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The Effect of MTHFR Gene C677T Polymorphism on the Diagnosis of St-Elevation Myocardial Infarction

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Study Area: Ahvaz, Iran

Coordinates: 31°19'13"N; 48°40'09"E

Keywords: Hypertension, Methylenetetrahydrofolate Reductase, Cardiovascular disease, CVD

The study protocol was reviewed and approved by the Human Research Ethics Committees (Project No. CVRC-971 and Ethic code: IR.AJUMS.REC.1397.597).

Introduction:

One of the most cosmopolitan cardiovascular diseases in the world is MI (Ezzat *et al.*, 2014; Haybar *et al.*, 2019). It plays an important role in emerging clinical problems and mortality (Slama *et al.*, 2014). It has been shown that thrombotic occlusion of coronary arteries due to atherosclerotic plaque disruption causes MI and activates coagulation processes (Uçar *et al.*, 2011). The complex pathogenic mechanism of MI involves the interaction of genetic factors and multiple environmental influences (Dogra *et al.*, 2012; Haybar & Zayeri, 2017). It has been reported that about 50% of the total risk of cardiovascular diseases in patients is dependent on the environmental factors including diet, dyslipidemia, obesity, hypertension, inflammation, diabetes, smoking, and alcohol consumption (Ezzat *et al.*, 2014; Onrat *et al.*, 2012; Nicoll *et al.*, 2015; Haybar *et al.*, 2018). Genetic susceptibility due to mutations and polymorphisms in a variety of genes can affect the regulation of blood pressure, blood coagulation, and metabolism of lipids, glucose, or homocysteine (Onrat *et al.*, 2012). Consequently, genetic factor was reported to be one of the remaining risk factors (Isordia-Salas *et al.*, 2010).

Abstract

Diverse studies have attempted to identify the association between Methylenetetrahydrofolate Reductase (MTHFR) gene C677T polymorphism and myocardial infarction (MI) which finally remain inconclusive. We aimed to investigate the possible associations between C677T polymorphism and ST-elevation myocardial infarction (STEMI). Study was conducted by considering case-control as 78 patients with STEMI and 77 other participants with no cardiovascular disease (CVD), ages 30 and higher. A polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) was performed to determine the C677T polymorphism in all participants. Moreover, a questionnaire was used to assess the demographic and clinical characteristics of all participants. This study showed no significant relationship between MTHFR gene C677T polymorphism and STEMI. However, a highly significant association was also evidenced in between this type of mutation and hypertension. The results of this study showed no significant relationship between MTHFR gene C677T mutation and MI risk. But the MTHFR gene C677T mutation polymorphism showed a significant relationship with hypertension.

The mechanism of vascular injury caused by the elevated homocysteine may include the following: promoting vascular remodeling, inhibition of endothelial growth, and post-injury repair induced endothelial dysfunction, as well as inflammatory mononuclear differentiation. The common causes of elevated homocysteine are folic acid, heredity, chronic renal insufficiency, vitamins B12 and B6 deficiency, and lifestyle factors. However, in developing countries such as in Northeast China, the data on genetic inheritance remains controversial and needs further discussion (Nicoll *et al.*, 2015).

Additionally, as per Haybar *et al.* (2018), reduction in enzyme activity results in hyperhomocysteinemia and is considered to be an independent risk factor for stroke, cardiovascular disease, as well as hypertension and venous thrombosis, including hypertension in pregnancy.

Mutation in the MTHFR gene associated with hyperhomocysteinemia and, in turn, the increased risk of MI, has received special attention in various studies (Slama *et al.*, 2014). MTHFR is a key enzyme in the homocysteine metabolism that converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, (which converts

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homocysteine to methionine (Rodwell *et al.*, 2015). To convert homocysteine to methionine, the effective form of folate in plasma that contributes to this process is 5-Methylenetetrahydrofolate, which is formed by the reduction of 5,10-methylenetetrahydrofolate (Zeng *et al.*, 2011; Gellekink *et al.*, 2005). A mutation in the MTHFR gene (resulting in an accumulation of 5,10-methylenetetrahydrofolate), as well as a reduction in Folic acid, severe deficiency in vitamin B12 and methionine, lead to hyperhomocysteinemia (Rodwell *et al.*, 2015). In another work, Lupi-Herrera *et al.* (2019) showed that the elevated homocysteine levels in the presence of the T allele in the C677T gene are associated with the acute myocardial infarction and massive and submassive pulmonary embolism.

Some previous studies have reported an association between the MTHFR C677T polymorphism and the risk of myocardial infarction (Ghazouani *et al.*, 2009; İlhan *et al.*, 2009; Güleç *et al.*, 2001). However, a few other studies did not report such a relationship (Rallidis *et al.*, 2008; Guerzoni *et al.*, 2009; Alam *et al.*, 2008). Since these findings are conflicting, then, the present study aims to investigate the possible association between C677T polymorphism and STEMI.

Materials and methods:

In this case-control study, 80 patients diagnosed with STEMI were categorized as the case group and 80 other additional participants with no CVD record were considered as the control group. Two and three individuals (orderly from the two groups) were eliminated from the study due to their unreadable slides. The remaining patients and participants were distinguished as 125 males and 30 females. They were randomly recruited from Golestan and Imam Khomeini hospital (located in the city of Ahvaz, Iran), between April 2018 and March 2019. The total number of patients in the case group was selected among those with STEMI; the age of 30 and higher. The control group was randomly selected from individuals having no record of cardiovascular disease, a family history of cardiovascular disease, and antihypertensive medication. Electrocardiography (EKG) and troponin tests were used to diagnose STEMI at zero and six hours. All patients were engaged in a careful measure of their blood pressure, while in the case group, the collected blood samples included cardiac and liver enzymes, lipid profile, blood glucose, BUN, Cr, and MTHFR gene mutation. A questionnaire including details of smoking, medication, personal, and family history was prepared and completed for each patient. The written informed consent was obtained from all subjects before their enrollment in this study.

To evaluate the MTHFR polymorphism, DNA was extracted by the salting-out method using the Qiagen kit (Hilden, Germany). The DNA purity was then determined

according to its absorption at 260-280 nm wavelengths. The acceptable ratio for DNA was 8.1 ± 1.0 . A fragment encompassing the region around nucleotide was amplified by the PCR with the 5'-TGAAGGAGAAAGGTGTCTGCGGGA-3', and 5'-AGGACGGTGCGGTGAGAGTG-3' primers (Isordia-Salas *et al.*, 2010). The PCR fragmented products that were prepared on 1.5% agarose gel (Sinaclone, Iran) were analyzed using electrophoresis (Fig.-1). The higher bands were digested with HinfI and Buffer Tango enzymes (Sinaclone, Iran) over 24 hrs at a temperature of 37°C. The analysis of the digested PCR products (Fig.-2) was electrophoresed on 2.0% agarose gel (Sinaclone, Iran) and visualized under ultraviolet light. Using genotyping PCR, the information was copied into an Excel file.

In this study, the continuous data were expressed as the Mean $\pm$ SD while the categorical data were expressed according to their frequency and percentage. The total number of patients or participants in each group was selected according to the Cochran formula. This formula allows the least number of patients or participants in each group to be equal to 60 (in this work, a total number of 80 individuals were selected in each group). The main tests performed for the assessment of normality were Kolmogorov-Smirnov (K-S) and quantile-quantile (Q-Q) diagrams. For the univariate analysis, t-test, chi-square, and Fisher's exact tests were employed. Multivariable analysis was performed by logistic regression and ordinal regression. P-values of <0.05 were considered as statistically significant. All statistical analyses were performed using the SPSS statistical software package v. 22.

Results:

The baseline information of the case and control groups are shown in Table-1. The population study included 78 patients at the ages of 30 and higher, diagnosed with STEMI, and 77 participants with no record of CVD. Among these, 125 individuals were male (group) category, while the remaining 30 were of the female group. Results indicated that the body mass index had no significant relationship with STEMI ($p>0.704$). However, in the univariable analysis, the incidence of risk factors among the patients with STEMI was high; that is, smoking (38.5 vs. 5.2%, OR 11.41; 95% CI 3.78-34.44, $p<0.001$), familial history of cardiovascular disease (24.4 vs. 1.3%, OR 24.48; 95% CI 3.18-188.12, $p<0.001$), diabetes mellitus (21.8 vs. 3.9%, OR 6.87; 95% CI 1.92-24.56, $p<0.001$), dyslipidemia (30.8 vs. 13% OR 2.98; 95% CI 1.31-6.76, $p<0.007$), and hypertension (65.4 vs. 49.4%, OR 1.94; 95% CI 1.023-3.70, $P=0.044$). In multivariable analysis, the independent risk factors for STEMI were found to be as follows: smoking (OR 10.52; 95% CI 2.79-39.71, $p<0.001$), familial history of cardiovascular disease (OR 27.98; 95% CI 2.16-362.01, $p<0.011$), and dyslipidemia (OR 4.89; 95% CI 1.56-15.36, $p<0.007$). In the control and case groups, orderly 43 (58.8%) and 50 (64.1%)

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Table-1: Demographic and clinical characteristics of participants in the case-control study

#	Control gr.	Case gr.	OR (95%CI)	OR(95%CI)
Number	77	78		
Age (M±SD)	Univariable (p<0.001) Multivariable (p<0.001)			
	48.18±8.98	56.74±8.98	1.69(1.38-2.08)	1.84(1.40-2.42)
Sex	Univariable (p<0.001) Multivariable (p<0.001)			
Male	67(87%)	58(74.4%)	Reference	Reference
Female	10(13%)	20(25.6%)	2.31(1.00-5.33)	1.98(0.62-6.31)
Smoking habit	Univariable (p<0.001) Multivariable (p<0.001)			
No	73(94.8%)	48(61.5%)	Reference	Reference
Yes	4(5.2%)	30(38.5%)	11.41(3.78-34.44)	10.52(2.79-39.71)
Family hist.: CVD	Univariable (p<0.001) Multivariable (p<0.001)			
No	76(98.7%)	59(75.6%)	Reference	Reference
Yes	1(1.3%)	19(24.4%)	24.48(3.18-188.12)	27.98(2.16-362)
Family hist.: DM	Univariable (p<0.001) Multivariable (p<0.055)			
No	74(96.1%)	61(78.2%)	Reference	Reference
Yes	3(3.9%)	17(21.8%)	6.87(1.92-24.56)	5.14(0.97-27.31)
Family hist.: DLP	Univariable (p<0.007) Multivariable (p<0.007)			
No	67(87%)	54(69.2%)	Reference	Reference
Yes	10(13%)	24(30.8%)	2.98(1.31-6.76)	4.89(1.56-15.36)
Family hist.: HTN	Univariable (p<0.044) Multivariable (p>0.062)			
No	39(50.6%)	27(34.6%)	Reference	Reference
Yes	38(49.4%)	51(65.4%)	1.94(1.023-3.70)	2.93(0.96-8.97)
Family hist.: MTHFR	Univariable (p>0.29) Multivariable (p>0.06)			
No	43(55.8%)	50(64.1%)	Reference	Reference
Yes	34(44.2%)	28(35.9%)	1.41(0.74-2.69)	2.92(0.95-9.00)
Family hist.: BMI	Univariable (p>0.704) Multivariable (p>0.852)			
(M±SD)	25.96±2.52	25.81±2.50	0.88(0.47-1.65)	0.92(0.37-2.29)

Table-2: Demographic and clinical characteristics of the patients with non-hypertension and hypertension

#	Non-Hyp.	Hyperten.	OR (95%CI)	OR(95%CI)
Number	66	89		
Age (M±SD)	Univariable (p>0.564) Multivariable (p>0.446)			
	51.95±10.19	52.89±9.76	1.05(0.9-1.23)	1.08(0.88-1.33)
Sex	Univariable (p>0.750) Multivariable (p>0.971)			
Male	54(81.8%)	71(79.8%)	Reference	Reference
Female	12(18.2%)	18(20.2%)	1.14(0.51-2.57)	1.02(0.38-2.72)
Smoking habit	Univariable (p>0.331) Multivariable (p>0.148)			
No	54(81.8%)	67(75.3%)	Reference	Reference
Yes	12(18.2%)	22(24.7%)	1.48(0.67-3.25)	2.0(0.78-5.13)
Family hist.: DM	Univariable (p>0.223) Multivariable (p>0.817)			
No	60(90.9%)	75(84.3%)	Reference	Reference
Yes	6(9.1%)	14(15.7%)	1.87(0.68-5.15)	1.16(0.33-4.11)
Family hist.: DLP	Univariable (p>0.172) Multivariable (p>0.305)			
No	55(83.3%)	66(74.2%)	Reference	Reference
Yes	11(16.7%)	23(25.8%)	1.74(0.78-3.89)	1.65(0.63-4.32)
Family hist.: MTHFR	Univariable (p<0.001) Multivariable (p<0.001)			
No	59(89.4%)	34(38.2%)	Reference	Reference
Yes	7(10.6%)	55(61.8%)	13.63(5.59-33.29)	16.51(6.44-42.37)
Family hist.: BMI	Univariable (p>0.187) Multivariable (p>0.074)			
(M±SD)	25.58±2.58	26.11±2.43	1.54(0.81-2.95)	2.12(0.93-4.82)

Figure-1: The PCR products of MTHFR (434 bp). 1) Normal Homozygote (CC) 392+41 bp. 2) Heterozygote (CT) 392+227+165+41 bp. 3) Mutant Homozygote (TT) 227+165+41 bp. 4) Normal Homozygote (CC) 392+41 bp

Figure-2: The analysis of the digested products of MTHFR C677T

individuals did not have any MTHFR gene mutation, although 34 (44.2%) and 28 (35.9%) patients in the corresponding groups, experienced this mutation. There was no statistically significant relationship between MTHFR gene C677T polymorphism mutation and STEMI (p<0.062).

Table-2 presents the demographic and clinical characteristics of all patients with non-hypertension and hypertension. In the univariable analysis, patients with MTHFR gene mutation (61.8, vs. 10.6%, OR 13.63; 95% CI 5.59-33.29, p<0.001) who had a higher blood pressure displayed a statistically significant relationship. In multivariable analysis, MTHFR showed a significant relationship with hypertension (OR 16.51; 95% CI 6.44-42.37, p<0.001). However, no significant correlation was found between hypertension and age, as well as sex, smoking, diabetes mellitus, dyslipidemia, and body mass index.

Discussion:

STEMI is the main thrombosis complication of CVD (Yusuf *et al.*, 2004). Furthermore, high levels of homocysteine have been accompanied by an increased risk for MI (Kölling *et al.*, 2004). Many factors such as nutrition and genetic effects have an impact on the level of homocysteine. Among these factors, the MTHFR gene mutation results in reduced enzyme activity and hyperhomocysteinemia (Onrat *et al.*, 2012). The present study was aimed to investigate the

association between MTHFR gene C677T polymorphism and STEMI. As per results, the relationship between the two was statistically insignificant. Previous studies have shown a relationship between MTHFR C677T and the increased risk for MI (Ghazouani *et al.*, 2009; Güleçl *et al.*, 2001; Kölling *et al.*, 2004), while some other surveys have not been able to identify this association (Isordia-Salas *et al.*, 2010; Hmimech *et al.*, 2016). This leads to an outcome that is still debatable. Isordia-Salas *et al.* (2010) conducted a case-control study to evaluate the association between MTHFR gene C677T polymorphism and STEMI in young Mexican people in which there was no interaction between the two, while the other factors such as smoking, family history of CVD, and dyslipidemia were indicated as the independent risk factors for STEMI. Their results are similar to the findings obtained in the present work. Kölling *et al.* (2004) concluded that MTHFR gene C677T polymorphism (but not A1298C polymorphism), is an independent risk factor that affects the plasma homocysteine levels. Additionally, they found that other factors such as age, plasma folate, vitamin B12, and serum creatinine were strongly associated with the plasma homocysteine levels. Their investigation also showed a significant relationship between MTHFR gene C677T polymorphism and low folate concentration. Kölling *et al.* (2004) conducted a review and meta-analysis survey to examine the association between MTHFR C677T as well as A1298C polymorphisms and found no significant relationship between the latter and the overall MI risk. However, they perceived an association between MTHFR C677T and MI in the African people. This association was absent in Asian, European, and North American populations. In a meta-analysis study, Xuanet *et al.* (.....?) revealed that the MTHFR C677T polymorphism is accompanied with the risk of MI in the young and middle-aged white people but not in the elderly males, females, South Asians, Caucasians, East Asians, and African-Americans. Consequently, it appears that several factors such as age, sample size, ethnicity, and environmental factors may cause these contrarities invariant studies.

In brief, hypertension is one of the risk factors that play an important role in the development of MI (Anand *et al.*, 2005; De Lorgeril *et al.*, 1999; Yusuf *et al.*, 2004). In the present work, the association between some risk factors and MTHFR with hypertension was investigated. Results showed that patients with MTHFR mutations had higher blood pressures. This association was also confirmed in previous studies (Yang *et al.*, 2014; Wu *et al.*, 2014). Furthermore, although the results of this work showed a significant relationship between MTHFR gene C677T polymorphism and hypertension, yet, no significant association was found between the MTHFR C677T and MI risk. Since various factors with different degrees of intensities may influence the occurrence of MI, then, from the genetics point of view, it is necessary to perform further surveys to investigate these correlations in more detail.

Acknowledgement:

The authors would like to thank all of the physicians and staff of Imam Khomeini and Golestan hospitals for their collaboration and assistance in this research.

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