CLINICAL STUDY

TNF receptor type 1 and TNF receptor type 2 mRNA expression was not associated with coronary artery disease in a group of Iranian Turks

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ABSTRACT

OBJECTIVES: Present investigation was carried out to evaluate the mRNA level of TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2) in peripheral-blood cells in patients with premature CAD over healthy controls.
BACKGROUND: TNFα as a pleiotropic cytokine could be concerned in cardiovascular pathophysiology regarding its special effects on endothelial cells. TNFα exerts its activities through its receptors, TNFR1 and TNFR2.
METHODS: Totally, 40 patients with premature CAD and 40 healthy controls were studied. The qRT-PCR technique was used to determine the mRNA level of TNFR1 and TNFR2 in tested groups.
RESULTS: The results of this study show that the relative expression was 1.32 ± 0.34 in cases and 1.11 ± 0.16 in controls for TNFR1. The relative expression was 0.96 ± 0.13 in cases and 1.49 ± 0.41 in controls for TNFR2. There is no significant difference in the level of gene expression in the studied groups regarding TNFR1 and TNFR2 genes ($p > 0.05$).
CONCLUSION: It can be concluded that the mRNA levels of TNFR1 and TNFR2 were not associated with CAD risk. Studies with more details, larger sample size, and new risk loci are necessary to reveal disease-causing mechanisms in the pathogenesis of CAD *(Fig. 3, Ref. 21)*. Text in PDF *www.elis.sk*.
KEY WORDS: gene expression, TNFR1, TNFR2, premature CAD.

Introduction

Cardiovascular disease is the most common cause of death in developing countries (1). The mortality rate from cardiovascular disease is increasing in Iran (2). Approximately one-third of all vascular diseases are related to coronary artery disease (CAD) (3). The traditional risk factors for this disease include lipid disorders, smoking, diabetes, high cholesterol, social and psychosocial factors and stress, lack of daily use of fruits and vegetables, lack of mobility and regular physical activity, family history of CAD and high blood pressure (4). Atherosclerosis is the main reason for the onset of CAD. The incidence of symptomatic CAD in people under 55 years of age, in men and women under 65 years of age is called premature CAD (4.5). CAD is primarily caused by the interaction of gene-gene or genetic-environmental factors (6). CAD is a chronic inflammatory disease, and its major cause is arteriosclerosis and the pathological formation of arthroplastic plaques in one or more coronary artery (7). Considering that there is a correlation between the elevation of serum alkaline phosphatase (ALP) and myocardial infarction and coronary death (8), and also because tumor necrosis factor-alpha receptors are the most important factors regulating inflammatory responses; a study of genetic changes and expression of the receptors of the Tumor Necrosis Factor-alpha (TNFα) in patients with CAD are very valuable (9). According to the above, and also the lack of a similar studies in this field, this study was designed to analyze the expression of TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2) in peripheral-blood cells in patients with premature CAD.

Materials and methods

In general, 80 individuals were studied in two groups. The first group included 40 patients with premature CAD (under 50 years old), while the second group consisted of 40 healthy controls. This study was carried out after approving and obtaining necessary permissions from the Urmia University of Medical Sciences's Ethics Committee at the specialized cardiology cen-

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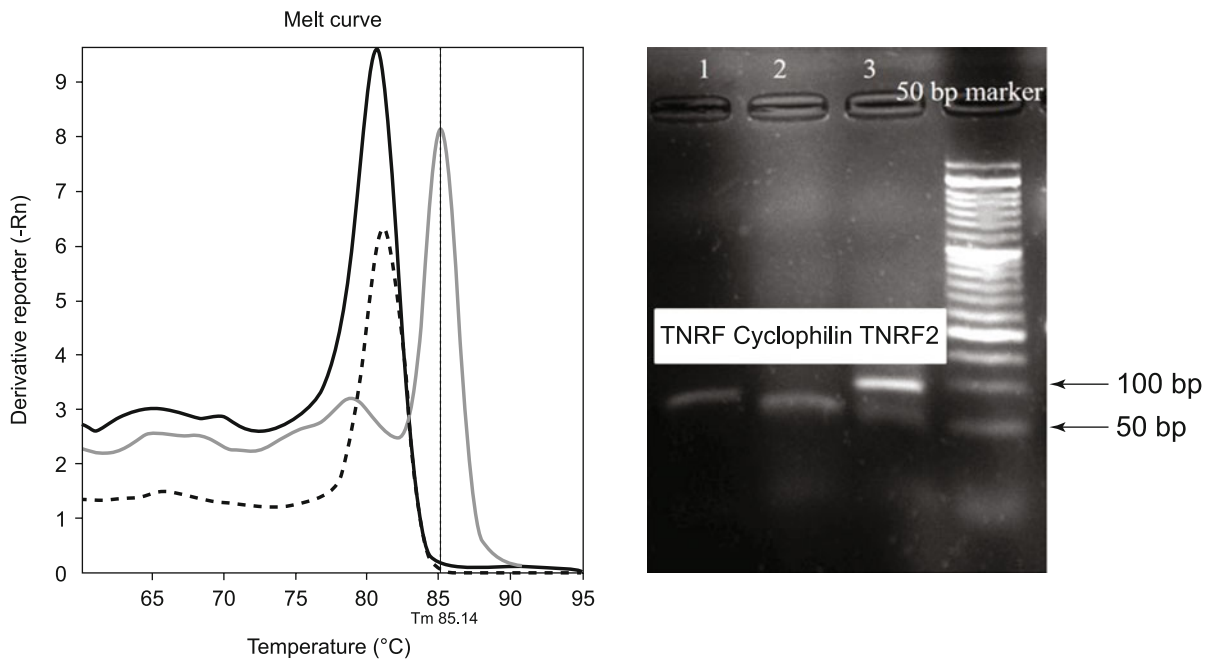Phone: +98.44.32770969, Fax: +98.44.32250730

Fig. 1. Melting curve and agarose gel analysis of TNFR1 (76 bp), TNFR2 (99 bp) and Cyclophillin (57 bp) genes on 2.5% agarose gel in this study.

ter of Sayyid Shohadah and the Cellular and Molecular Medicine Institute, Urmia, Iran. Written consent was obtained from all participants. CAD predisposition less than 50 years old was defined as premature (5). Information about age, sex, family history of CAD, dyslipidemia, diabetes mellitus, hypertension or the use of antihypertensive drugs, cigarette smoking were collected. Clinical,echocardiogram, electrocardiogram and coronary angiography findings were evaluated by an expert cardiac specialists (10). CAD is generally defined as the presence of a more than 50% stenosis at angiography (11). 2–3 ml blood sample was collected in a Falcon tube containing EDTA. The RNX Plus Solution Kit (SinaClon) (Catalog Number: RN7713C) was used to extract RNA from the specimens according to manufacturer's design with a few changes. Before performing other tests, the quality and purity of the RNA was confirmed. The spectrophotometer (Eppendorf AG, Germany) measured the absorbance of OD of RNA solution and not exceeded by 260/280<1.6OD. Then, in order to synthesize cDNA, the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit was used in accordance with the manufacturer's guide. The qRT-PCR technique was used to determine the level of gene expression in the samples. The sets of primers 5'-cgctaccaacggtggaagtc-3' and 5'-caagctccccctcttttttcag-3', 5'-caagccagctccacaatgg-3' and 5'-tgaccgaaaggcacattcct-3', and 5'-tgccatcgccaaggagtag-3' and 5'-tgcacagacggccactcaaa-3' were used for TNFR1 (76 bp), TNFR2 (99 bp) and Cyclophillin (57 bp), respectively. The results of Real time PCR were analyzed using the $2^{-\Delta\Delta Ct}$method (12).

Statistical analysis

Data analysis was performed using SPSS 20 software. The Kolomogorov-Smirnov test was used to investigate the normal distribution of data. In the case of normal distribution of data, for comparing the mean of data between two groups, t-test for 2-Independent-samples was performed. In the case of non-normally distributed data, for comparing the mean of data between two groups, Mann–Whitney U-test was used for non-parametric variables. A $p < 0.05$ is considered significant. The data were reported based on Mean ±SE.

Results

The results of this study are summarized in Figures 1 to 3. In this study, 80 patients were studied in two groups of patients (40 patients with an average age of 45 years) and control (40 healthy subjects with an average age of 44 years). The relative expression
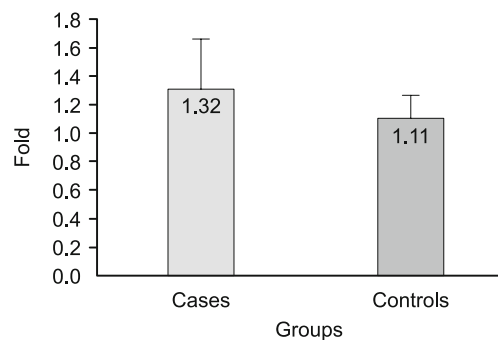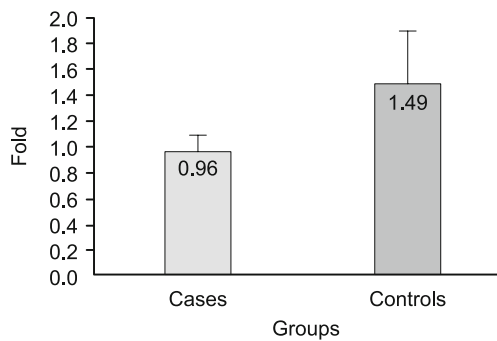

Fig. 2. Mean expression of TNFR1 (fold) in cases and controls. The fold-change of TNFR1 gene in the tested groups was not significant (p = 0.6).

Fig. 3. Mean expression of TNFR2 (fold) in cases and controls. The fold-change of TNFR2 gene in the tested groups was not significant (p = 0.2).

was 1.32 ± 0.34 in cases and 1.11 ± 0.16 in controls for TNFR1. The relative expression was 0.96 ± 0.13 in cases and 1.49 ± 0.41 in controls for TNFR2. Analysis of the findings of this study shows that there is no significant difference in the level of gene expression in the studied groups regarding TNFR1 and TNFR2 genes (P- value > 0.05). Figure 1 shows melting curve and agarose gel analysis of TNFR1, TNFR2 and Cyclophillin genes in the present examination.

Discussions

Cardiovascular disease is a public health problem and the main cause of morbidity and mortality in the world [14]. TNFα is a strong pleiotropic cytokine that has adverse pro-inflammatory effects. TNFα could be concerned in cardiovascular pathophysiology regarding its special effects on endothelial cells [15]. In the vascular system, TNFα causes a lot of changes in endothelial function and vascular smooth muscle, as well as influences the blood cells. Such changes in vascular dysfunction, the onset and progression of atherosclerosis are very important. TNFα plays a major role in the pathogenesis and progression of atherosclerosis. The production and release of TNFα and its related downstream signaling mediators after activation of its receptors, TNFR1 and TNFR2 have a special effect on the cardiac and vascular systems [16,17]. Both are expressed in myocytes of the heart [17]. Circulating levels of TNFα can be seen in patients with cardiomyopathy, myocardial infarction and chronic heart failure [18]. The findings of several studies indicated controversial results [16-19]. Hassan-Nejhad et al (2018) failed to show any association between TNFα gene expression and premature CAD in Iranian Turks. They proposed that other mechanisms in the pathophysiology of premature CAD might be evaluated regarding more deitailes and genes such as TNFR2 [19]. In this regard, it has been shown that the ratio of TNF/TNFR can be a predictor of the pathogenesis of the disease [20]. In the present study, the expression of TNFR1 and TNFR2 genes was investigated in patients with CAD and healthy controls and the analysis of the findings showed that there are no significant differences in the relative expression (fold-changes). Genetics of CAD is complex. The influence of environmental factors increases the CAD complexity while traditional risk factors remain vital.

About 60 SNPs with minor allele frequency > 0.05 have been associated with CAD risk [21]. Our study had some limitation regarding low sample size and poor quality of medical records in registry systems. Further studies with more details are necessary to reveal the role of genes and other mediators relevant to vessel wall biology and immune responses.

Conclusion

It can be concluded that the mRNA levels of TNFR1 and TNFR2 were not associated with CAD risk. Studies with more details, large sample size, gene-gene interactions, and new risk loci are necessary to reveal disease-causing mechanisms in the pathogenesis of CAD.

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