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EXPERIMENTAL STUDY

Evaluation of interleukin-17 receptor (IL-17RA) gene expression in PBMCs of patients with premature coronary artery disease

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ABSTRACT

OBJECTIVES: Present study has been carried out to analyze the IL-17R gene expression in PBMCs of patients with premature coronary artery disease (CAD) in comparison to normal controls.

BACKGROUND: Premature CAD results in disability and lack of quality of life over the years and consequent mortality. Cardiovascular disease (CVD) has global distribution. In 2022, CAD is the leading cause of mortality in the United States and Iran. IL-17 cytokine family plays an important role in promoting inflammation and producing pro-inflammatory cytokines, chemokines, and matrix metalloproteinases.

METHODS: Entirely, 60 subjects were entered into this examination. The case group consisted of 30 subjects with CAD as well as the control group which consisted of 30 healthy persons. The real-time quantitative reverse transcription PCR assay was used to find out, the relative expression (fold) level of IL-17R gene.

RESULTS: Our findings indicated that, the relative expression (fold) level of IL-17R gene in the patients group showed an increased level as compared to the control group. The analysis of findings obtained in this study showed that the patient group is significantly different from the control group regarding the IL-17R mRNA level (fold) ($p = 0.035$).

CONCLUSION: It has been concluded that IL-17R plays an important role in the pathogenesis of CAD. It follows that superior understanding of IL-17/IL-17R signaling way will be vital for innovating novel therapeutic targets that will facilitate the designing of new drugs for the management of patients *(Ref. 40)*. Text in PDF *www.elis.sk*

KEY WORDS: gene expression, premature CAD, IL-17R.

Introduction

Coronary artery disease (CAD) results in disability and lack of quality of life over the years and consequent mortality. Cardiovascular disease (CVD) has global distribution and its survival rate is low (1). In 2022, CAD accounts for around 610,000 deaths yearly (expected 1 in 4 deaths) and is the leading cause of mortality in the United States (1) as well as in Iran (2). CAD is the third foremost cause of mortality worldwide and is allied with 17.8 million deaths yearly (1). Healthcare services for CAD are estimated to cost more than 200 billion dollars annually in the United States. It accounts for almost 50 percent of all deaths per year in Iran (2). While CAD is a significant cause of death and disability, it is preventable (1, 2).

CAD is defined as the occurrence of atherosclerosis within coronary arteries and it may be asymptomatic (3). Atherosclerotic plaques as the hallmark of atherosclerosis are the main reason for the onset of CAD. They reduce the coronary artery lumen and impair myocardial blood flow. The blood flow decline in coronary arteries may be symptomatic or asymptomatic. It may occur any time, and may lead to a myocardial infarction (4).

It has been demonstrated that CAD has several variable and invariable risk factors. Many articles indicate that age (over 35 years in both, men and women), sex (men), ethnicity (Blacks, Hispanics, Latinos, and Southeast Asians) and family history are known as invariable risk factors, while variable risk factors include: hypertension, hyperlipidemia, diabetes mellitus, obesity, cigarette smoking, fat intake, lack of nutritious food, domestic and family violence, and inadequate housing. Additionally, new risk factors have been identified, namely non-alcoholic fatty liver disease (NAFLD)(5), chronic kidney disease (CKD) with GFR of 15–59 (6), systemic lupus erythematosus (SLE) (7), rheumatoid arthritis (RA) (8), inflammatory bowel disease (IBD) (9), human immunodeficiency virus (HIV) (10), thyroid disease (11), low

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testosterone due to aging (12), vitamin D deficiency (13), and socioeconomic status (14). The occurrence of traditional and new risk factors in patients with a family history of premature cardiac disease at age younger than 50 years showed an increased risk of CAD (15, 16).

Numerous inflammatory cytokines contribute in every part of atherosclerosis (17-25). In this regard, the roles of chemoattractants including chemokine 2 (CCL2) and CCL5, which promote the recruitment of monocytes (18), as well as secretion of pro-inflammatory cytokines: IL-1 β, IL-6, and TNF- α in NF-κB signaling in inflammation (19) have been identified. Recent studies indicated that there is a genetic association between pro-inflammatory cytokine gene production and CAD (26,27), but the results of some of the studies take a contrary stand (28, 29).

The level of interleukin (IL)-17 gene expression has been analyzed in patients with CAD in different groups, but these studies failed to find a positive association (29,30). IL-17 belongs to the IL-17 cytokine family. IL-17 plays a vital role in promoting inflammation in human diseases. IL-17 has been reported to regulate the expression of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases. But the role of IL-17 receptor is not well understood. The expression of IL-17 receptors is dissimilar among cell types, and this may lead to different immune responses (31). The present study has been carried out to analyze the IL-17R gene expression in PBMCs in patients with premature CAD in comparison to normal controls.

Methods and materials

The Ethics Committee of Urmia University of Medical Sciences has approved all stages of the study (Ir.umsu.rec.1394.138). Entirely, 60 subjects were entered in this examination. The case group consisted of 30 subjects with CAD, as well as the control group which consisted of 30 healthy persons. CAD is regarded premature when its onset takes place at age younger than 55 years for males and 65 years for females (29). Healthy subjects and patients with CAD were identified by an expert specialist in Seyyed-al Shohada University Hospital (Urmia, Iran) according to strict criteria as we described previously (32). Informed written consent form was obtained from all participants in this project or their guardians. A volume of 4 ml of peripheral blood was collected from cases and related controls in Falcon tubes containing 500 microliter EDTA (0.5 M) as anticoagulant and stored in a freezer at –80 °C until the day of the test. We used RNX Plus Solution Kit (SinaClon) (Catalog Number: RN7713C) for RNA extraction. Volumes of 10 µl from each sample were converted to cDNA with a cDNA synthesis kit (Thermo Scientific RevertAid Reverse Transcriptase (RT) (Catalog Number: #k1622). The real-time PCR assay was applied to find out the mRNA level using IL-17R (5¢-AGACACTCCAGAACCAATTCC-3′ and 5′-TCTTAGAGTTGCTCTCCACCA-3′) and GAPDH genes (33). A volume of 2 µL cDNA was amplified using Thermo scientific SYBER Green/ROX qPCR Master Mix (2X) by Mic qPCR Cycler (Bio Molecular System). The real-time PCR program included 95 °C for 10 minutes; 40 cycles at 95 °C for 15 s and 60 °C for 60 s. The expression level of target gene was normalized to the relative gene expression of GAPDH in that sample. All tests were done in duplicate reactions. The Livak method was used for gene expression analysis in tested groups via the 2(-Delta Delta C(T)) (34).

Statistical analysis

Statistical analysis was performed using REST 2009 software. The p value less than 0.05 was considered significant. The data were reported based on mean ± SE.

Results

In this study, the mean age (± SE) was 45 (± 5) in cases and 44 (± 4) in controls. Our findings indicated that the relative expression level of IL-17R gene (fold) in the patient group showed an increased level as compared to the control group. The analysis of findings showed that the patient group is significantly different from the controls group regarding IL-17R mRNA level (fold) by a mean factor of 27.64 ($p = 0.035$).

Discussion

Premature CAD is known as a complex of diseases with different etiologies under control of several risk factors (11). The majority of CAD risk factors are associated with inflammation and atherosclerosis. Atherosclerosis is triggered by the cytokine cascade (15). Cytokines and cytokine genes polymorphisms have been associated with pathobiology of human diseases (3, 22, 28, 29, 32).

IL-17 may play a vital role in the intonation of macrophage-derived cytokine expression and may function as an important pro-inflammatory cytokine (35). IL-17 activates several signaling pathways such as nuclear factor (NF)-κB, and leads to the expression of pro-inflammatory cytokines genes including IL-1, IL-6, IL-8, TNF-α, chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7), granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and matrix metalloproteinases (such as MMP1, MMP3, MMP9, etc) (3, 36). IL-17 exerts its action by way of interaction with IL-17R on target cells to impel their biological actions.

IL-17R is expressed in a variety of human cell populations. The array of cells consists of epithelial, smooth muscle and vascular endothelial cells, fibroblasts, etc. IL-17R as a type I receptor, is a 130 kDa protein. This macromolecule crosses the width of the membrane once and has an amino domain on the extracellular surface. Blockage of IL-17R with antibodies was used to block the production and secretion of IL-6. The IL-17R family has unique properties with distinct and signaling pathway from other cytokines (37). In this study, the relative expression (fold) level of IL-17R gene in the patients group showed a significant increase as compared to the control group. Our finding was in conformity with Iranian research (38) and a Chinese group (39), but also in disagreement with a group of Caucasians (40). We recognized that the alternative expression levels of cytokines are mainly influenced by race differences and time of sample collection.

The IL-17, along with expression of its different receptors on various cells and tissues, complicate the immune response as well as the process as to how its impaired regulation may lead to the production of pro-inflammatory cytokines and consequently atherosclerosis. However, considering the complexity of pro-inflammatory and tissue protective roles of IL-17 family in human diseases, it is not clear how therapeutic developments namely anti-ligand/receptor antibodies, may impinge on patients. Regarding to these incompatibilities, in the future, it is necessary to design new studies with larger sample sizes as well as investigate other genes such as vascular cell adhesion molecule 1 (VCAM-1) with more details.

Conclusion

IL-17R plays an important role in the pathogenesis of CAD. It follows that superior understanding of IL-17/IL-17R signaling way will be vital for innovating novel therapeutic targets that will facilitate the designing of new drugs for patients who do not respond to conventional therapies.

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