Association of tRNAThr 15927G→A Mutation with the Incidence of Coronary Artery Disease

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ABSTRACT

One of the leading causes of death in the world are cardiovascular diseases, among which coronary artery disease (CAD) is the most common one. It occurs as a result of narrowing of the arteries which supply blood to the heart due to a theroma plaque formation. This kind of heart disease can be considered as a multifactorial one as genetic and environmental factors are involved in its incidence. The already conducted studies have investigated the relationship between some of polymorphisms in different loci and CAD with the aim of on-time diagnosis of this disease, which may lead to its prevention and appropriate treatment. The aim of this study was to examine the association of tRNA Thr 15927 G → A mutation with the risk of CAD incidence to offer programs for early diagnosis and treatment of this disease in Iran. Fifty patients with CAD were included in the patient group, and fifty healthy participants were selected for the control group. 5mL of peripheral blood samples drawn from subjects in patient and control groups were collected in the tubes containing Ethylenediaminetetraacetic acid (EDTA). After DNA extraction from blood with the employment of DNA extraction kit, its quality and quantity were measured using electrophoresis and NanoDrop devices, respectively. Then, the isolated DNA with the implementation of amplification-refractory mutation system (ARMS) and restriction fragment length polymorphism (RFLP) techniques was examined to determine and evaluate the target polymorphism in the intended locus. Sequencing method was also used to confirm the findings. To this end, 4 samples were randomly selected and sequenced.In the control group, 6 screened patients had the mutation while the others did not. Similar result was observed in the patient group. The findings of the present study reveal that there was not any significant relationship between tRNAThr 15927G → A mutation and risk of CAD incidence.

Keywords: Angiogenesis; atherosclerosis; Coronary Artery Disease (CAD)**;** heterogeneity; thrombosis

INTRODUCTION

Heart disease can be considered as the leading cause of sudden death all over the world. Its most prevalent type is coronary artery stenosis or CAD. According to the statistics presented at the 11th Recent Findings of Cardiology Congress administered in June 2009, 38 to 40% of deaths are caused by cardiovascular diseases in Iran. Furthermore, 198 Iranian individuals die from heart attack per day [1-5]. CAD is a multifactorial disease, in which the significant role of factors such as environmental conditions, life style, and nuclear genetics has been acknowledged [6, 7]. As heart requires ionic balance in its muscle to regularly pump and circulate blood in the body, and since the proper functioning of mitochondria is regarded as the balancing factor, the significant role of mitochondrial genetics in CAD becomes apparent [8, 9]. High blood pressure is specified as a major risk for the incidence of CAD diseases. A good number of studies have been conducted with respect to the congenital blood pressure revealing the role of mitochondria in disease transmission from mother to child. It is well acknowledged that mitochondrial genome is inherited only from mother [10-14]. Each mitochondria contains multiple copies of double-stranded and circular mitochondrial DNA known as mtDNA. Human mtDNA encodes 13critical subunits of the inner membrane, which are

responsible for oxidative phosphorylation. For the translation of RNA in mitochondrial protein synthesis system, 22mt-tRNA produced by mtDNA are required. The unique structure of mt-tRNAs distinguishes them from cytoplasmic tRNAs. Coding Mt-tRNA genes are highly mutatable and are regarded as the main factors causing malfunction of the mitochondria [15-17]. Knowing that one or more mutations accompany a disease paves the path of preventing the risk of the mentioned disease incidence [18, 19]. Officially, 32 mutations have been reported in genes relevant to mt-tRNA, of which only two have been specified to be pertinent to CAD [20]. In another analysis conducted on patients with congenital heart disease, 65 mitochondrial mutations were found while 13 of them were quite new ones [21]. However, by far, few statistical studies have been conducted to test the association of the mentioned mutations with heart diseases. Zidong Jia et al. (2013) can be considered as the first group of researchers examining the relationship between the point mutation known as tRNA-Thr 15927 and CAD disease. They focused their study on four generations of a Chinese family, in which 13 out of 32 adults suffered from congenital CAD. It was found that a mutation from G to A (G → A) took place at 15927 region of the amino acid threonine tRNA gene. As a result of the mentioned mutation, reduction in the efficiency occurs in the amino acetylated tRNA-Thr, which ultimately reduces 53% of mitochondrial translation in the mutant cells [22]. In this regard, the aim of the present study was to investigate the association of RNA-Thr15927 single nucleotide mutation with CAD disease in Iran's statistical population.

MATERIALS AND METHODS

To investigate the association of tRNA Thr 15927G → A mutation with CAD disease, individuals whose disease were proved employing WHO indices were chosen. Fifty patients with CAD were included in the patient group, and fifty healthy participants were selected for the control group. For this study, all individuals filled a written informed consent form. Both males and females with an average age of 58-59 years and all above 40 years of age took part in the study. 5ml of blood was drawn from each person and kept in tubes containing EDTA at -20° c for the intended tests to be performed on them. First, a standard column kit was used to extract DNA from the samples. Then, in order to verify the quality of the extracted DNA, its purity was approved, using gel electrophoresis and NanoDrop device. The obtained OD spectrum (using NanoDrop device) at a wave length of 260nm to280nm was between 1/77 and 1/90. For a pure sample of DNA, the ratio should be about 1/8. In the next step, a primer was designed with the implementation of Primer3Plus and OligoAnalizer softwares to perform PCR-RFLP and PCR-ARMS techniques. Regarding PCR-ARMS, two forward primers, i.e. TACGGYTACCTTGTTACGAC and TACACCAGTCTTGTAAACCA and one reverse primer, i.e. TGGCAGTAATGTACGAAATAC were employed. PCR product along with the mentioned primers made a fragment with 202 base pair length. To perform PCR-RFLP, forward primer called ATCATTGGACAAGTAGCATC and reverse primer known as TGGCAGTAATGTACGAAATAC were employed. As a result of these two primers' patterning, the PCR product had the length of 319 base pair. In the application of PCR-RFLP technique, after implementation of PCR, enzymatic digestion of the amplified fragment was done, then it was transferred onto the gel for verification of the band pattern. To address this objective, HpaII enzyme with the CCGG recognition sequence was used in this study. If the under investigation individual has nucleotide A in his 15927 mtDNA, only a 319 base pair fragment will be present after enzyme digestion by *Hpa*II enzyme as sequencing of a CCGG enzyme has not taken place and no incision has been performed. However, if the individual in his 15927 mtDNA has nucleotide G, two 135 and 184 base pair fragments will be present after enzyme digestion by *Hpa*II enzyme. In the last step and for the final confirmation of the presence or absence of tRNA Thr 15927G→A mutation, the ARMS technique was implemented on samples. In the application of this technique, if nucleotide G is in the region of SNP, TACACCAGTCTTGTAAACCG forward primer will yield product in PCR reaction, and if nucleotide G is in the region of SNP,

TACACCAGTCTTGTAAACCA forward primer will yield product in PCR reaction. At the end, for ultimate confirmation of the results obtained through the implementation of the two methods, 4 samples were quite randomly selected and sent for sequencing the intended region. The accuracy of the results was ensured after examining the received results and checking their compliance with ARMS and RFLP results.

RESULTS

Considering the results obtained by performing agarose gel electrophoresis of PCR product, it was found that out of 100 involved cases,12 had polymorphism A, 6 of which belonged to the patient group and the other 6 were members of control group.

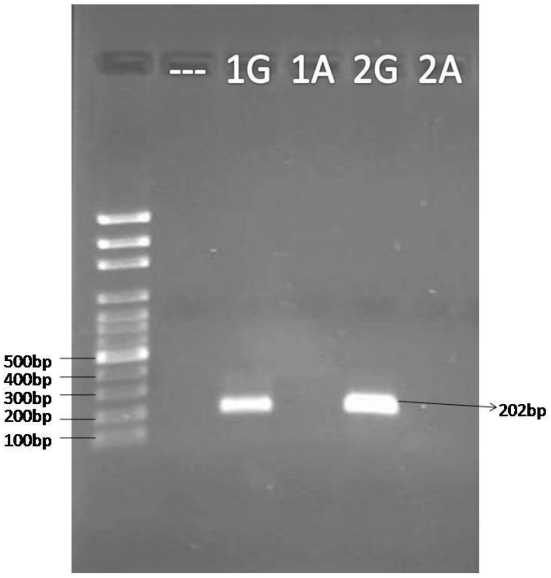

Figure 1. Electrophoresis of PCR product in ARMS technique is negative with no band, which indicates the authenticity of the study and the absence of contamination in the product 1G and 2G present samples having forward primer, which amplify the strand containing G allele. 1A and 2A are samples having forward primer, amplifying the strand containing allele A. Reverse primer is similar for both samples. In this figure, both of the investigated samples have allele G.

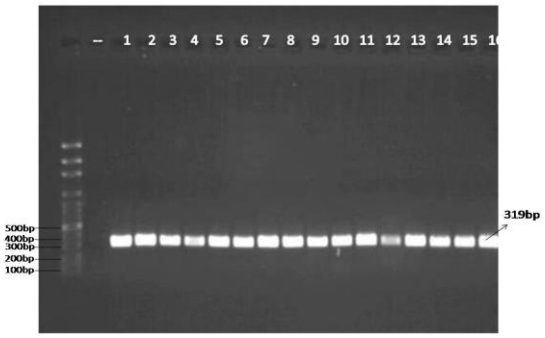

Figure2. Electrophoresis of PCR product in RFLP technique. is negative with no band, which indicates the authenticity of the study and the absence of contamination in the product.1 to16 contain PCR product of 16 involved cases, and single band was observed in all cases, which reveals the accuracy of the study and appropriate performance of the designed pair primers.

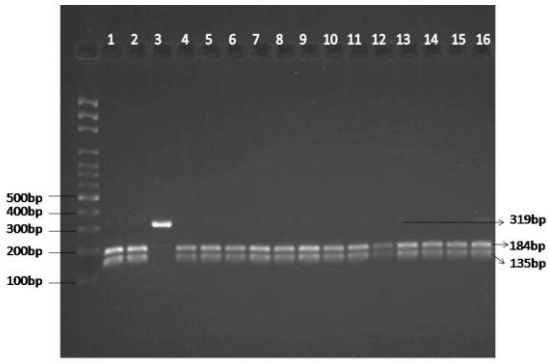

Figure 3. Electrophoresis of enzyme-digested product in RFLP technique. These are the same 16 cases, which were mentioned in the previous figure. After enzyme digestion by *Hpa*II enzyme, only sample 3 had allele A, which was not identified and cut by the enzyme. The remaining 15 samples all had allele G, which were identified and cut by the *Hpa*II enzyme and were shown as two bands in the figure.

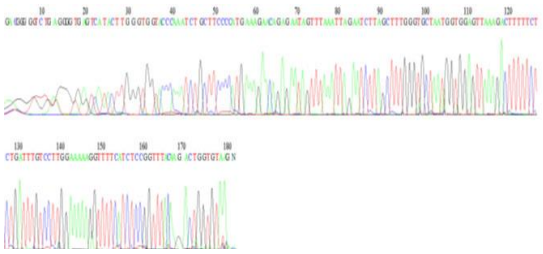

Figure 4. Sequencing Results: The presented nucleotide is target polymorphisms; here, it is nucleotide G.

DISCUSSION

Deposition of fatty substances on the inner lining of the coronary arteries is the major cause of heart embolism; in fact, CAD occurs due to the accumulation of atherosclerotic plaques in the coronary arteries. As a result of the accumulation of atherosclerotic plaques in coronary arteries, blood and eventually oxygen supply to the heart decrease. Severe reduction in the blood flow to the heart, due to CAD disease, may lead to the loss of heart cell activities, and if this trend continues, heart cells die. This phenomenon is known as a myocardial infarction or heart attack. CAD and MI can be considered as the main causes of death all over the world. According to the World Health Organization, by 2030 more than 23 million people will die as a result of heart diseases. In this regard, many countries such as America have allocated a billion-dollar budget for conducting prevention and treatment studies with respect to heart diseases. So far, a good number of the studies have been conducted addressing association of genetics with susceptibility to CAD with a focus on the nuclear genome. Ozaki et al.(2002) [23] using 92,788 gene-based SNP markers have conducted the first genome wide genomic association study in the world with regard to this disease. Their target population was Japanese patients. The obtained results revealed that two SNPs in *lenfotoksin* A (LTA) in 6p21 locus were significantly associated with the increased risk of MI in the Japanese population [23]. In another study, Helgadottir et al. (2007) [24] focused on 4587patient cases (positive angiography) and 12767 control cases; they determined the genotype of 305,953 cases. All the studied cases were of European ancestry. For the first time, the obtained results demonstrated a strong association between CAD and variants in 9p21 locus. They revealed that allele G in rs10757278 polymorphism had strong relationship with MI. In another research, McPherson et al.(2007) [25] identified two SNPs (rs10757274 and rs2383206) on chromosomal region 9p21, which were associated with 15 to 20% increased risk of CAD in 50% of heterozygous cases, and 40% increased risk in 25% of homozygous cases. Moreover, in the study carried out by the Consortium WTCCC involving 1926 patient cases and 2938 control cases, significant association was observed between SNPs of chromosome 9p21 and CAD (Burton et al., 2007) [26]. In another study involving 875 patients and 1644 healthy German participants using Affymetrix kit, the strongest association was noticed between CAD and rs1333049 locus. [27]. A number of studies have also been conducted addressing haplotype determination and their association with CAD disease. The first study with respect to haplotypes was carried out by WTCCC group examining MI on the German population, in which SLC22A3-LPAL2-LPA gene cluster on chromosome 6q26-27 was identified as the potential locus for CAD (OR = 1.8 [28]. Another study was conducted in China to investigate whether mitochondrial DNA defects were associated with the risk of CHD. A systematic and continuous screening of mitochondrial mutations was conducted on a large group of Chinese patients with CHD. In their mitochondrial DNA mutation analysis, a G→A 15927 tRNAThr mutation was recognized (88). This G→ A 15927 tRNA Thr mutation was observed in the anticodon stem of the tRNAThr protected region [29 ,30].

CONCLUSION

ARMS and RFLP techniques were employed in this study and sequencing was used to confirm the results. As no significant difference was observed between the two studied groups with respect to the prevalence of alleles A and G, and P_{val}< 0/5 was obtained using SPSS(Ver. 15), the present study reported no association between the mentioned polymorphism and coronary artery disease(CAD).

"The authors declare no conflict of interest

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