatory response in ischemic stroke, *IL-6* is linked to atherosclerosis and cardiovascular disease [7]. The single nucleotide polymorphism (SNP) G174C (rs1800795) in the *IL-6* functional founder has previously been discovered and may be connected to increased *IL-6* levels [8]. Those with cardiovascular illnesses had greater mortality rates when their *IL-6* levels are elevated [9].

## 2. Materials and Methods

### Sampling and data collection

The study was conducted on 60 subjects, including 40 Atherosclerosis patients and 20 healthy controls. The subjects were collected from patients who had joined AL- Najaf Center for Cardiovascular Surgery and Cardiac Catheterization. The subjects of this study, and their ages ranged from 40-60 years, as the collection of samples began from January 2023 to March 2023 after obtaining official approvals and the approval of the two study groups. 2 ml of blood was drawn between the hours of (8.30-11) transferred to tubes containing EDTA.

Purification of Genomic DNA was executed by "Favor prep DNA Purification Kit" (FAVORGEN). The *IL-6* 174 G/C gene polymorphism was detected using the Tetra ARMS PCR Method using a standard PCR thermocycler (Labnet/USA). The primer pair synthesized by Macrogen/China is shown in (table 1 and table 2) which lists the results of the Tetra ARMS PCR protocols for the *IL-6* gene polymorphism at location -174 G/C. The PCR products were separated using electrophoresis and a ladder marker (Promega, USA). The product was placed onto a 2% agarose

plate and operated at 70 volts for 1.5 hours with a Red safe dye. Using a photo documentation method,

the DNA bands were captured after being made visible by a UV transilluminator.

Table 1: Sequences of the *IL-6* polymorphism primers.

Gene	Primers sequences		PCR product bp	Ref.
<i>IL-6</i> -174G/C (rs1800795)	F Outer	5'GACTTC AGCTTT ACTCTTTGTCAAGACA -3'	326bp	(12)
	R Outer	5'GAATGAGCCTCAGACATCTCCAGTCCTA-3'		
	F inner (G allele)	5'GCACTT TTCCC CTAGTTGTGTCTTCCG-3'	205bp	(13)
	R inner (C allele)	5ATTGTGCAATGTGACGTCCTTTAGCTTG-3	184bp	

Table 2: The T-ARMS PCR technique was used to analyze the *IL-6* (-174 G/C) gene polymorphism in samples from patients with atherosclerosis and control subjects.

Target gene	Steps	Temperature (co)	Number of cycles	Time (seconds)
<i>IL-6</i> -174 G/C gene	Pre-denaturation	95		600
	Initial denaturation	95		30
	Annealing	54	40	30
	Extension	72		30
	Final Extension	72		300

### 3. Statistical analysis

Percentage frequencies were used to detect Genotypes of *IL-6* SNP G174C (rs1800795), and significant variations were utilized to assess their distributions in arteriosclerosis patients and healthy additionally, it was used on multiple regression tests and Odd ratio by using SPSS version 23 software.

### 4. Results

The allele and genotype frequency distributions of IL6 in 40 Atherosclerosis patients and 20 control are shown in Table 3. Importantly, patients with atherosclerosis and controls both had a higher prevalence of the two alleles G/C for the *IL-6* gene

polymorphism at locus -174 G/C with genotypes GG, and CC (Figure 1), by Tetra ARMS -PCR technique. A higher frequency of GG genotype was found in Atherosclerosis patients (72.5%) as compared to healthy subjects (65%). When analyzing these results, significant differences were observed between study groups which were (p-value =0.016, OR =2.231, 95%CI=1.160-4.291). (table 3). As regards GC genotype, there was no significant difference among patients (27.5%) as compared to healthy subjects (35%), which was (p-value =0.350, OR =1.571, 95%CI=0.609-4.054). The CC genotype was absent in both study groups. A statistically significant difference was seen in the G allele among study groups which was higher in patients as compared to healthy subjects (p-value=0.001).

Table 3: The prevalence of genotypes and allelic for the *IL-6* gene (-174 G/C) in patients with atherosclerosis and controls.

Genotype	Atherosclerosis patients (N=40)	Healthy controls (N=20)	Odds Ratio	CI	Sig
GG	29 (72.5%)	13 (65%)	2.231	1.160-4.291	0.016
GC	11 (27.5%)	7 (35%)	1.571	0.609-4.054	0.350
CC	0	0			
G	69 (86.25%)	33 (82.5%)	0.176	0.176-0.107	0.001
C	11 (13.75%)	7 (17.5%)			

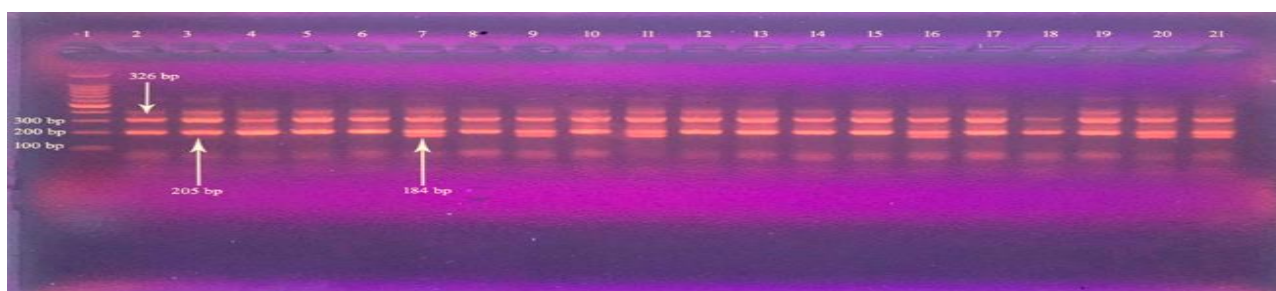


Fig1: The electrophoresis image (2% at volts for 1.5 hours) of Tetra ARMS-PCR reaction for interleukin-6 174 G/C (rs1800795) gene polymorphisms arteriosclerosis patients. These bands showed from G in 205 bp and C in 184 bp which stained with Red Safe, Ladder (100-1000) visualized by U.V light.

### 5. Discussion

Atherosclerosis is an inflammatory disease characterized by a chronic inflammatory process mediated by several proinflammatory mediators, including cytokines and chemokine, the disease's lethal endpoints are myocardial infarction, stroke, or

sudden cardiac death at all stages. [14]. IL-6 is regarded as a coordinator of the inflammatory response and a major factor in human atherosclerosis among other cytokines [15]. By causing endothelial dysfunction, increased production of adhesion molecules, Smooth muscle cell proliferation, leukocyte recruitment, and matrix degradation, IL-6 has been demonstrated to play a crucial role in

atherogenesis [16]. According to studies, IL-6 can also encourage the formation and rupture of atherosclerotic plaques, hastening the size and instability of these plaques [17]. The findings revealed that the G allele predominated in both the genotype distribution and allele frequencies of the IL-6 G174C SNP. (GG: 72.5%, GC: 27.5%, CC 0; G-allele: 86.25%, C-allele: 13.75%).

The allelic frequencies in our study participants are more similar to those observed in populations from Asia. Results from populations of Africans, East Asians, and Malaysian Malays have been similar [18]. Our results concurred with a prior study conducted in Indonesia that examined various ethnic groups and discovered that the GG genotype is more prevalent than the CC genotype [19]. On the other hand, prior studies among European Caucasians have discovered that the GC genotype is the most common [20].

## 6. Conclusion

The findings of the present investigation demonstrated a link between atherosclerosis and the GG genotype of the IL6 gene located in region 174 (rs1800795). It suggested that might be a substantial risk factor for atherosclerosis patients in the Iraqi population.

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