

METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM, HOMOCYSTEINE AND FOLATE LEVELS IN YOUNG PATIENTS WITH MYOCARDIAL INFARCTION

*Dr. Mehmet Kanadaşı, Dr. Yurdaer Dönmez, Dr. Murat Çaylı, Dr. Mustafa Demirtaş, Dr. Kahraman Tanrıverdi, Dr. Mesut Demir, Dr. Cumhuri Alhan

Çukurova Üniversitesi Tıp Fakültesi Kardiyoloji ABD, *Çukurova Üniversitesi Tıp Fakültesi Hematoloji ABD , Adana

Bu çalışmada Türkiye'nin güney bölgesinde akut miyokard infarktüsü (AMİ) geçiren genç hastalarda metilentetrahidrofolat redüktaz (MTHFR) gen polimorfizmi, plazma homosistein ve folat düzeylerinin araştırılması amaçlandı.

55 yaştan küçük AMİ geçiren 96 hasta ve 77 sağlıklı birey çalışmaya alındı. Plazma lipid, homosistein, folat ve vitamin B12 düzeylerini ölçmek ve MTHFR gen polimorfizmini saptamak için açlık venöz kan örneği alındı. MTHFR genotipleri normal (CC), heterozigot (CT) ve homozigot (TT) olmak üzere üç gruba ayrıldı.

TT genotip sıklığı, plazma homosistein ve folat düzeyleri hasta ve kontrol gruplarında benzerdi. Plazma homosistein ve folat düzeyleri 4 çeyrek dilime ayrıldı. Plazma homosistein düzeyleri yüksek

olanlarda AMİ sıklığı anlamlı olarak artmıştı, fakat farklı folat seviyeleri ile AMİ sıklığı arasında anlamlı ilişki bulunmadı. Plazma homosistein seviyesinin 16.05 µmol/l'den fazla olmasının AMİ için anlamlı bir risk faktörü olduğu saptandı.

Bölgemizde MTHFR gen polimorfizmi ve plazma folat düzeyleri genç yaşta akut miyokard infarktüsü ile ilişkili değildir. Bununla birlikte, artmış plazma homosistein seviyesi genç yaşta akut miyokard infarktüs riskini arttırmaktadır.

Anahtar kelimeler: Metilentetrahidrofolat redüktaz, Homosistein, Folat, Akut miyokardiyal infarktüs

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INTRODUCTION

It is known that mild to moderately elevated plasma homocysteine (tHyc) level is an independent risk factor for early coronary artery disease (CAD)¹. Genetical and nutritional factors are the main determinant factors for the plasma tHyc level. One of the most important genetic factors that influences the plasma tHyc level is the 5', 10'-methylenetetrahydrofolate reductase (MTHFR) genotype². The enzyme MTHFR reduces 5', 10'- methylenetetrahydrofolate to 5'- methylenetetrahydrofolate, the main circulating form of folate, which is a cosubstrate in the remethylation of tHyc to methionine. It has been reported that mutation in MTHFR enzyme might be associated with the relative risk for acute myocardial infarction (AMI)³. However, the impact of this mutation on the incidence of AMI is reported to vary among different populations⁴. It has been shown that mutation of MTHFR gene is more significant in

populations with low plasma folate levels⁵. Although Turks have relatively low plasma cholesterol levels, the Turkish population has a high prevalence of CAD⁶. It has been shown that reduced plasma folate levels is a major cause of elevated plasma tHyc levels in the Turkish population⁷. We investigated the relationship of MTHFR enzyme genotype, and plasma tHyc and folate levels with the incidence of first AMI in patients aged <55 years in Southern Turkey.

MATERIAL and METHODS

Patients and controls: In this study, we included 96 patients aged <55 years who were admitted to the coronary care unit with AMI, as evidenced from the presence of typical chest pain and ST elevations on the presentation electrocardiogram and elevated cardiac enzymes on admission. Further, we also included a control group of 77 healthy subjects <55 years, randomly selected from the same population, and who did not have a history or clinical evidence of cardiovascular disease. All conventional coronary risk factors such as age, gender, hypertension (blood pressure >140/90 mmHg or prior antihypertensive therapy), diabetes mellitus (fasting blood glucose >126 mg/dl or oral

Adress for Correspondence: Murat Çaylı, MD
Çukurova University, School of Medicine, Department of Cardiology
01330 Balcalı/Adana
Tel: + 90 322 338 60 60/3191
Fax: + 90 322 338 70 17
E-mail: drçayli@yahoo.com

Table 1: Distribution of coronary risk factors between patient and control groups

	Control (n=77)	Patient(n=96)
Male/Female (n)	45/32	68/28
Age (year)	42.4±5.8	43.8±5.0
Smoking (n)	30	46
Hypertension (n)	11	31*
Family history of CAD	13	40*
Total cholesterol, mg/dl	194.5±34.7	204.8±47.7
LDL cholesterol, mg/dl	114.1±30.9	129.6±40.4*
HDL cholesterol, mg/dl	44.9±9.0	41.8±9.3*
Triglyceride, mg/dl	180.0±135.5	168.9±108.3

CAD: Coronary artery disease, LDL= Low density lipoprotein, HDL= High density lipoprotein * p< 0.05

hypoglycemic or insulin therapy), family history of CAD (presence of a first degree relative with cardiovascular disease before the age of 55 years for men and 65 years for women), smoking (current smoker or stopped smoking within the past 6 months) and dyslipidemia (total cholesterol>200 mg/dl, or LDL cholesterol >130 mg/dl, or HDL cholesterol <40 mg/dl, or triglyceride > 200 mg/dl) were recorded for both groups. After 12 hours fasting, venous blood samples were taken for evaluation of plasma lipid (LDL, HDL, total cholesterol and triglyceride), tHyc, folate and Vitamin B12 levels, and MTHFR polymorphism. As recent results from the National Cholesterol Education Program showed that as a risk factor, diabetes mellitus is equivalent to having coronary artery disease, we excluded patients with diabetes mellitus from the study⁸. Triglyceride, HDL and total cholesterol levels were determined by enzymatic colorimetric tests using the GPO/PAP and CHOD/PAP methods (Olympus 5240). LDL cholesterol level was calculated by the Friedwald formula. Faculty ethical committee approval and written informed consent from each patients and controls were obtained.

Determination of Homocysteine level and MTHFR Polymorphism: Venous blood samples were collected for determination of plasma tHyc level and MTHFR polymorphism. Each sample was drawn after overnight fasting, and was centrifuged for 15 min at 2000 g within 1 h of collection. Plasma aliquots were immediately frozen and kept at -20°C until they were analyzed. Plasma levels of tHyc were measured by fluorescence polarization immunoassay in an IMx Analyzer (Abbott Diagnostics). Briefly, bound tHyc (oxidized form) is reduced to free tHyc by dithiothreitol and the free tHyc is enzymatically converted to -S-adenosyl-l-homocysteine (SAH) by SAH hydrolase and excess adenosine. The immunoassay is based on the competition between SAH and the fluorescein-labelled-S-adenosyl-cysteine for binding to a

mouse monoclonal antibody. Bound antibody is then detected as a function of the intensity of the polarized fluorescent light measured. Compared to high-performance liquid chromatography, the performance characteristics of this method were satisfactory, with a coefficient of variation of 5.2% and a correlation coefficient of 0.989. The reference value for plasma tHyc is 5-15 µmol/l.

Identification of the 677C to T transition in the MTHFR gene was carried out as follows: A 2-ml sample of K3EDTA-anticoagulated venous blood was collected from each subject for genomic DNA analysis. The genomic DNA was isolated from whole blood using the MagNa Pure LC Nucleic Acid Purification System (Roche). The isolated DNA was then stored at -20°C until mutation analysis was performed. Detection of MTHFR C677T mutations was carried out according to the previously described RealTime PCR technique using a LightCycler device¹⁴. The subjects MTHFR genotypes were classified as normal (CC), heterozygous (CT) and homozygous (TT).

Statistical analysis: SPSS 9.0 for Windows was used for statistical analysis. Data were presented as mean standard deviation. Among CAD risk factors, age and lipid profile were analyzed by the Student's t test, while all the other risk factors were analyzed by the chisquare test. Allele genotype frequencies in both groups were analyzed by the chi-square test and the eligibility of the distribution of mutations was tested according to the Hardy-Weinberg equality. Plasma tHyc, folate and Vitamin B12 levels for different genotypes were compared with the ANOVA test. The range of plasma tHyc and folate levels were divided into four quartiles and univariate logistic regression analysis was used to determine the impact of the plasma tHyc and folate levels in each quartile on the relative risk of AMI for the whole study population. Age, gender, family history of CAD,

Table 2: Adjusted risk of acute myocardial infarction between patients and controls associated with quartiles of plasma homocysteine and folate levels

	Quartile			
	I	II	III	IV
Homocystein				
Patients/control(n)	15/26	20/23	24/15	31/9
Range, µmol /l	1.00-10.37	10.38-13.90	13.91-16.04	16.05-43.00
OR (95% CI)	reference	1.17 (0.54-2.54)	1.67(0.70-4.00)	2.67(1.01-7.01)
Folate				
Patients/control(n)	23/17	24/19	23/18	22/20
Range, nmol/l	0.30-6.85	6.86-10.10	10.11-14.25	14.26-26.00
OR (95% CI)	reference	1.00 (0.37-2.67)	1.04(0.40-2.74)	0.77 (0.28-2.10)

OR: odds ratio, CI: confidence interval p= 0.04, quartile IV versus I

Table 3: Comparison of plasma homocysteine, folate and Vitamin B12 levels in different MTHFR genotypes (patients and controls)

	MTHFR Genotype		
	CC	CT	TT
Vitamin B12, pg/ml	386.7±199.4	433.8±277.8	408.0±214.1
Folate, nmol/l	11.1±5.6	11.4±6.5	8.6±3.6
Homocysteine, µmol /l	12.4±5.2	13.9±6.7	18.6±9.9*
Plasma homocysteine in patients with plasma folate <11.9 nmol/l	12.5±5.2	13.4±7.1	22.2±10.7*
Plasma homocysteine in patients with plasma folate >11.9 nmol/l	12.2±5.2	15.0±3.9	13.2±4.2

MTHFR: 5, 10-methylenetetrahydrofolate reductase enzyme, CC: normal genotype, CT: heterozygous genotype, TT: homozygous genotype * p < 0.01

smoking, hypertension, dyslipidemia, elevated plasma tHyc level and MTHFR genotypes were selected as potential risk factors for AMI. Independent predictors of AMI were determined by using multiple stepwise logistic regression analysis. For each odds ratio (OR), two-tailed p values and 95% confidence intervals (CI) were calculated. A p value <0.05 was considered statistically significant.

RESULTS

The coronary risk factors of all subjects are shown in Table 1. Hypertension, family history of CAD, and LDL-cholesterol level were significantly higher in the patient group. The mean HDL cholesterol level of the patient group was lower than that of the control group. Genotype distribution was similar in both groups. There was no increase in the relative risk of AMI with the MTHFR gene polymorphism (OR: 1.1 95% CI, 0.82-1.58, p= 0.45).

The mean plasma tHyc level was determined 14.3±8.2 µmol/l and 13.2±5.4 µmol/l in patient group and the control group, respectively (p>0.05). The mean plasma folate level was also comparable in both groups (11.1±6.2 nmol/l in patients, 10.9±5.6

nmol/l in controls). The plasma tHyc and folate levels were divided into four quartiles to examine the influence of these substrate levels on AMI risk for our sample population. The frequency of AMI significantly increased for higher tHyc levels (p= 0.01) but there was no significant association for different folate levels (p>0.05). Plasma tHyc level higher than 16.05 µmol /l was found to be a significant risk factor for AMI (Table 2). In both groups, we compared plasma tHyc levels corresponding to different MTHFR genotypes. Plasma tHyc level was significantly higher in the TT genotype compared to the other genotypes (Table 3). Folate levels were similar in different genotype groups. The mean folate level was determined in order to investigate the effect of tHyc levels on the relative risk for AMI in the study population. TT genotype carriers with lower plasma folate levels had the highest tHyc levels (Table 3). The tHyc levels of our subjects in TT genotype were inversely proportional to their folate levels (r= -.630, p=0.03).

The significance of age, gender, hypertension, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and folate, Vitamin B12 and tHyc levels and MTHFR genotype for the risk of AMI were

Table 4: Predictors of acute myocardial infarction for study population

	OR (95% CI)	P
Family history of CAD	3.3 (1.34-4.04)	0.009
Hypertension	3.2 (1.18-8.81)	0.02
HDL cholesterol	0.9 (0.88-0.99)	0.04
Homocysteine	2.3 (0.92-5.62)	0.07
LDL cholesterol	1.0 (0.61-1.49)	0.21
TT genotype	1.1 (0.82-1.58)	0.32
Smoking	1.8 (0.74-2.52)	0.59

CAD: Coronary artery disease, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TT: homozygous genotype, OR: odds ratio, CI: confidence interval

analysed by multivariate stepwise logistic regression analysis. Among these risk factors hypertension, family history of CAD and HDL-cholesterol were determined as the strong independent predictors of AMI, whereas plasma tHcy level emerged as a weak predictor of AMI. Thus, MTHFR polymorphism was not significant predictors of AMI (Table 4).

DISCUSSION

We found a similar distribution of MTHFR gene mutation among young AMI patients and healthy control subjects in our population. Also, MTHFR enzyme polymorphism was not a significant risk factor for the risk of AMI.

Several studies investigated the effect of TT genotype for risk of AMI in mainly elderly AMI patients. In a retrospective study which investigated 190 patients with AMI aged over 55 years it was reported that MTHFR polymorphism did not significantly alter the relative risk for AMI¹⁰. Dilley et al¹¹. found a TT genotype frequency of 1.8% in a study involving 111 black American patients with a median age of 55. They reported that MTHFR mutation frequency in black Americans was lower than white Americans although this did not significantly alter the risk for AMI. Two other studies investigating the effect of MTHFR polymorphism with younger and older patients, respectively, showed that there was no significant increase in risk for MI^{12,13}. Schwartz et al¹⁴. investigated 79 women patients < 45 years with AMI and they found a similar TT genotype frequency compared to a control group of healthy subjects. But the prevalence of conventional risk factors in the patient group was strikingly different than the control group included in that study.

In contrast, Nakai et al¹⁵. reported that HDL-cholesterol, triglyceride, diabetes mellitus, hypertension and TT genotype were significant and independent factors for risk of AMI in Japanese patients with pre-

vious AMI. Their patient group had an average age of 59 years. Mager et al¹⁶. found a higher TT genotype ratio in AMI patients aged <45 years compared to AMI patients > 45 years or a control group of healthy subjects (respectively %28, %13 ve %14). But the authors acknowledge that the prevalence of coronary risk factors in their patient group was lower than in their control group, so that the multivariate regression method in this case was not a suitable method for the analysis of different genotypes.

Wu and Tsongalis reported a TT genotype frequency of 13.1% in a meta-analysis of 10 studies involving 5644 patients¹⁷. The homozygous mutant MTHFR frequency reported by Mager et al. (%28) is interestingly higher than the frequency of this mutant gene in other populations. Güleç et al¹⁸. stated that TT genotype frequency of patients with AMI aged <45 years was higher than the control subjects (15.6% vs. 5%; OR 5.94; %95 CI: 1.96-18.02, p=0.0016) in their sample of Middle Anatolian population. This result is essentially dissimilar to ours, although this difference may be attributed to the fact that the population analysed in the former study possibly had a different ethnic background. Recently, a large study investigating the influence of nine different polymorphisms of genes encoding hemostasis factors (which include the MTHFR gene) on myocardial infarction showed that there was no apparent relationship between these polymorphisms and the occurrence of premature myocardial infarction¹⁹.

As discussed above, there are many conflicting reports regarding the relationship between TT genotype and CAD in the epidemiological studies performed to date. Some studies demonstrate a significant relationship between TT genotype and CAD^{13,14,16}, while others do not¹⁰⁻¹². We think that there are two main reasons for these conflicting results. Firstly, the objective of these studies and the methods used differ from study to study. In some

studies, the relationship between MTHFR polymorphism and risk for CAD was investigated in groups of patients with established coronary artery disease (history of old myocardial infarction or coronary atherosclerosis diagnosed via the coronary angiography)^{8,11}. Further, as there is no age restriction in most of the studies^{8,9,13}, it is not possible to judge the impact of MTHFR polymorphism on the manifestation of early CAD.

It is reported that each 5 µmol/l increase in tHyc level increases the CAD risk in the same way as 0.5 µmol/l increase in total cholesterol level²⁰. Genetic factors and nutrition conditions are the most important factors affecting plasma tHyc levels². We found the plasma tHyc level in TT genotype group significantly higher than that of the other genotype groups. In addition, the plasma tHyc levels were higher in patients with folate levels lower than 11.9 nmol/l compared to patients with higher folate levels in our study. According to another study from Turkey, tHyc levels over 15 µmol/l are associated with a higher risk of CAD but the TT genotype does not have a significant impact⁷. The same authors also report that plasma tHyc levels are elevated in patients with lower plasma folate levels.

Verhoef et al²¹. showed that there was no difference in the frequency of TT genotype when a group of patients with significant coronary atherosclerosis on their coronary angiogram were compared with a control group of healthy subjects. The authors also reported that the plasma tHyc levels were elevated in patients with lower plasma folate levels, but that this had no significant effect on the risk for CAD. In our study, when analyzed alone, the plasma tHyc levels over 16.05 µmol/l increased the risk of AMI significantly. But, when other coronary risk factors were taken into account by means of a multivariate logistic regression analysis, this risk ratio decreased and became weakened. Tsai et al²². investigated the effect of plasma tHyc level on atherosclerosis in patients with a high profile of cardiovascular risk (conventional risk factors >3) and a low cardiovascular risk profile (conventional risk factors <3). The authors reported that the relationship between plasma tHyc level and atherosclerosis was weakened when the number of risk factors were increased. Analogously, our patient group has a higher risk factor ratio than our control group, and therefore also a higher profile of cardiovascular risk. It is possible that the effect of elevated plasma tHyc levels on the relative risk for AMI may be diminished because of this.

CONCLUSION

Hypertension, positive family history of CAD and

lower plasma HDL-cholesterol levels significantly increase the risk for early AMI. MTHFR gene polymorphism itself does not have any apparent effect on the risk for early AMI. Furthermore, elevated tHyc levels weakly augment the risk for early AMI. It may also be suggested that both lower plasma folate levels and MTHFR 677CT gene mutation play a definite role in elevated plasma tHyc levels in our population.

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