

## INTERLEUKIN-6 –174 G/C AND –572 G/C NUCLEOTIDE POLYMORPHISMS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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### Abstract

**Background.** In the past decades there has been a growing search point out that immune system plays an evident role at every stage of cardiovascular disease and their compliances, such as myocardial infarction (MI). Therefore, some of cytokines, such as interleukin-6 (IL-6) has been studied as potential new risk factors. In this study, we investigated the association of the IL-6 nucleotide polymorphisms –174 G/C and –572 G/C and traditional cardiovascular risk factors in patients with acute myocardial infarction (AMI) in emergency department localized in the southern part of Turkey.

**Methods.** Eighty-four patients (71 male, 13 female) and 85 healthy controls (30 male, 55 female) were included in this study. The mutations were assessed by Light Cycler Real-Time PCR mutation detection kits.

**Results.** IL-6 single nucleotide polymorphisms, –174 G/C and –572 G/C were not significantly associated with AMI ( $p=0,664$  and  $p=0,491$ , respectively). 0% and 3.6% of subjects had mutations in –174 G/C and –572 G/C in patients with AMI; respectively.

**Conclusions.** The present study confirmed that IL-6 -174 G/C and -572 G/C polymorphisms can not be considered as risk factors in AMI. However this study is including a small population, more detailed and large numbers is needed to study.

**Keywords:** Interleukin-6, acute myocardial infarction, single nucleotide polymorphisms, cardiovascular disease, genetics

### Introduction

Increasing rate of cardiovascular disease (CVD) has become a common problem in most of the countries. It was estimated that 17,5 million people died from CVD in 2005, representing 30% of global deaths. Among these deaths, 7.6 million were related with Myocardial Infarction (MI)<sup>(1)</sup>.

Nowadays, evidence has accumulated that inflammation has a critical role in the development and progression of CVD<sup>(2,3,4)</sup>. So, some of cytokines, such as interleukin-6 (IL-6) has been studied as potential new risk factors<sup>(2)</sup>. IL-6 is a multifunctional cytokine which is an important mediator of acute inflammatory responses. Moreover, it plays an anxious role in differentiation and growth factor in various cells such as hematopoietic precursor cells, B cells, T cells, mesangial cells, keratinocytes, neuronal cell, osteoclasts, some tumor cells and endothelial cells<sup>(5,6)</sup>.

Recent studies focused on that increased plasma levels of IL-6 may be a prediction of feature risk of MI<sup>(7,8,9,10)</sup>. The IL-6 has been described to play an important role in cardiovascular

diseases<sup>(2,3)</sup>. It's thought that IL-6 may affect the progression and the healing process of MI<sup>(9)</sup>. IL-6 also considered as having functions on destabilization of atherosclerotic plaque<sup>(11,12)</sup>.

As mentioned above, although several studies have proposed that increased plasma levels of IL-6 may be related to CVD, phenotype markers could be affected by different factors such as gender, age, diet even environmental situations. The gene encoding IL-6 has been mapped to chromosomal region 7p21<sup>(13)</sup> and IL-6 gene is a single chain protein with a molecular mass ranging from 21 to 28 kDa, related with the cellular source<sup>(14)</sup>. After discovery of single nucleotide polymorphisms (SNPs) in the IL-6 gene, certain alleles may become informative in evaluating the risk of MI. SNPs in the IL-6 gene have been examined in recent studies with MI and CVD. In these studies both positive<sup>(15,16,17)</sup> and negative reports<sup>(18,19,20)</sup> decelerated about these polymorphisms.

Since the recent studies concerning IL-6 as one of the cardiovascular risk factors, we attempted to search whether IL-6 -174 G/C and -572 G/C gene polymorphisms were associated in patient with AMI in a group of Turkish population.

## **Materials and Methods**

### **Study Population**

We investigated the association of the IL-6 nucleotide polymorphisms -174 G/C and -572 G/C in patients admitted to emergency department with chest pain complaint and diagnosed AMI. The diagnosis of AMI was confirmed according to the World Health Organization criteria. 84 patients [71 male (84.5%), 13 female (15.5%)] and also 85 healthy controls [30 male (35.3 %), 55 female (64.7%)] were included in the study. The protocol of this study was approved by the Ethics Committee School of Medicine, Cukurova University. The investigation conforms to the principles outline in the declaration of Helsinki.

A questionnaire was applied to the all subjects in order to interview about history and actual presence of coronary risk factors such as arterial hypertension, hypercholesterolemia, cigarette smoking, alcohol intake, medication and diabetes. The patients under antihypertensive therapy or with a systolic and diastolic blood pressure over 140/90 mmHg were considered to be hypertensive. Subjects with diabetes mellitus or receiving drugs related with lipid parameters were not included in this study. Besides SNPs, biochemical analyses were also studied.

### **Biochemical Analyses**

All biochemical parameters were carried out on auto analyzer using kits supplied by Boehringer Mannheim, Germany. Serum total cholesterol (Total Chol) and triglycerides were measured by CHOD-PAP and GPO-PAP methods, respectively. High-density lipoprotein cholesterol (HDL Chol) was estimated by direct nonprecipitating CHOD-PAP method. Low-density lipoprotein cholesterol (LDL Chol) was calculated using the Friedewald formula.

### **DNA extraction and genotyping of Interleukin-6 -174 G/C and -572 G/C**

Peripheral blood for genetic analysis was collected into evacuated tubes containing EDTA. DNA was extracted from circulating leukocytes by using a high pure PCR template preparation kit (Roche diagnostics, GmbH, Mannheim, Germany) according to the instructions of the manufacturer. IL-6 -174 G/C and -572 G/C mutation were determined by Light Cycler Real-Time PCR mutation detection kits (Roche diagnostics, GmbH, Mannheim, Germany).

### **Statistical analysis**

Statistics were carried out using the SPSS 11.5 statistical packet program. Chi-Square test was used for comparisons between groups for gene polymorphism and other parameters. Chi-Square test was also used for logistic regression analyses. Allele frequencies were estimated by the gene counting method, and Hardy–Weinberg equilibrium was checked by a Chi-Square test. Shapiro-Wilks test are used to determine whether lipid parameters is normally distributed or not and all parameters are normally distributed. The student t-test was used for parameters normally distributed; Mann-Whitney U test was used for parameters non-normally distributed. A value of  $p < 0.05$  was considered to represent a statistically significant result.

## Results

As given in Table 1 gender and smoking were significantly associated with MI ( $p=0.0001$  and  $p=0.001$ , respectively). There was a significant association only with increased level of HDL-cholesterol ( $p=0.0001$ ) where as no difference was observed regarding other keys characterizing the MI such as total cholesterol, LDL cholesterol and triglycerides.

The genotype and allele frequencies of IL-6 -174 G/C and -572 G/C polymorphisms did not differ between patients with AMI and controls. The distribution of IL-6 genotype and alleles in both patients and controls were given in Table 2.

For the IL-6 -174 G/C SNP, 52 (61.9%), 29 (34.5%) and 3 (3.6%) carried GG, GC and CC genotypes and for the IL-6 -572 G/C SNP, 70 (83.3%), 14 (16.7%) and 0 (0%) carried GG, GC and CC genotypes, respectively in patients with MI. 29.4% of subjects were heterozygote and 2.4% of subjects had mutations in IL-6 174 G/C; 15.3 % of subjects were heterozygote and 1.2% of subjects had mutations in IL-6 -572 G/C in controls.

When the IL-6 -174 G/C and -572 G/C polymorphisms were evaluated, the stepwise logistic regression analysis showed that carrying C allele is not a significant independent predictor of MI. However, age, male gender, smoking, LDL and HDL cholesterol were found to be independent predictors of MI.

## Discussion

There are substantial evidence and knowledge about the role of cytokines in patient with MI<sup>(3)</sup>.<sup>21)</sup> Science several studies have searched the pathophysiological effects of IL-6 for MI, the part of this cytokine in the progression of MI is still unsettled.

In this study, we attempted to establish an association between -174 G/C and -572 G/C polymorphisms in the promoter region of the IL-6 gene, known to decrease IL-6 protein expression. Over all we demonstrated that the SNPs of IL-6 were not associated with AMI.

In contrast to our results, in the ECTIM study, Georges et al. searched -597 G/A, -572 G/C and -174 G/C SNPs in MI and the IL-6 SNPs were found to be associated with an increased cardiovascular morbidity. In this study, it was reported that MI patients carrying the C-allele has a significant risk. Georges et al. also found out a nearly complete association between IL-6 174G/C and 596 G/A polymorphism<sup>(22)</sup>. Furthermore, Fedezt et al. countenanced this data by pointing out the complete linkage disequilibrium between the -174 and the -597 alleles<sup>(23)</sup>.

Chiappelli et al. also revealed that IL-6-174 G/C SNP increased the risk of MI in men and they also found that CC genotype or C allele was more represented in patients older than 60 years with MI. According to this data, the association appeared to be age dependent<sup>(24)</sup>. Besides, Humphries et al. showed a modest but a significant association of IL-6 -174 G/C polymorphism, with risk of coronary heart disease and systolic blood pressure in healthy men ( $n=2751$ )<sup>(25)</sup>.

It was established that with about a 40 % prevalence, the IL-6 174 G/C SNP is functionally important in Caucasians<sup>(26,27)</sup>. The IL-6 -174 G/C is evidently associated with several features consistent with cardiovascular risk in healthy elderly Brazilian females<sup>(28)</sup>. The CC genotype of

the -174 G/C SNP was related with increased IL-6 levels and even as with atrial fibrillation in patients with coronary arter disease<sup>(17)</sup>. On the other hand, Sie and et al. performed meta analysis (n= 6434) in order to provide a better overview of outcomes of previous studies but they also did not show a clear association between the IL-6-174 G/C polymorphism and risk of CVD<sup>(29)</sup>.

Our results at IL-6 gene polymorphism -174 G/C were similar to the findings of Nauck et al.<sup>(18)</sup> and Lieb et al.<sup>(19)</sup>. Nauck et al. investigated the IL-6 -174 G/C polymorphism in patients with MI (n=1365) and they did not find a significant association of the -174 G/C genotype<sup>(18)</sup>. However, in agreement to our results, Lieb et al. also did not find a significant association with MI and traditional cardiovascular risk factors such as BMI, systolic and diasystolic blood pressure, total cholesterol, HDL cholesterol, LDL cholesterol, diabetes and IL-6<sup>(19)</sup>.

In the HIFMECH study, Kelberman et al. reported that IL-6 genotypes (-174 G/C, 572- G/C) were not significantly affected levels of IL-6 or other inflammatory markers such as CRP and fibrinogen in MI survivors on healthy individuals in the North or South of Europe. These results may be related the aspirin medication (91.4% in the North, 83.7% in the South) and statin therapy (26.7% in the North, 27.2% in the South) which would reduce inflammation and thus of the post infarction plasma IL-6 concentration<sup>(30)</sup>.

Bennermo et. al. demonstrated that the early IL-6 response during MI is associated with progression in patients with Q-wave MI where as IL-6 -174 G/C genotype has no association with MI, and IL-6 -572 G/C polymorphism showed a borderline significant increase in risk in univariate analysis<sup>(20)</sup>. However, in the WOSCOPS study, there was no significant association IL-6 -572 G/C polymorphism with coronary heart disease<sup>(31)</sup>.

Shin et al. observed associations between IL-6 -572 C/G promoter polymorphism and IL-6 levels, especially in G/G genotype in healthy smokers. So it was thought that individuals with the G/G genotype of the IL-6-572 C/G polymorphism (7%) have a high inflammatory response to cigarette smoking<sup>(26)</sup>.

There is an apparent distinctness in results relating to the IL-6 -174 G/C and -572 G/C SNPs between our study and the previous studies. The conflict between these studies might be relevant to population stratification, sex, disease status and inter ethnic variation in the allele frequency.

As a conclusion, in the present study, we failed to reveal any impact of the IL-6 -174 G/C and -572 G/C variants on neither MI nor with actual risk factors of MI such as fasting serum lipids. This study included a small population but could be carrying on more subjects or other cytokines for further studies.

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**Table 1.** Clinical features of patients with MI and controls

	<b>Patients (n=84)</b>	<b>Controls (n=85)</b>	<b>P value</b>
Age (years)	53.1 ± 10.3	49.9 ± 11.4	0.052
Male / Female	71 / 13	30 / 55	0.001
Hypertension (n,%)	31 (38.9)	34 (%40)	0.401
Smoking (n,%)	50 (%59.5)	22 (%25.9)	0.001

Total -C (mg/dL)	192.4 ± 52.4	193.9 ± 50.5	0.857
HDL-C (mg/dL)	36.9 ± 12.8	44.6 ± 13.5	0.001
LDL-C (mg/dL)	133.3 ± 46.2	120.9 ± 38.0	0.059
TG (mg/dL)	143.1 ± 110.6	137.8 ± 76.8	0.723

Values are expressed as mean ± standard deviation.

Total C; Total cholesterol, HDLC; high density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, TG; triglyceride.

**Table 2.** Distribution of IL-6 genotypes in patients and controls

		<b>Patients n (%)</b>	<b>Controls n (%)</b>	<b>Odds ratio (CI 95%)</b>	<b>p</b>
<b>IL-6 174</b>	GG genotype	52 (61.9)	58 (68.2)	Reference	
	GC genotype	29 (34.5)	25 (29.4)	0.756 (0.401-1.427)	0.389*
	CC genotype	3 (3.6)	2 (%2.4)	0.651 (0.106-3.996)	0.643**
	G allele	133 (%79.2)	141 (%82.9)	Reference	
	C allele	35 (%20.8)	29 (%17.1)	1.279 (0.741-2.209)	0.376
<b>IL-6 572</b>	GG genotype	70 (83.3)	71 (83.5)	Reference	
	GC genotype	14 (16.7)	13 (15.3)	0.986 (0.438-2.219)	0.973*
	CC genotype	0 (0.0)	1 (1.2)	Can not be calculated	
	G allele	154 (%91.7)	155 (%91.2)	Reference	
	C allele	14 (%8.3)	15 (%8.8)	1.065 (0.497-2.280)	0.872

\* GG versus GC+CC

\*\* GG+GC versus CC

IL-6; Interleukin 6, CI; confidence interval