

# Interleukin-17 is Not Associated with Risk of Premature Coronary Artery Disease in Iranian Turks

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## ABSTRACT

Cardiovascular diseases are one of the most important causes of death globally. Results of recent studies have indicated that cytokine dysregulation was associated with premature coronary artery disease (P-CAD). The majority of cytokine gene polymorphisms influence the level of cytokine production and secretion. This study aimed to analyse IL-17 gene expression in patients with P-CAD and healthy individuals in an Iranian population. This case-control study, conducted in Urmia University of Medical Sciences, compared patients with P-CAD hospitalised for risk of coronary artery stenosis, those admitted for medical cares and healthy normal controls. Thirty patients with P-CAD and 30 healthy individuals entered the study. The tested individuals were selected according to strict criteria such as clinical, echocardiogram, electrocardiogram and coronary angiography findings. Individuals with diabetes type 1 or 2 were excluded from the study. Reducing the diameter of at least one of the coronary arteries with more than 50% obstruction was selected as P-CAD. The qRT-PCR technique was used to determine the level of IL-17 gene expression in the studied groups. IL-17 gene expression was compared between the tested groups using t-test or Mann-Whitney U-test. Subjects' mean age ( $\pm$ SE) was 45( $\pm$ 5) and 44 ( $\pm$ 4) among tested cases and related controls, respectively. The relative mRNA expression was 4.04 $\pm$ 2.4 in patients with P-CAD and 2.75 $\pm$ 1.3 in controls for IL-17. IL-17 gene expression was not significantly different in the tested groups ( $P > 0.05$ ). IL-17 is not associated with risk of P-CAD in Iranian Turks.

**Keywords:** gene expression, interleukin-17, premature coronary artery disease.

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## INTRODUCTION

Cardiovascular disease is one of the most important causes of death in the United States and Iran (1). Premature coronary artery disease (P-CAD) accounts for about one third of all deaths in people over 35 in Western countries. Approximately 15.5 million people in the US have P-CAD. Cardiovascular disease-related mortality has been increased in the USA with a lifetime risk of 37.5% for males and 18.3% for females (2). Coronary artery disease (CAD) accounts for almost 50 percent of all deaths in Iran each year, leading to disability, mortality and morbidity. The risk factors of CAD include diabetic mellitus, smoking, and family histories of cardiovascular disease. Given that Iranian population is exposed to a high level of CAD risk factors, managing these risk factors is necessary to reduce the risk of CAD. Solving this problem will lead to improving health care services for people who are at risk of CAD (3). The disease has several traditional risk factors, including lipid disorders, smoking, diabetes, high cholesterol level, obesity, lack of physical activity, unhealthy diet and life style, and stress (4). In 30–50% of patients with heart failure, sudden death or acute asymptomatic heart failure occurs (5). Atherosclerosis is the main cause of CAD (6). Several risk factors have been identified for coronary artery disease in young people. The role of inflammation in the pathogenesis of P-CAD is well accepted. Inflammation has an important role in atherogenesis regarding the accumulation of foam cells and plaque formation. Cardiovascular risk factors have a tendency to make chronic inflammation through impaired nitric oxide production (7). Interleukin 17 (IL-17) as a pro-inflammatory cytokine is secreted from TH17 cells and it mainly affects the production and secretion of other cytokines. These inflammatory mediators play an important role in absorbing and invoking lymphocytes and endothelial trafficking molecules (8). CAD is defined as an inflammatory disease (9). The clinical role of cytokines remains incompletely explored and more studies are further needed to explore details. As to our knowledge there are no similar study in the population aged under 50, the present study was designed to analyse IL-17 gene expression in pa-

tients with P-CAD and healthy individuals in an Iranian population. □

## MATERIALS AND METHODS

The Ethics Committee of Urmia University of Medical Sciences has approved the study (Ir.umsu.rec.1394.138). Totally, 60 subjects were studied in two groups. The case group included 30 subjects with P-CAD and the control group 30 healthy individuals. P-CAD was recognized within the age of CAD onset under 55 years for men and under 65 for women (10). CAD is known in patients with acute myocardial infarction and registered in coronary angiography.

Subjects were selected based on strict criteria such as clinical, echocardiogram, electrocardiogram and coronary angiography findings. Reducing the diameter of at least one of the coronary arteries with more than 50% obstruction was selected as CAD (11-14). All participants were evaluated by an expert cardiologist and individuals with high blood pressure, diabetes, and smoking were excluded from the study. Subjects who signed the written informed consent were enrolled in the study.

Two milliliters of whole blood were obtained from participants and saved in 3 mL vacutainer tubes with EDTA. All samples were stored at -80°C prior to RNA extraction. RNX Plus Solution Kit (SinaClon) (Catalog Number: RN7713C) was used for RNA isolation. Amplified RNA samples (5 µL from each) were converted to cDNA using a cDNA synthesis kit (Thermo Scientific RevertAid Reverse Transcriptase (RT)). The qRT-PCR technique was used to determine the level of mRNA expression regarding IL-17 (124 bp) (TCT GGG GGG CAA AGT GCC GC and GGG CAG TGT GGA GGC TCC CT) and beta-actin (210 bp) (GGC GGC ACC ACC ACC ATG TAC CC and GAC GAT GGA GGG GCC CGA CT) genes (15). Thermo scientific SYBER Green/ROX qPCR Master Mix (2X) and 2 µL template cDNA were used for Real Time PCR. The PCR program included 95°C for 10 minutes; 40 cycles 95°C for 20 s and 60°C for 30 s. All analyses were carried out in duplicate reactions. The results of Real time PCR were analyzed using the 2- $\Delta\Delta$ Ct method.

## Statistical analysis

Statistical analysis was carried out using SPSS 16 software. The Kolomogorov-Smirnov test was

used to investigate the normal data distribution. In the case of normal data distribution, t-test, 2-independent-samples were performed to compare the mean of data between the two groups. In the case of non-normally distributed data, Mann-Whitney U-test was used for non-parametric test to compare the mean of data between two groups. The P-value < 0.05 was considered significant. The data were reported based on mean±SE. □

**RESULTS**

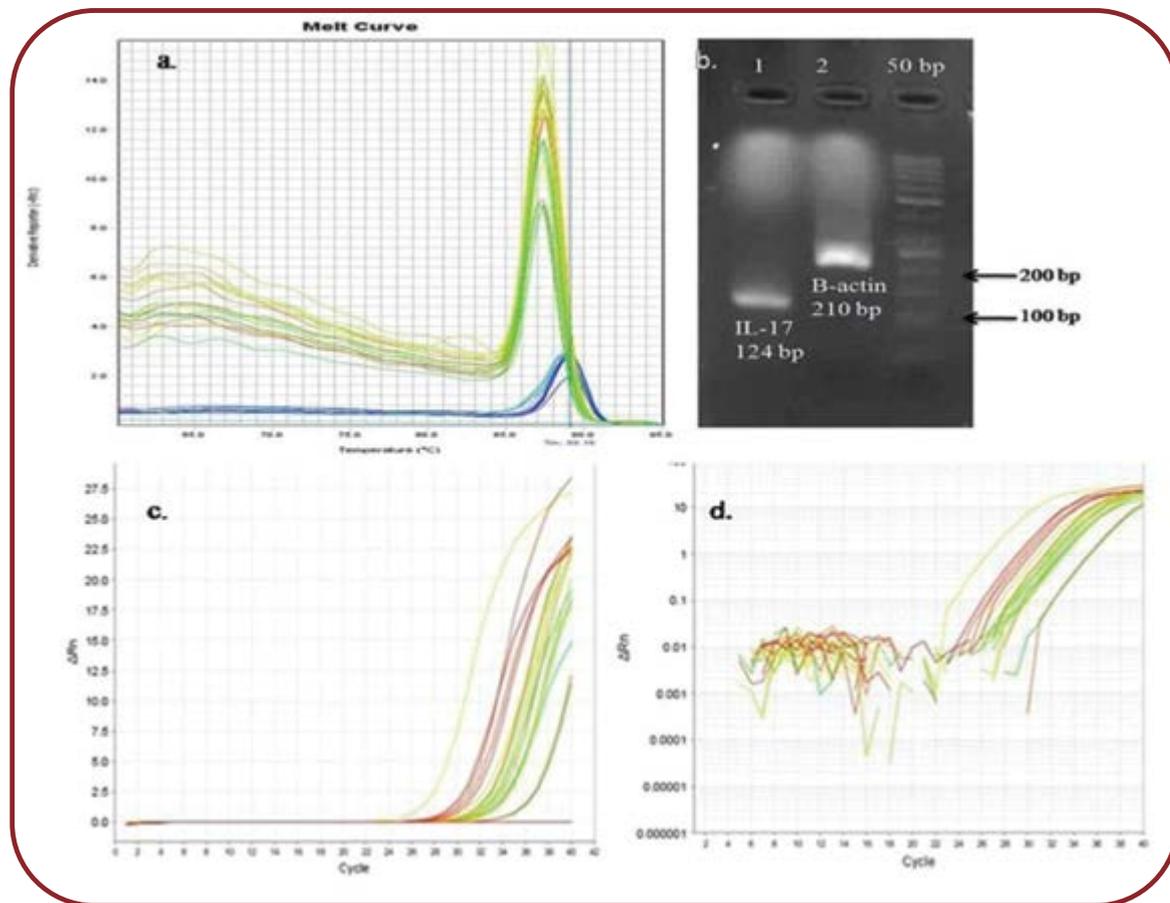
In this study, 30 patients with an average age of 45 years and 30 healthy subjects with an average age of 44 years were evaluated. The mean age (±SE) was 45 (±5) and 44 (±4) in tested cases and controls, respectively. The fold of relative mRNA expression was 4.04±2.4 in cases and 2.75±1.3 in controls for IL-17. Analysis of findings obtained in this study showed that there was no significant difference between the case and con-

trol groups regarding the level of IL-17 gene expression (P value 0.5). Figure 1 shows the analysis of amplified fragments on 2% agarose gel. □

**DISCUSSION**

P-CAD as a complex disease has several genetic and environmental risk factors. The role of some risk factors such as gender, age, educational status, hypertension, high cholesterol, obesity and diabetes has been studied (4). Inflammation and atherosclerosis play a central role in the development of P-CAD (16). The role of cytokines and cytokine gene polymorphisms could be highlighted. Cytokines are low molecular weight proteins that regulate immune responses. The results of recent studies have proven that the level of cytokine expression in various diseases has undergone significant changes (17-19).

In this study, the level of IL-17 gene expression has been analyzed in patients with P-CAD and



**FIGURE 1.** Data analysis in this study: a) melting point analysis curve for IL-17 gene; b) RT-PCR for β-actin (210 bp) and IL-17 (124 bp); c) linear amplification curves for IL-17 gene; and d) logarithmic amplification curves for IL-17 gene

healthy subjects among Iranian Turks. Our findings showed that IL-17 was not associated with the risk of P-CAD in the tested population, which had been also reported by Khojasteh-Fard *et al* in 2012 (20). Khojasteh-Fard *et al* (2012) studied the expression profiles of IL-17, IL-23 and TGF- $\beta$ 1 in individuals with and without CAD using Real-time PCR. In their study, CAD+ subjects had a mean age of 60 years and IL-17 and TGF- $\beta$ 1 gene expressions were similar in cases and controls. But a significant decrease was found in patients with CAD regarding IL-23 gene expression. It has been demonstrated that IL-17F rs763780 polymorphism was associated with myocardial infarction (21). Shuang *et al* (2015) suggested that the carriers of AA and GA+AA genotypes had an increased risk of CAD regarding of rs2275913 polymorphism. In our study, we failed to find any positive

association between IL-17 and P-CAD risk among Iranian Turks. A study with more details about genetic polymorphism and mRNA level may be informative for identifying which individuals are exposed to a high risk of P-CAD.  $\square$

## CONCLUSIONS

It can be concluded that the IL-17 was not associated with P-CAD risk. Further detailed studies are necessary to reveal disease-causing mechanisms in the pathogenesis of P-CAD.  $\square$

*Conflicts of interest: none declared.*

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