

Methylenetetrahydrofolate Reductase (677C/T) Polymorphism in Myocardial Infarction and Hypertension

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ABSTRACT

Hyperhomocysteinemia is a well-established risk factor for cardiovascular disease and hypertension. This study aimed to assess the MTHFR gene polymorphisms (677C/T) as a potential genetic risk factor for hypertension and Myocardial Infarction (MI) in Egypt. Myocardial infarction and hypertensive diagnosed patients were divided into two groups; first comprised 50 patients aged < 45 years, second comprised 47 patient aged > 45 years and a third control group comprised 84. CT genotype was significantly higher in the first group (48.8%) versus control group (30%) and second group (29.4%) with (OR 2.35, 95% CI 1.3-4.2, p = 0.006). The percentage of TT genotype was 4.7, 11.8 and 15% in the three groups respectively. The percentage of MTHFR 677C→T polymorphism was significantly higher in patients with hypertension than normotensive. MTHFR 677C→T polymorphism in Egyptian MI and hypertensive patients has no direct role in developing such diseases but in combination with high levels of cholesterol, LDL-C and triglycerides might constitute a probable potentiating factor for MI and hypertension among Egyptian patients and could not be used as a predictor for early diagnosis without considering other factors.

Keywords: MTHFR 677C→T Gene Polymorphism, Hyperhomocysteinemia, Hypertension, Myocardial Infarction, Egyptian Patients

1. INTRODUCTION

Myocardial Infarction (MI) and Hypertension are well-known prevalent disorders among Egyptians. Inadequate data were published concerning Coronary Artery Disease (CAD) susceptibility in Egyptian patients in terms of their genetic background.

The aim of this work is to study the presence of MTHFR gene polymorphisms as a genetic risk factor for hypertension and MI among Egyptian patients.

Morbidity and mortality due to Essential Hypertension (ET) and CAD are significant more frequent in developing countries with associated several risk factors such as advancing age, male gender, hyperlipidemia, diabetes and insulin resistance (Laraqui *et al.*, 2007). Acute Myocardial Infarction (MI) among young adults is about 5 and 10% of

all MIs (Choudhury and Marsh, 1999; Doughty *et al.*, 2002). Young patients with managed MI are characterized by family history of CAD, heavy smoking and apparently normal angiographically (Zimmerman *et al.*, 1995; Panagiotakos *et al.*, 2007). MI in young patients is resulted from multiple pathogenetic mechanisms; coronary spasm, coronary embolism and thrombus formation resulted from non-obstructive plaque rupture and endothelial layer erosion separately or in combination (Kardasz and Caterina, 2007; Verheugt *et al.*, 1987; Romagnoli and Lanza, 2007).

MTHFR gene common polymorphism remains controversial in its atherosclerotic potential, although Hyperhomocysteinemia is being established as CAD risk factor (Choudhury and Marsh, 1999; Zimmerman *et al.*, 1995; Panagiotakos *et al.*, 2007; Kardasz and Caterina, 2007; Zee *et al.*, 2007; Ogawa *et al.*, 2003; Doughty *et al.*,

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2002). About 10 mutations in the MTHFR locus were identified and the most common missense mutation, a 677 C→T substitution (alanine to valine) resulting in reduced remethylation of homocysteine leading to a thermo labile enzyme variant which develops Hyperhomocysteinemia (Sadeghian *et al.*, 2006; Nikfardjam *et al.*, 2001; Payne *et al.*, 2001).

Many reported conflicting data regarding the association between the MTHFR 677C→T polymorphism and the risk of CAD (Zee *et al.*, 2007; Schmitz *et al.*, 1996; Jee *et al.*, 2000). A case-control meta-analysis showed a higher risk of CAD of individuals with the MTHFR 677TT genotype, while another meta-analysis is carried out later did not support this relation in North America, Australia and Europe, (Klerk *et al.*, 2002; Lewis *et al.*, 2005). Hyperhomocysteinemia due to MTHFR gene T allele homozygosity in hypertensive patients could be an independent risk factor for early atherosclerotic organ damage development (Ravera *et al.*, 2001).

2. MATERIALS AND METHODS

Patients were recruited from consecutive admission to the coronary care unit, Suez Canal University Hospital, Ismailia, Egypt. All prerequisites demanded by the Faculty research ethical committee were considered and fulfilled for our patients.

A-Priori test was performed to identify the requested numbers of subjects in each group using anticipated effect size (Cohen's d) (0.5), Desired statistical power level (0.8) and Probability level (0.02). The obtained calculations were Minimum total sample size (one-tailed hypothesis) (138), Minimum sample size per group (one-tailed hypothesis) (69); Minimum total sample size (two-tailed hypothesis) (164), Minimum sample size per group (two-tailed hypothesis) (82).

The recruited subjects (total 181) were subdivided into two main groups; patients (total 97) and control group (total 84). The patients group was subdivided into two main subgroups, the first group comprised (50) young adults aged < 45 years and the second one comprised (47) older adults aged ≥ 45 years.

On admission, the following data were fulfilled for all recruited groups: age, smoking, history of Diabetes Mellitus (DM) and Hypertension (HTN), family history of MI and stroke. Clinical examination: Blood pressure examination and Body Mass Index (BMI) calculation were carried out and for patient groups (1 and 2) a short outcome prognosis was done using left ventricular Ejection Fraction (EF). Routine laboratory investigations for all recruited groups including fasting and postprandial glucose level, Triglycerides, total Cholesterol, HDL-C and LDL-C were carried out using Hitachi 912 auto-analyzer (Roche Diagnostics GmbH, Str. Mannheim, Germany).

2.1. MTHFR (677C/T) Polymorphism Genotyping

Diagnosis of patients with acute MI was based on full history taking, clinical examination, resting ECG, cardiac enzymes and echocardiography. MI was defined either by presence of ST-Segment Elevation (STE) or depression (non-STE) at the J-point ≥1 mm in two or more contiguous leads, In leads V2 and V3, 2 mm of ST elevation in men and 1.5 mm in women was required, new left bundle branch block or development of pathologic Q and a rise and/or fall in cardiac troponin with at least one value above the 99th percentile of the upper reference limit (troponin I levels > 0.1 ng/ mL) and elevated CK-MB is enzyme (Tàssies *et al.*, 2009; Thygesen *et al.*, 2007).

Hyperlipidemia was defined as total Cholesterol >220 mg/dL, Triglycerides >150 mg/ dL or treatment with lipid lowering drugs. Diabetes was defined as patients with random glycemia >200 mg/ dL at admission or FBG >125 mg/dL in two determinations or receiving insulin or oral hypoglycemic drugs; hypertension was defined as repeated blood pressure ≥140/90 mmHg or previous treatment with antihypertensive drugs (Chobanian *et al.*, 2003).

Genomic DNA was extracted from peripheral blood leucocytes using silica membrane based DNA purification method (Qiamp DNA blood kit, Qiagen, Germany). Real time PCR Rotor-Gene™ 6000 (Corbett Research, Mortlake, NSW, 2137, Australia) was used for MTHFR (677C→T) genotyping.

Primers 5'-AAAGCGGAAGAATGTGTCAGC-3' and (5'-GACATCTGTGTGGCAGGTTACC-3') were used yielding 97bp amplicon.

Two TaqMan probes were designed and used (5'-TGATGATGAAATCGACTCCCGCAGA-FAM-3') for T-allele and (5'-TGATGATGAAATCGGCTCCCGC-TET-3') for C-allele. PCR reaction mixture consisted of 0.1µg of DNA; QPCR master mix provided from Qiagen, Germany was used. 400 nM from each primer and 150 nM from each probe were added in final 25 µl reaction volume. PCR cycles were optimized as follow: 95°C for 15min. for initial enzyme activation and DNA denaturing; 40 cycles (95°C for 15 sec., 58°C for 60 sec); final cycle 72°C for 10 min.

2.2. Statistical Analysis

Baseline characteristics of the study population were presented as frequencies and percentages (%) or mean values and Standard Deviations (SD). Age, smoking, history of DM, HTN, stroke were treated as categorized variables. Age, cholesterol, triglycerides, HDL, LDL, FBG, PPBG, BMI, EF, systolic and diastolic blood pressure were treated as continuous variables. Differences between frequencies in both groups were compared by Chi-square test when all expected values in 2×2 table > 5 or Fisher exact test when one of the expected values in 2×2 table <5. Differences between means in both groups were compared by Student's t-test.

Table 1a. Data characteristics among patients and control groups

Characteristic	Control (n = 84)	Patients (n = 97)	p-value
Smokers (%)	32 (38.1%)	64 (66%)	<0.0001**
Cholesterol (mg/dL)	177.96±26.34	240.12±72.7	<0.0001**
Triglycerides (mg/dL)	93.77±26.94	159.73±51.57	<0.0001**
HDL-C (mg/dL)	56.92±14.16	35.37±5.42	<0.0001**
LDL-C (mg/dL)	124.73±24.94	169.24±72.7	<0.0001**
Systolic BP (mmHg)	114.63±9.34	141.32±12.87	<0.0001**
Diastolic BP (mmHg)	77.92±9.79	89.11±8.47	<0.0001**
RBS	91.4±12.7	279.7±104.2	<0.0001**

**Significant p-value (2-tailed) ≤0.05

Table 1b. Data characteristics among younger and older patients

Characteristic	Younger age (n = 50)	Older age (n = 47)	P value
Smokers (%)	33 (66%)	31 (66%)	1.0000
DM (%)	26 (52%)	31 (66%)	0.1600
HTN (%)	29 (58%)	27 (57.4%)	1.0000
MI (%)	30 (60%)	32 (68.1%)	0.4100
Stroke (%)	0 (0%)	0 (0%)	1.0000
BMI	27.75±1.89	28.08±1.77	0.3800
Cholesterol (mg/dL)	266±85.58	212.6±41.54	0.0002**
Triglycerides (mg/dL)	178.10±49.96	140.19±46.21	0.0002**
HDL-C (mg/dL)	35.70±5.1	35.02±5.78	0.5400
LDL-C (mg/dL)	192.02±86.72	145±43.08	0.0012**
Systolic BP (mmHg)	138.85±9.8	143.83±15.08	0.0550
Diastolic BP (mmHg)	87.81±8.44	90.43±8.39	0.1300
FBS (mg/dL)	143.34±46.67	161.02±55.99	0.0940
PPBS (mg/dL)	220.80±83.21	250.23±98.18	0.1100
Ejection fraction	42.56±6.52	44.94±7.61	0.1000

**Significant p-value (2-tailed) ≤0.05

Differences between multiple groups were compared by Analysis Of Variance (ANOVA) test. A p value of <0.05 was considered significant. The Relative Risk (RR) for MI (estimated as Odds Ratios (OR) and 95% Confidence Intervals (CI)) for those homozygous for the TT allele was compared with the risk for those who carried the CC allele. With a total of 43 younger and 34 older cases, it is estimated that for a statistical power of 80%, OR ≥ 2 will be significant at the 5% level. Pearson correlation coefficient test was used after controlling of age variable to correlate the dependent variable (MTHFR 677C→T genotypes) and other independent variable (predictors). Logistic regression was performed to estimate the effect of the MTHFR 677C/T polymorphism. Logistic regression with adjustment for age and sex was performed on each variable to estimate OR and p-value. The Chi-square test was used to evaluate deviations of genotype distribution and Hardy-Weinberg equilibrium was tested for all groups.

3. RESULTS

The systolic and diastolic blood pressure, smoking, FBG, PPBG, Cholesterol, Triglycerides, HDL-C and LDL-C showed significant difference between patients groups and control group (**Table 1a**). Cholesterol, Triglycerides and LDL-C showed significant difference between young patients group and old patients group (**Table 1b**).

Genotyping of the control group and patients group showed wild CC allele 56 versus 58.8%, CT allele 35.7 versus 34% and TT allele 8.3 versus 7.2% respectively. While Genotyping of patients two subgroups showed younger patients group was significantly different than older patients group for both CT and TT alleles (OR 2.06, 95% CI 1.13-3.75, p = 0.02 and OR 7.17, 95% CI 0.83-61.01, p = 0.047 respectively) showing higher tendency for CT genotype in young group and TT genotype in older group, patients in young group with CT genotype 42 versus 25.5% in the older group and 35.7% while, patients in young group with TT genotype was 2 versus 6% in the older group and 8.3% in controls (**Table 2a-c and 3a, b**). Correlations between genotypes and other variables after controlling of age showed no significant correlation between MTHFR 677C→T polymorphism and DM, HTN, Cholesterol, TG, HDL-C, LDL-C, FBG, PPBG, SBP, DBP and smoking. Logistic regression analysis test with MTHFR 677C→T polymorphism as dependent variable showed that DM, HTN, Cholesterol, TG, HDL-C, LDL-C, FBG, PPBG, SBP, DBP and smoking were found to be insignificant independent risk factor for the development of MI. Percentage of MTHFR 677C→T polymorphism showed significant increase in patient with cholesterol and hypertension than in normotensive patients. However no significant difference was found in this polymorphism associated with other risk factors (**Table 4**). Hardy-Weinberg equilibrium showed consistent equilibrium at p>0.05 (**Table 5a-d**).

Table 2a. Genotype of MTHFR gene polymorphism among younger, older patients groups

Genotype	Younger age (n = 50)	Older age (n = 47)	p-value
Wild (CC)	28 (56%)	29 (61.7%)	0.57
Hetero (CT)	21 (42%)	12 (25.5%)	0.02*
Homo (TT)	1 (2%)	6 (12.8%)	0.047*

*Significant p-value (2-tailed) ≤ 0.05

Table 2b. Genotype of MTHFR gene polymorphism among younger patients and control groups

Genotype	Younger age (n = 50)	Control (n = 84)	p-value
Wild (CC)	28 (56%)	47 (56%)	1.00
Hetero (CT)	21 (42%)	30 (35.7%)	0.48
Homo (TT)	1 (2%)	7 (8.3%)	0.05*

*Significant p-value (2-tailed) ≤ 0.05

Table 2c. Genotype of MTHFR gene polymorphism among older patients and control groups

Genotype	Older age (n = 47)	Control (n = 84)	p-value
Wild (CC)	29 (61.7%)	47 (56%)	0.52
Hetero (CT)	12 (25.5%)	30 (35.7%)	0.23
Homo (TT)	6 (12.8%)	7 (8.3%)	0.54

Table 3a. Odds ratio of genotypes of MTHFR gene polymorphism among younger and older age patients groups

Genotype	Younger age (n = 50)	Older age (n = 47)	OR	CI		p-value
				Lower	Upper	
Wild (CC)	28 (56%)	29 (61.7%)	0.79	0.35	1.78	0.57
Hetero (CT)	21 (42%)	12 (25.5%)	2.06	1.13	3.75	0.02*
Homo (TT)	1 (2%)	6 (12.8%)	7.17	0.83	61.01	0.047*

*Significant p-value (2-tailed) ≤ 0.05

Table 3b. Odds ratio of genotypes of MTHFR gene polymorphism among patients and control groups

Genotype	Patients (n = 97)	Control (n = 84)	OR	CI		p-value
				Lower	Upper	
Wild (CC)	57 (58.8%)	47 (56%)	1.12	0.62	2.03	0.70
Hetero (CT)	33 (34%)	30 (35.7%)	0.93	0.50	1.71	0.81
Homo (TT)	7 (7.2%)	7 (8.3%)	0.86	0.29	2.54	0.78

Table 4. The percentage of MTHFR 677C→T polymorphism in DM, SBP, DBP, Total cholesterol and Triglycerides

		Wild (n = 57)		Hetero/Homo (n = 40)		p-value
		No.	(%)	No.	(%)	
DM	Non-Diabetic	25	43.9	15	37.5	0.53
	Diabetic	32	56.1	25	62.5	
SBP (mmHg)	<140	17	29.8	21	52.5	0.02*
	≥140	40	70.2	19	47.5	
DBP (mmHg)	<90	15	26.3	23	57.5	0.002*
	≥90	42	73.7	17	42.5	
Cholesterol (mg/dL)	<220	35	61.4	16	40.0	0.037*
	≥220	22	38.6	24	60.0	
Triglycerides (mg/dL)	<150	24	42.1	17	42.5	1.00
	≥150	33	57.9	23	57.5	

*Significant p-value (2-tailed) ≤ 0.05

Table 5a. Hardy-Weinberg equilibrium for patients group

Genotypes	Patients Observed #	Expected #	X ²	P value
Wild (CC)	57	57	0.39	0.82
Hetero (CT)	33	35		
Homo (TT)	7	5		

Consistent with Hardy-Weinberg equilibrium when $P > 0.05$

Table 5b. Hardy-Weinberg equilibrium

Genotypes	Control observed #	Expected #	X ²	P value
Wild (CC)	47	47	0.40	0.82
Hetero (CT)	30	32		
Homo (TT)	7	5		

Consistent with Hardy-Weinberg equilibrium when $P > 0.05$.

Table 5c. Hardy-Weinberg Allele Frequencies in Patients populations

Allele frequencies in patients populations					
	A (p)	a (q)	Total	X ²	P value
Observed	147	47	194		
Expected	149	45	194	0.81	0.91

Consistent with Hardy-Weinberg equilibrium when P> 0.05.

Table 5d. Hardy-Weinberg Allele Frequencies in Patients populations

Allele Frequencies in control populations					
	A (p)	a (q)	Total	X ²	P value
Observed	124	44	168		
Expected	126	42	168	0.81	0.90

Consistent with Hardy-Weinberg equilibrium

4. DISCUSSION

The present study aimed to assess the presence of Methylene tetrahydrofolate reductase 677C→T gene polymorphism and its relation to hypertension and Myocardial Infarction (MI) among Egyptian population. Genotyping of the control group and patients group showed wild CC allele 56 versus 58.8%, CT allele 35.7 versus 34% and TT allele 8.3 versus 7.2% respectively. Genotype of CT and TT alleles between patients subgroups showed younger patients group was significantly different than older patients group for both CT and TT alleles (OR 2.06, 95% CI 1.13-3.75, p = 0.02 and OR 7.17, 95% CI 0.83-61.01, p = 0.047 respectively) showing higher tendency for CT genotype in young group and TT genotype in older group indicating that younger patients with CT allele are more affected with predictors for both MI and hypertension than older group. In Greece patients with premature MI the prevalence of TT homozygosity compared to controls was (27.1 Vs. 14.6%) (Ravera *et al.*, 2001). In a Southern Texan heterozygosity and homozygosity alleles were 39 and 7%, respectively (Payne *et al.*, 2001), while (Thygesen *et al.*, 2007) meta analysis reported frequency of heterozygosity to be 34-59% and homozygosity to be 6-30% that came in match with current study results.

The current study showed significant higher prevalence of smoking, hypertension, DM, cholesterol, Triglycerides, HDL-C and LDL-C in MI and hypertensive patients than in controls which were in agreement with study carried out by another group in Greece (Ravera *et al.*, 2001)

Patients with the TT genotype showed higher baseline Diastolic Blood Pressure (DBP) than those with

the CC and CT genotypes (p = 0.018) (Xu *et al.*, 2012). Some studies found associations of TT homozygosity with premature CAD (Chobanian *et al.*, 2003; Rallidis *et al.*, 2008; Brattstrom *et al.*, 1998) while others were negative (Gallagher *et al.*, 1996; Mager *et al.*, 1999; Gulec *et al.*, 2001). Data from meta-analysis studies on cardiovascular disease risk assessment related to tHcy concentrations and MTHFR 677C→T genotypes are sometimes discordant. For example, a meta-analysis revealed that TT genotype was associated with a homocysteine concentration 25% higher than the CC genotype, but not with an increased risk of cardiovascular events (Thygesen *et al.*, 2007). Another (Schwartz *et al.*, 1997) meta-analysis reported an association between ischemic heart disease in European and 677C→T polymorphism but not detected in North American populations. Low dietary folate could aggravate the atherothrombotic potential effect of TT homozygosity by increasing the levels of homocysteine (Ravera *et al.*, 2001). These discrepancies might be due to methodological aspects; First, the definition of premature CAD varied across these studies since the cut-off age ranged from 45 (Rallidis *et al.*, 2008; Brattstrom *et al.*, 1998; Gallagher *et al.*, 1996) to 60 years (Mager *et al.*, 1999); Second, the studied populations had different ethnic or geographic origin, i.e., Irish (Chobanian *et al.*, 2003), Israeli Jews (Rallidis *et al.*, 2008), Turkish (Brattstrom *et al.*, 1998) suggesting that different nutritional habits might confound the results of genotype studies. While in Tunisia MTHFR gene C677T genotypes were not associated with MI in the Tunisian male population (Mourali *et al.*, 2012) and matches with report from Mexico stating it is not possible to establish an association between the 677CT polymorphism of the MTHFR gene and the presence of heart disease in the Mexican mestizo population (Sanchez-Urbina *et al.*, 2012).

The current study showed significant increase in the percentage of MTHFR 677C→T polymorphism (CT allele) in Egyptian young patients with MI and hypertension in comparison with old patients group but was insignificant compared to control group. This outcome might be explained by the inability of homocysteine remethylation yielding a thermo labile enzyme variant leading to Hyperhomocysteinemia which develops atherothrombosis (Panagiotakos *et al.*, 2007; Kardasz and Caterina, 2007; Christensen *et al.*, 1997). The European Concerted Action Project, a multicenter study of 750 patients with vascular disease and 800 control subjects, confirmed that Hyperhomocysteinemia is associated with an increased risk of vascular disease, however there was poor association between MTHFR 677C→T polymorphism and MI (Pinto *et al.*, 2001; Klerk *et al.*, 2002) which confirms this study results.

The TT genotype of the 677C/T MTHFR polymorphism is associated with Essential Hypertension (EH) and CAD. TT genotypes had higher plasma Hcy levels in CAD patients compared with CC and CT genotypes. The authors concluded that that MTHFR gene polymorphism is an independent risk factor for EH but not for CAD (Lentz, 2005).

This study showed that MTHFR 677C→T polymorphism among the Egyptian MI and hypertension patients was in accordance with the international percentage. Although no difference were detected among patients and controls but the existing CT genotype in combination with high levels of cholesterol, LDL-C and triglycerides might constitute a probable potentiating factor for MI and hypertension in younger patients while, the existing TT genotype in combination with high levels of cholesterol, LDL-C and triglycerides might constitute a probable potentiating factor in older patients.

5. CONCLUSION

MTHFR 677C→T polymorphism in Egyptian MI and hypertensive patients has no direct role in developing such diseases but in combination with high levels of cholesterol, LDL-C and triglycerides might constitute a probable potentiating factor for MI and hypertension among Egyptian patients and could not be used as a predictor for early diagnosis without considering the other factors.

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