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ORIGINAL ARTICLE

Study of Genetic Polymorphisms of MTHFR C677T as a cause of Cardiovascular Disease in the Population of Tabriz, Iran

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ABSTRACT
In this study, 208 patients without clinical signs of cardiovascular disease and a history of venous thrombosis in different parts of the city of Tabriz, Iran were studied with different races. In this study, the distribution of the mutant allele MTHFR C677T in different breeds of Tabriz, Iran has been investigated and the results are compared with studies. To assess the distribution of polymorphisms of MTHFR C677T, one of the genetic factors of cardiovascular diseases (CVD), Of Tabriz in reverse hybridization method was used for the rapid and accurate diagnosis. By Multiplex PCR and hybridization analysis on the test strip tape parallel lines oligonucleotide probes containing allele-specific. The allele frequencies of MTHFR C677T mutation in the 0/24 investigation with results reported by Gulbahar and colleagues from Iran (0/24) corresponded perfectly. The frequency of MTHFR C677T mutation in less than Europe, but most of the frequency reported from India and most countries in the region.
Keywords: *venous thrombosis, polymorphism, methylene tetrahydrofolate reductase, Tabriz, Iran*

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INTRODUCTION

Cardiovascular disease refers to a group of multiple failure in which the heart, arteries and veins lose their normal function. Among these diseases, thrombosis IV, the third most common cardiovascular disease in the world that involves the 1 year 1 in 1,000 people [1]. The main route of the coagulation cascade protective precise regulation of the interaction between the components of the vessel wall, platelets and plasma proteins in blood loss limit is [2]. The lack of regulation of the activity of this cascade may lead to the formation of arterial and venous thrombosis and life-threatening event. Venous thrombosis may be the result of a genetic disorder or as a result of changes in the coagulation process or the interaction between the two [3]. These complications are a major cause of death in developed countries and a major cause of morbidity and mortality during pregnancy, childhood malignancies [4]. Methylation of homocysteine to methionine by the enzyme 5-methylene tetrahydrofolate reductase 10 (MTHFR; 1p36.3) catalyzes the highest form of folate in the blood circulation. The DNA sequence of this enzyme is 2.2 kb in length, contains 11 exons [5]. Figure 5-methyl folate plays a role in the transfer of carbon that is part of the various processes, such as making nucleotides, making the S-adenosylmethionine, methylation of homocysteine to methionine, methylation of DNA, proteins, neurotransmitters and phospholipids [6]. MTHFR activity to maintain normal folate and methionine help blood circulation and prevents the formation of homocysteine. Abnormalities of fetal structural abnormalities in the metabolism of folate can lead to toxic metabolites and toxic homocysteine methylation and inadequacy direct role in this [7].

MTHFR gene polymorphisms of enzymes that are involved in the operation, are well known. Two common polymorphism C677T MTHFR gene are and A1298C. Mutation C677T (alanine, valine ←) to produce an unstable enzyme leads to an increase in homocysteine blood and venous thrombosis [8] . C677T allele encoding an enzyme that is active at 37°C and further reduced and therefore the

conventional enzyme is unstable. The results show that activity in homozygous MTHFR C677T, 50-60% at 37 ° C and 46 ° C at 65% compared with the control group decreased [9].
C677T mutation Ast12-10 known risk factor for cardiovascular disease. The individual homozygous for the polymorphism C677T and C677T and A1298C a compound heterozygous for two mutations may reduce the enzyme activity by more than 50% [8].
The results of several studies suggest that the risk of spina bifida in carriers homozygous C677T allele increases three times, and the child with the parent homozygous lead [13,14]. Gene polymorphisms C677T and A1298C MTHFR, due to their role in the metabolism of folate and homocysteine, a risk factor for recurrent spontaneous abortion of the fetus in early pregnancy (6 to 20 weeks) is considered. High incidence of spontaneous abortion fetal genotype of MTHFR mutant protective role of folic acid supplements before pregnancy, more clear [15]. The relationship between environmental factors and cardiovascular disease has been examined in several times, but the role of genetic factors in the pathogenesis of these diseases as the major cause is still unknown in Iran. Very few studies on the prevalence of C677T MTHFR mutation in the population there. The purpose of this study was to determine the frequency of polymorphisms in the population is referred to as genetic factors influencing cardiovascular diseases specified in Tabriz Epidemiologic data also provide for future studies.

MATERIALS AND METHODS

In a recent study, 208 healthy individuals without symptoms of cardiovascular disease and a history of venous thrombosis, including 103 men and 105 women, aged 18 to 55 years, holding the center of the city of Tabriz purify the blood and cancer genetics respectively. Individuals were selected in such a way that all the geographic areas covered in Tabriz. Peripheral blood samples were obtained from mixed with sodium EDTA. Using isolated by phenol-chloroform and ethanol, DNA was extracted from the genome. The carriers were not detected by reverse hybridization method in which the kit CVD Strip Assay (ViennaLab Austria) was used. The new kit for the detection of common mutations in different populations is designed. In this method, a mutation can be examined simultaneously. Different gene sequences by PCR Multiplex propagated and amplified by a process linked to biotin. The PCR reaction consisted of / 10 mg of genomic DNA is mixed with 15 ml of PCR amplification is already initialized, it will be added. This mix includes primers that target sequences identified, dNTPs and Taq polymerase is an enzyme unit. The distribution terms are as follows: initial denaturation step at 94 ° C for 2 min, followed by 35 cycles of amplification (denaturation at 94 °C for 15 seconds, 30 seconds, annealing at 58 ° C and 30 s extension at 72 °C) and a final extension at 72 ° C for 3 min. The amplified products were denatured and test strips were hybridized individually. The allele-specific oligonucleotide probes containing bar (wild type and mutant) have been established in parallel on tape. Biotin with streptavidin-alkaline phosphatase method sequences attached to the substrate color was detected. Hardy-Weinberg equilibrium was assessed by a careful examination of the program Arlequin [16].

RESULTS

208 people from different parts of the city of Tabriz with different breeds were studied. Polymorphism genotype and allele frequencies of the different races Mekhlef points 1 and 2 are listed in the table. The most common allele was seen in the recent review of the MTHFR C677T, the frequency was 75%. Homozygous for the mutant genotype frequency of the MTHFR C677T 7/6%, respectively. Generally, the frequency of the mutant allele T 'in the West, South, West, North and Central Iran, 32%, 25%, 27% and 22% were determined (Table 1). Gulf of different races, Lor, said, Gilaki, Baluchi, Azeri (AST) and studied Arabic, mutated allele T 'in the race lor, Persian and Gilaki had the highest prevalence (30%, 29% and 27 %) and Baloch race and had the lowest frequencies (respectively 11% and 19%) (table 2). Hardy-Weinberg equilibrium was studied population.

Table 1: genotypes (%) MTHFR C677T polymorphism and allele frequency in different parts of Iran.

Sum	West	South West	South East	South	North West	North East	North	Center	Genotype
57/2	72/4	48/7	60/7	100	58/8	23/1	48/3	64/5	**CC**
36/1	20/7	38/5	39/3	-	41/2	53/8	48/3	25/8	**CT**
6/7	6/9	12/8	-	-	-	23/1	3/4	9/7	**TT**
0/753	0/680	0/917	0/804	1	0/794	0/5	0/725	0/774	**C**
0/247	0/320	0/256	0/196	-	0/206	0/5	0/275	0/226	**T**

Table 2 genotypes (%) MTHFR C677T polymorphism and allele frequencies in different ethnic population.

Sum	Arab	Gilaks	Baloch	Kourd	Lor	Azeri	Persian	Genotype
57/2	52/6	50/0	60/9	77/8	61/1	58/6	53/3	**CC**
36/1	42/1	46/2	39/1	22/2	16/7	41/4	36/0	**CT**
6/7	5/3	3/8	-	-	22/2	-	10/7	**TT**
0/753	0/737	0/731	0/805	0/889	0/695	0/793	0/713	**C**
0/247	0/263	0/269	0/195	0/111	0/305	0/207	0/287	**T**

DISCUSSION

Sudden onset of cardiovascular disease and a major health problem in developing countries, such as Iran. With a 39% incidence of such diseases is the leading cause of death in 8. Only in America every year more than 300,000 people die from sudden cardiac causes. Although the techniques have evolved and improved cardiovascular health its incidence is still increasing trend [8]. MTHFR C677T, Factor 5 Leiden and prothrombin G20210A, with a frequency of 5%, 10% and 3% heterozygous carriers, three are the most common causes of thrombophilia polymorphism in Caucasian populations are known. The three polymorphisms of the derivation of species of Asian Caucasian population, ie about 20,000 to 34,000 years ago, have been formed. FV mutation prevalence in the general population of 2 to 14% have been reported on the basis of racial and geographical differences that result from genetic drift[8], immigrant[9] population or the natural choice, different [17]. Polymorphisms of MTHFR, due to an increase in plasma homocysteine, folate, particularly when the level is below average, causing premature cardiovascular disease in homozygous carriers of the C677T 18. As can be seen in Table 3, the high frequency of MTHFR C677T allele in this study (0/24) similar to the frequencies reported from Turkey and Azerbaijan, but almost double the frequencies reported from Pakistan, India and Saudi Arabia [19-22] .

Allele frequency		Population (Reference)
T	C	
%40	%60	**(29)** Greece
%11	%89	**(30)** Lebanon
%25	%75	**(21)** Turkey
%23	%77	**(21)** Azerbaijan
%16	%84	**(20)** Pakistan
%4	%60	**(30)** Arabia
%2	%98	**(30)** Bahrain
%14	%86	**(22)** India
%40	%60	**(22)** East Asia
%25	%75	Tabriz, Iran

Table 3: MTHFR C677T polymorphism and allele frequency in different populations with Iran.

Results obtained in this study with the allele frequencies of MTHFR C677T allele frequency 0/24, obtained from 391 healthy individuals from the population (Gulbahar et al. 2005) in perfect harmony. Many researchers have examined the prevalence of MTHFR C677T alleles in different populations have. Among other Asian countries have the lowest rate of black Africa (52%), African Americans (0/11) and the Brazilian Indians (0/14) reported. The highest values of the Columbia (0/487), Italy (0/40) and Greece (0/40), respectively. C677T allele distribution in the Arab population reported from different countries including Lebanon (0/11), Tunisia (0/09), Bahrain (0.02) and Saudi Arabia (0.04).

Recently published reports indicate that the association between MTHFR C677T allele in homozygous state and the incidence of heart attack, even in young people with the normal function of the coronary arteries. In a recent study of the prevalence of MTHFR C677T mutation in the study population% homozygosity for 6/7 the table (1). The frequency of MTHFR C677T gene mutation in America homozygosity % 10-13 / 1, Canada, 10-14%, China% 12 / 3-12 / 8, Slovakia% 5 / 8-12 / 1, Europe (10-14%), Pakistan% 3/1 and 3% have been reported in India. Despite the relatively high frequency of allele MTHFR C677T, especially in homozygous state in population, to determine whether this polymorphism in patients who are at increased risk of venous thrombosis and plasma homocysteine levels are higher than normal as well as cancer patients, especially those with low serum folate levels , it seems. Laboratory detection of polymorphisms in individual susceptibility to venous thrombosis are usually diagnosed on the basis of Each polymorphism separately with simple procedures followed by enzymatic digestion or PCR is used to direct the production. Reverse hybridization method using a commercial kit, checking 12

different common polymorphisms in cardiovascular diseases simultaneously provides. In fact, in this way, amplification and detection for all desired sequences can be done simultaneously. The method is rapid, practical and relatively automatic and accurate results in less than 6 hours to arrive. The results of this method with other diagnostic tests to confirm the accuracy of this method.

CONCLUSION

The data reported in this study and in the neighboring countries of Iran, Turkey, Azerbaijan, Pakistan and Arabic countries, it seems that there is a reduction in the frequency of the West to the East. Due to higher allele frequencies of MTHFR C677T mutation in the population of the city of Tabriz, Iran, in comparison with the surrounding countries, it seems that the association of MTHFR C677T with inherited risk factors such as disease Cardiovascular, arterial and venous thrombosis, is relatively high and the need for further studies to determine the association between MTHFR C677T polymorphism with these diseases in our population.

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