Genetic Variants of Toll Like Receptor-4 in Patients with Premature Coronary Artery Disease, South of Iran

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Abstract

Introduction: Ischemic cardiovascular diseases are the leading causes of morbidity and mortality in most developed and developing countries including Iran. Premature myocardial infarction has a polygenic base with a complex relation with environmental factors. Since expression of different inflammatory genes especially toll like receptor-4 (TLR4) has increased considerably in human atherosclerotic plaques, we have decided to study variants of TLR4 in premature coronary artery disease in patients in Jahrom city, Iran.

Methods: In this case-control study, 100 patients with a history of premature coronary artery diseases and 100 healthy control subjects referred to health centers in Jahrom city were studied. Target sequences of TLR4 gene were amplified by PCR amplification and digestion was done by StyI restriction enzyme (PCR-RFLP method).

Results: There was no significant difference regarding age (P>0.05). The distribution of TC heterozygote genotype in the premature myocardial infarction group is significantly higher than in the healthy group (P<0.05) but the homozygote mutated genotype showed no significant difference (P>0.05). In addition, the genotype carrying the mutated allele (TC+CC) showed a significant difference when compared to TC variant (P < 0.05). The genotype distribution in rs1927911 in both genders shows no concomitance between males and females (P>0.05).

Conclusion: According to the results derived from this study, it seems like the existence of the genotype carrying the mutated allele (TC+CC) in rs1927911's mononucleotide polymorphism of TLR gene is associated with an increased risk of premature myocardial infarction.

Key words: Premature coronary artery disease – TLR4 gene - rs1927911 polymorphism

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Introduction

Despite improvements in diagnosis and treatment of coronary artery disease (CAD), it is still among the leading causes of death and disability in the world. Cardiovascular diseases are the most common life threatening diseases in industrial societies and a rapidly growing problem in developing countries (1). MI is a complex multifactorial and polygenic disorder (2). There are several environmental risk factors correlating with CAD such as obesity, diabetes mellitus, hypertension, family history and smoking (1). Twenty percent of acute myocardial infarction patients are referred to as premature MI (3), which is defined as the first attack occurring in males aged 50 years and younger and females aged 55 years and younger (4). Premature CAD is known to be the most aggressive form of the disease (5). In recent decades, the idea of the inflammatory nature of atherosclerosis has been strongly propounded and therefore serum levels of inflammatory markers for risk stratification of cardiovascular events have been considered (6, 7). Inflammatory cells, especially macrophages, are present in atherosclerotic plaques (8).

There is a family of receptors that present in phagocytic cells like macrophages which are named as Toll like receptors (TLR) (9).

When TLRs on macrophages are activated, these lead to activation of the nuclear factor kappa B (NFkB) pathway which results in production and expression of pro inflammatory molecules (10).

TLR4 is one of the important members that is expressed by macrophages and endothelial cells in human atherosclerotic lesions (11).

Some clinical studies have demonstrated that the effects of polymorphisms of genetic variants of the human TLR4 gene, located on chromosome 9, on the progression of the atherosclerosis, is controversial (12-14).

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in a population (15). There are 10 SNPs in the genotyping system of TLR4: Re10759930, rs2737191, rs2770150, rs1927914, rs1927911, rs5030728, rs11536889, rs1554973, rs11536897, rs11536891 (16). The rs1927911 SNP is located within the intron – coding region of the TLR4 gene on chromosome 9 (17).

Due to the lack of data about the role of TLR4 gene polymorphism in premature CAD in the literature, this study was conducted to determine the association between polymorphism in variants of TLR4 gene and occurrence of premature MI.

Materials and Methods

Design and participants

The study was retrospective, observational, and cross-sectional. One hundred patients with a history of premature coronary artery diseases and 100 healthy control subjects referred to health centers in Jahrom city were invited to participate in the study. All participants signed an informed consent approved by the Institutional Ethical Committee after a detailed orientation of the study requirements, possible risks, and benefits. The information and data about the patients were extracted without name by using codes and were kept confidential. This study was approved by the Research Ethics Committee of Jahrom University of Medical Sciences (ethic code: JUMS.REC.1394.62.9).

Demographic information

Demographic information was collected from case and control groups. This study was conducted based on the declaration of Helsinki and approved by the ethics committee of Jahrom University of Medical Sciences. All individuals had consent to participate in study and based on the testimonial they could leave the study.

Extraction of DNA and PCR

Five ml of venous blood was taken and collected in tubes containing EDTA as an anticoagulant then stored in -20 ˚C in order to extract DNA. Extraction of DNA was done by commercial kit (Cinagen Co., Tehran, Iran).

Genotyping of rs1927911 polymorphism in TLR4 gene

Genotyping of rs1927911 polymorphism was performed using restriction fragment length polymorphism (RFLP).

Amplification of DNA was done by polymerase chain reaction (PCR) in premix pipes (Bioneer Co. Daejeon, Korea). Selection of forward and reverse primers was done according to related articles. Gene sequence accuracy was confirmed by gene bank information website (http/ncbi.nlm.nih.com). Also primers gene sequence was re-checked with Gene runner software and blast program primer sequences were F: TCACTTTGCTCAAGGGTCAA R: AAACCTGCATGCTCTGCAC

To detect the rs1927911 polymorphism, StyI restriction enzyme (Frementase Co) was used. 3% Agarose gel electrophoresis was done for endorsement of the dissected sequence.

Statistical analysis

Correlation between occurrence of acute premature coronary syndrome and rs1927911 polymorphism TLR-4 gene in the case and the control groups was determined with Odds ratio (OR), Chi-square and Fisher exact tests. In the deductive part of the study, the differences in biochemical markers and demographic information were evaluated with T test (p value< 0.5 defined as significant). All analyses were done by SPSS version 15.

Results

Participants' ages were in the range of 30-50 years old. Mean of age in the case group was 41.5±4.9 years and mean of age in the control group was 42.5±6.6 years with no significant difference (P=0.197). Gender (P=0.876) and smoking (P=0.323) in case and control groups had no significant differences.

Results of study showed that 70% (70 people) of the case group had a family history of CAD and 91% of the control group had no family history of CAD. There was a noticeable difference between case and control group (P=0.000) that clarifies the obvious role of family history in occurrence of CAD. In the case group 25% of participants had hypertension (HTN), 23% had hyperlipidaemia (HLP) and 25% had diabetes mellitus (DM). There were significant differences between groups in cardiovascular risk factors: HTN (0.001), HLP (0.07), DM (0.010) (Table 1).

Table 1. Demographic data of both study groups

	NGT subjects	Premature Coronary Artery Disease	P
n (males/females)	30/70(%30/70)	32/68 (%32/68)	0.76
Age (y)	6.6±42.5	4.9±41.5	0.197
Smoking (n)	27	25	0.321
DM (n)	11	25	0.010
FHX (n)	0	2	0.000
HLP (n)	9	23	0.070
HTN (n)	8	25	0.001

FHX: family history of CAD, DM: diabetes mellitus, HLP: hyperlipidaemia, HTN: hypertension

According to the results of study there was no significant difference between CC genotype mutant of TLR-4 gene and occurrence of premature CAD (p value: 0.435) but in mix genotype CC+TC vs TT there was a significant difference between premature CAD and healthy subjects (p value: 0.021), and C-allele frequency distributions were not significantly different (P;0.093) (Table 2).
Difference between alleles of TLR-4 gene (C and T) and occurrence of
premature CAD in case and control groups is shown in Table 2 (p value: 0.013).

Table 2: Frequencies of genotypes and alleles in participants

	Controls N =100	Premature Coronary Artery Disease N =100	Value of P*	OR
TT	67(67.0%)	50(50%)	Reference	
TC	5(5.0%)	23(23.0%)	0.001	0.162(0.058-0.456)
CC	28(28%)	27(27%)	0.435	0.774(0.407-1.472)
TC+CC	33(33.0%)	50(50.0%)	0.021	2.030(1.146 -3.598)
TT	67(67.0%)	50(50%)		
Allele frequency				
allele T	123(62.5%)	139(%69.5)	0.093	0.701(0.463-1.061)
allele C	74(%37.6)	61(%30.5)		

Discussion

MI is the leading cause of mortality in developed countries and the second leading cause in developing countries (1). Expression of different inflammatory genes, specifically TLR4, has increased significantly in human atherosclerotic plaques (18). 1927911 SNP located on chromosome 9 is one the polymorphisms that has always been investigated in CVDs (19).

According to the results found in this study, distribution of heterozygous genotype (TC) was meaningfully higher than that in the healthy group control but the mutated homozygous genotype did not show a meaningful difference. Besides that, when compared to TC state, the genotype carrying the mutated allele (TC+CC) did not show a meaningful difference.

Even though the distribution of the mutated C allele was higher in the healthy control group compared to the premature MI group, this difference was not meaningful.

The Logistic regression analysis of distribution of genotype in rs1927911 in both genders shows that there is no meaningful concomitance in men and women, even though the mutated C allele was meaningfully more in females than in males.

Results of a study conducted by Yanmin Song et al. in the southern Chinese province of Hunan in 2014 showed that for rs1927911 there is a meaningful difference between acute cardiac ischemia (ACI) patients and the control groups from a genotype and allele distribution but hypertension, fasting blood sugar and serum fat level with different genotypes in both ACI patients and control groups had no meaningful difference (20).

In a study done by Daniel A. Enquobahrie et al. sweeping changes of gene in PPARA (peroxisome proliferator activated receptor alpha) and TLR4 gene was accompanied by MI. A minor allele of PPARA SNP, rs4253623, was accompanied with an increased risk of MI and a minor allele of TLR4 SNP, rs1927911, with an increased risk of MI. rs1927911 minor allele, a part of TLR4-D haplotype, is accompanied with a 12% risk of MI (21).

Conclusion

According to the findings of this study, it seems like the presence of the carrying genotype of mutated allele (TC+CC) in rs1927911 single nucleotide polymorphism (SNP) of TLR4 gene is associated with an increase of premature MI.

Considering the breadth of polymorphisms of TLR4 gene and role of genetics in premature MI, in order to establish this polymorphism as a risk factor, further studies in larger populations in this area is proposed.

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