Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(2):528-536

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Lack of Association between OX40L gene polymorphism rs3850641 and the risk of premature myocardial infarction in Iranian population

Running title: rs3850641 OX40L gene polymorphism and premature myocardial infarction.

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Category: Original article. The area of the manuscript is diagnostic genetics.

ABSTRACT

Background: *Tumor necrosis factor (TNF) is one of the inflammatory cytokines which has an important role in inflammation and migration of other inflammatory cells to the atherosclerotic plaques. OX40 ligand (OX40L) is a member of the TNF super family receptor protein. OX40 and OX40L are co-stimulators for T-cells and can increase inflammatory response in atherosclerotic plaques. The aim of this study was to determine the association of rs3850641 polymorphism in OX40L gene with premature myocardial infarction (MI) in Iranian population.*
Methods: *This case control study was done on 100 patients with premature MI and a similar number of sex, age and some other cardiovascular risk factor matched healthy people. The OX40L rs3850641 polymorphism was genotyped, using PCR-RFLP method.*
Results: *A-allele frequency of rs3850641 SNP was lower non-significantly in Premature MI, compared to healthy subjects (57.5% vs. 58.8%). The analysis of rs3850641 (A/G) polymorphism showed an odds ratio of 0.980 (95% CI: 0.473-2.030; P= 0.957) for the GG genotype and 1.127 (95% CI: 0.635-1.999; P= 0.00) for the AG genotype, compared to the AA genotype.*
Conclusion: *The results of this study indicate that the rs3850641 SNP of OX40L gene is not associated with premature MI in the Iranian population.*
Keywords: *premature myocardial infarction, OX40L, Gene polymorphism.*

INTRODUCTION

Ischemic heart diseases are the most common cause of mortality in the world (1, 2). The number of young patients with myocardial infarction (MI), as the most common subtype of coronary artery disease (CAD), is increasing (3). Previous study reveal about 18% of acute myocardial infarction patients are younger than 50 years old, defined as premature myocardial infarction(4). Patients with premature myocardial infarction show higher re-infarction rate,

more occurrences of severe heart failure and also significantly higher mortality rate due to cardiovascular events at young ages (5).

MI is a heterogeneous condition with a wide spectrum of underlying causes, such as hyperlipidemia, diabetes, hypertension, vasculitis, and atherosclerosis (6). Atherosclerosis, the predominant process underlying cardiovascular disease, is now considered an inflammatory disease (7, 8). Activated T-cells are shown to be in important cellular and molecular events in atherogenesis (9, 10). Moreover, there is evidence from family and twin studies that genetic factors play an important role in the pathogenesis of atherosclerosis (11). Although the mode of genetic transmission of the disorder is widely believed to be multifactorial, few candidate genes have been established (12).

OX40L (known as TNFSF4, CD252), the cognate ligand of OX40, is a member of the tumor necrosis factor superfamily. The OX40L gene is located on human chromosome 1 and encodes a type II glycoprotein which expressed not only on professional antigen-presenting cells (APCs), but also on CD4+T cells, CD8+T cells, vascular endothelial cells, mast cells and activated NK cells (13-15). The interaction between OX40 and OX40L provides a costimulatory signal that strongly regulates the proliferation and survival of T lymphocytes, modulates NKT cell and NK cell function, and contributes to the differentiation and activity of regulatory T cells (15). OX40L is considered to have the potential to enhance inflammatory response in atherosclerosis plaques that could be related to the immunoregulatory role of this pathway (16). Some studies presented evidence that OX40L is the gene underlying the atherosclerosis susceptibility locus 1 (Ath1) in mice, earlier identified by Paigen and colleagues (17), and shown that genetic variants in the OX40L locus are associated with MI and severity of coronary artery disease (CAD) in human (18, 19).

Some studies showed that members of OX40L gene involve in the atherosclerotic process of which the single nucleotide polymorphism (SNP) rs3850641 in OX40L is believed to be significantly associated with the risk of MI (18, 20). However, little is known about the role of the rs3850641 SNP in OX40L gene with the premature MI. Since evidence regarding this issue is scarce, we sought to determine if the rs3850641 polymorphism in OX40L gene is associated with premature MI in Iranian population.

METHODS

Study design:

The target subjects of present based descriptive cross-sectional survey consisted of women and men aged 30 to 50 years residing in Jahrom, Iran between the spring of 2014 and the fall of 2015. Of them, one hundred adults with

premature MI were compared with 100 non premature MI ones as controls. Control subjects were frequency matched to patients by sex and age. All of case subjects were selected from patients who admitted to clinical centers in Jahrom, and control subjects were randomly selected from the parent study. Two independent cardiologists corroborated the diagnose of CAD according to the World Health Organization criteria for MI such as chest pain, cold sweating, cardiac enzyme elevation in serum and diagnostic change in ECG. None of the patients and control subjects had physical or mental impairment that make participants unable to answer the questionnaire, past medical history of hospital admission due to cardiovascular disease (CVD), and had no significant renal dysfunction and liver damage. All of the participants consented to donate biological specimens for present study. The study protocol was approved by the Ethics Committee of Jahrom University of Medical Sciences and all of the participants gave their written informed consent.

Blood Samples:

Three ml of venous blood taken and collected in tubes containing EDTA as an anticoagulant then stored at -20 ˚C in order to extract DNA.

Extraction of DNA and PCR:

Extraction of DNA was done by DNP ™ kit based on the instruction of the product. The part of GATA2 gene [A transcription factor GATA2 (GATA-binding protein 2), which regulates several endothelial-specific genes, as a novel susceptibility gene for Coronary Heart Disease], including the rs3850641 Polymorphism multiplied by polymerase chain reaction (PCR) by master mix tube of Bioneer Company (South Korea). The reaction, containing MgCl2 solution Mμ 1.5, dNTP each 250Mμ, and 0.2 μL (1 unit) Taq polymerase enzyme, DNA template 2.5μL, 10 pmol primers each, and sterile water to 20 μL total reaction volume. PCR was done using thermal cycler device.

PCR cycles were explained as below:

PCR conditions for genotyping using either set of primers included an initial denaturation at 94 ˚C for 5 minutes followed by 35 cycles and 94 ˚C for 30 seconds, 55 ˚C for 30 seconds, 72 ˚C for 1 minute and finally 72 ˚C for 10 minutes. Final PCR product was assessed in agarose gel containing Ethidium bromide.

Specific primers that used are mentioned in table below:

Sequence	**Primer**	**SNP**
TGA CTG GAA GAG ATA GAT TGAGA	Forward	rs3850641
AGA GGC ACA GCT TCT AGA TCT TTTT	Reverse	

Gene runner software used to determine the duplicated parts of primers. Gene sequences were confirmed by blast program. These primers yielded a PCR product of 188 base pairs (bp) spanning the Mun I (Fermentase Company, concentration: 10 U/ul), polymorphic site. Amplification products were digested with the restrictive enzyme Mun I wild type (genotype AA), the PCR product was digested into DNA fragments of 160 and 28 bp. The mutant G allele did not undergo digestion with the enzyme Mun I. DNA fragments obtained after restrictive enzyme digestion and the DNA size marker were electrophoresed on a 3% gel agarose and stained with ethidium bromide.

Statistical analysis:

Results were reported as mean ± standard deviation (SD) or median for quantitative variables, and percentages for categorical variables. A 2-sided of P values of 0.05 or less was considered statistically significant. All the statistical analyses were performed using SPSS version 14.0 (SPSS Inc, Chicago, IL, USA) for Windows. With the observed number of events/nonevents or cases/noncases and the assumption of 2-sided values of P values of 0.05 or less, we calculated the hazard ratio/odds ratio which could be detected in present studies at 90% power. The groups were compared using the Student's t-test and the chi-square test (or Fisher's exact test if required) for continuous and categorical variables, respectively. Allele frequencies were calculated for each genotype by allele counting.

RESULT

One hundred patients with type premature MI and 100 controls without premature MI were genotyped for the rs3850641 gene polymorphisms in OX40L. All participants were aged 30 to 50 years. No significant differences between demographic characteristics of control and patients groups were observed. Thirty two (32%) men had premature MI and 30 (30%) men were in control group. The average age of the participants with premature MI and controls were 41.5±4.9 and 42.5±6.6 years (P = 0.197), respectively. There was no significant difference between gender (P=0.876) and smoking (P=0.323) in case and control group. According to the results of study 70% (70 people) of case group had family history of CAD. In control group 90% of participants had no family history of CAD. There was a notable difference between case and control group (P < 0.001). In case group 25% of participants had hypertension (HTN), 23% had hyperlipidemia (HLP) and 25% had diabetes mellitus (DM). There were significant differences involving with HTN (0.001), HLP (0.07), and DM (0.010) in case group compared with control group. The clinical data and baseline characteristics of participants are shown in Table 1.

Table 1. Clinical data of the study subjects

Characteristics	Patients (n=100)	Control (n=100)	P value
	N[a] or Mean ± SD	N[a] or Mean ± SD	
Age (year)	41.5±4.9	42.5±6.6	0.197
Male	32	30	0.761
Smoking	25	27	0.321

Diabetes	25	11	0.010
Family history	70	10	<0.001
Hyperlipidemia	23	9	0.070
Hypertension	25	8	0.001

[a] **N: number**

A-allele frequency of rs3850641 SNP was lower non-significantly in Premature MI, compared to healthy subjects (57.5% vs. 58.8%). The analysis of rs3850641 (A/G) polymorphism showed an odds ratio of 0.980 (95% CI: 0.473-2.030; P= 0.957) for the GG genotype and 1.127 (95% CI: 0.635-1.999; P= 0.00) for the AG genotype, compared to the AA genotype. The results of this study indicate that the rs3850641 SNP of OX40L gene is not associated with premature MI in the evaluated population (Table 2).

Table 2. Genotype and allele frequency of rs3850641 polymorphism

	Control Group N =100	**Case group N =100**	**P value**	**Odds Ratio (confidence interval)**
AA	58.8%	57.5%	**Reference**	**Reference**
AG	0.0%	1.4%	0.00	1.127 (0.635-1.999)
GG	41.2%	41.1%	0.957	0.980(0.473-2.030)
AA	58.8%	57.5%	0.0886	0.459-1.959
AG+GG	41.2%	42.5%		
Allele frequency				
A	59%	57.7%		
G	41%	42.3%	0.794	0.948 (0.638-1.411)

DISCUSSION

The OX40/OX40L system, along with several other receptor-ligand pairs, has been suggested to be involved in the recruitment and activation of T-cells and is therefore tentatively implicated in atherosclerosis and acute coronary syndromes such as MI. We have previously demonstrated that OX40 haplotype is not associated with risk of premature MI (19). To our knowledge, present study is the first survey in the literature aimed to evaluate the role of rs3850641 SNP of OX40L gene in premature MI. Revealed data of the current study indicate that the rs3850641 SNP of OX40L gene is not associated with premature MI in the evaluated population.

Atherosclerosis is an important pathogenesis process for MI. There is evidence that OX40/OX40L signaling pathway is involved in T cells activation, survival, and the interaction between activated T cells and endothelial cells (13, 15, 21), indicating that OX40L gene plays an important role in the atherosclerotic process. In mice, OX40L expression is associated with increased atherosclerosis. In some independent human cohorts (18, 22),

the minor allele of SNP rs3850641 in OX40L is significantly more frequent in patients with myocardial infarction than in control group. However, the region containing the OX40L gene has been found to be associated with celiac disease, a chronic inflammatory disease with a strong immune component and systemic lupus erythematosus, an autoimmune disease, which are likely to share common inflammatory pathways with atherosclerosis, the main underlying cause of MI (23, 24). The fact that OX40L is expressed by several cell types suggests that OX40L has more functions than the originally reported involvement in T-cell activation (18, 25). Of cells present in the atherosclerotic lesion, both endothelial cells, macrophages, mast cells and SMCs express OX40L (26). OX40L expressed on endothelial cells was reported to mediate the adhesion of OX40L-expressing T-cells to vascular endothelial cells and the subsequent migration to distant inflammatory sites (27), suggesting an involvement of OX40L in lymphocyte recruitment as well. Unstable plaques are particularly rich in activated lymphocytes (28); therefore all these events, possibly triggered by OX40L, may favor destabilization and rupture of the plaque.

With respect to the SNP rs3850641 of the OX40L gene, we failed to show that it was associated with the risk of premature MI in the Iranian population. In contrast, The A allele carriers had a lower occurrence rate for premature MI group when compared to the control group. Bivariate non-condition logistic regression analysis showed that SNP rs3850641 had little association with premature MI, consistent with findings in other Asians (29). Similar to the study in Sweden, in which Olofsson et al. (30) showed that OX40L is expressed on antigen-presenting cells in human carotid atherosclerotic lesions but provides no evidence for an association of OX40L gene variation with the risk for ischemic stroke, we conclude that the rs3850641 SNP is not associated with the risk for ischemic disease. There were no study in the literature to show the role of SNP rs3850641 of the OX40L gene in premature MI. Perhaps we should have divided MI into several subtypes, such as large-artery atherosclerosis, small-artery occlusion, and other determined or undetermined etiologies. Human OX40L gene plays an important role in the atherosclerotic process and the minor allele of SNP rs3850641 was significantly associated with myocardial infarction (18), which is frequently arises as the result of an occluding thrombus at the site of a ruptured atherosclerotic lesion. These discrepancies may be related to the lower age groups of MI in our study, population differences, differences between methods performance of studies, and sample size which should be considered for future studies. Furthermore, abnormal splicing site leading to the negative result of the rs3850641 with premature MI in our study.

In conclusion, our data suggest that rs3850641 SNP of OX40L gene is not associated with the risk of premature MI in an Iranian population.

Acknowledgment

This article was partly extracted from the thesis written by Mohamed Amin Ghobadifar and was financially supported by Jahrom University of Medical Sciences.

Disclosure of interest

None of the authors have any conflict of interest to declare.

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