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Association of factor II G20210A, factor V G1691A and methylenetetrahydrofolate reductase C677T gene polymorphism with different forms of myocardial infarction: ST segment elevation and non-ST segment elevation

Povezanost polimorfizama gena za faktor II G20210A, faktor V G1691A metilentetrahidrofolatreduktazu C677T sa različitim formama infarkta miokarda: sa elevacijom ST segmenta i bez elevacije ST segmenta

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Abstract

Background/Aim. Coagulation Factor II G20210A and Factor V G1691A variants are moderately associated with coronary artery disease. Polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene C677T is associated with myocardial infarction (MI) in some ethnical groups. At the present time there are rare studies which try to differentiate two forms of MI, ST-elevation MI (STEMI) and non ST-elevation MI (NSTEMI) according to the genetic background. The aim of the study was investigate the association of polymorphisms of Factor II G20210A, Factor V G1691A and MTHFR C677T with different forms of MI: STEMI and NSTEMI. **Methods.** The study included 82 patients, divided into two cohorts: patients with STEMI (49 patients) and NSTEMI (33 patients). Genetic factors that would be different in those two entities, included in response to plaque rupture and occlusion of coronary artery, were examined. The peripheral blood lymphocytes were used as DNA source. Genotypes were determined on the polymerase chain reaction (PCR) based methodology. **Results.** The frequency of MTHFR C677T CT genotype was higher in the patients with NSTEMI in comparison with the patients with STEMI [odds ratio (OR) 3.33; 95% confidence interval (CI) 1.22–9.15; $p = 0.02$]. Logistic regression analysis shows MTHFR CT genotype as an independent prognostic factor for development of NSTEMI (OR 3.15; 95% CI 1.20–8.29; $p = 0.02$). There were no differences between two patients groups in frequency of Factor II G20210A and Factor V G1691A gene polymorphism. **Conclusion.** MTHFR C677T CT genotype was significantly associated with the NSTEMI development examined patients.

Key words:
genes; factor v; prothrombin; st elevation myocardial infarction; non-st elevated myocardial infarction; polymorphism, genetic; risk factor.

Apstrakt

Uvod/Cilj. Varijante faktora koagulacije II G20210A i faktora V G1691A umereno su udružene sa koronarnom arterijskom bolešću. Polimorfizam gena za metilentetrahidrofolat reduktazu [*methylenetetrahydrofolate reductase* (MTHFR)] C677T udružen je sa infarktom miokarda u nekim etničkim grupama. Retke su studije koje pokušavaju da diferentuju dve forme infarkta miokarda, sa elevacijom ST segmenta [ST-*elevation myocardial infarction* (STEMI)] i infarkta miokarda bez elevacije ST segmenta [*non ST-elevation myocardial infarction* (NSTEMI)], u odnosu na genetičku osnovu. Cilj rad bio je da se utvrdi povezanost polimorfizama faktora II G20210A, faktora V G1691A i MTHFR C677T sa različitim formama infarkta miokarda: STEMI i NSTEMI. **Metode.** Ispitivanjem su obuhvaćena 82 bolesnika podeljena u dve kohorte: bolesnici sa STEMI (49) i bolesnici sa NSTEMI (33). Ispitani su genetički faktori kod ova dva entiteta, uključeni u rupturu plaka i okluziju koronarne arterije. Korišćeni su limfociti periferne krvi kao izvor DNK. Genotipovi su određivani po metodologiji zasnovanoj na lančanoj rekciji polimeraze. **Rezultati.** Učestalost MTHFR C677T CT ge-

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notipa je bila veća kod bolesnika sa NSTEMI u poređenju sa bolesnicima sa STEMI [*odds ratio* OR) 3.33; 95% *confidence interval* (CI) 1,22–9,15; $p = 0,02$]. Logistička regresiona analiza pokazala je da je MTHFR CT genotip bio nezavisan prognostički factor za razvoj NSTEMI (OR 3,15; 95% CI 1,20–8,29; $p = 0,02$]. Nije bilo razlike između dve grupe bolesnika u učestalosti genskih polimorfizama faktora II G20210A i faktora V G1691A. **Zaključak.** MTHFR C677T CT genotip je značajno povezan sa razvojem NSTEMI forme infarkta miokarda kod ispitivanih bolesnika.

Ključne reči:
geni; faktor v; protrombin; infarkt miokarda sa st elevacijom; infarkt miokarda bez st elevacije; polimorfizam, genetički; faktori rizika.

Introduction

Myocardial infarction (MI), and an ischemic heart disease are the leading mortality factor world-wide [1]. According to the third universal definition of MI, this disease is diagnosed upon specific electrocardiogram (ECG) patterns, as ST-elevation MI (STEMI) and non ST-elevation MI (NSTEMI) [2]. The clinical characteristics are different in these two forms of MI, as well as the histology of coronary plaques. Patients with STEMI show more severe plaque rupture of coronary culprit lesions than patients with NSTEMI [3]. Also, fibroatheromas in NSTEMI were more calcified than in STEMI [4]. If patients have recurrent infarctions, the type of infarction is often the same implying that an individual patient is prone for developing of either STEMI or NSTEMI [5]. There are established 50 genetic risk variants in the genome-wide association studies (GWAS) for coronary artery disease (CAD) and MI [6]. Among these genetic risk variants for CAD, there are some genes that are more related to the plaque rupture and thrombosis than atherosclerosis. Unfortunately, in many genetic studies there are no clear distinction between different forms of CAD and MI, leading to complicated conclusions of the impact of genetics in development of the particular disease. The literature is poor of genetic risk factors which differentiate STEMI and NSTEMI [7]. Most of reported single nucleotide polymorphisms (SNPs) were demonstrated to be associated with CAD, and not specifically with MI, so we presumed that genetic risk factors involved in atherosclerosis development, common condition for CAD and MI, are the same in STEMI and NSTEMI. We tried to examine genetic factors that would be different in these two entities, included in a response to plaque rupture, and an occlusion of coronary arteries. We supposed that SNPs of coagulation factors II and V, and polymorphism in the gene involved in metabolism, methylenetetrahydrofolate reductase (MTHFR), also known as risk factor for CAD [8] and vein thrombosis [9], would have different distribution in STEMI and NSTEMI. Factor V single base polymorphism, G1691A (factor V Leiden) leads to change in a functional protein which reduce protein C cleavage sites from three to only one site, and leads to an increased thrombin production. Prothrombin G20210A polymorphism affects a single base, but in a promoter region of the gene. This polymorphism increases the prothrombin production to levels of 30% and 70% higher in the heterozygous and homozygous individuals, respectively, than in those who does not have it [10]. It is shown in meta-analysis that polymorphisms in factor V Leiden and factor II are associated with CAD, and that per-allele relative risk is 1.17 for factor V and 1.31 for factor II mutation [11]. MTHFR C677T gene polymorphism (alanine to valin substitution) which results in a thermo labile form of the enzyme, is established as a risk factor for CAD developing with clear evidence for the TT genotype, and a trend towards an increased risk, for the CT genotype [12]. There are some studies which demonstrated association of MTHFR polymorphism with early onset of CAD [13], and others do not [14]. The homozygous form of the MTHFR C677T gene polymorphism is associated with elevated homocysteine in plasma. Experimental data have demonstrated that homocysteine is involved in an endothelial dysfunction and injury, followed by activation of platelets and thrombus formation [15, 16]. Some studies have shown mean homocysteine concentrations modestly increased in CT heterozygotes in comparison with CC homozygotes [17, 18]. In this study we analyzed factor II G20210A, factor V G1691A and MTHFR C677T variants in patients with STEMI and NSTEMI who underwent percutaneous transluminal coronary angioplasty (PCTA) with a bare metal stent implantation. The aim of the study was to determine impact of genetic polymorphisms of factor V, factor II and MTHFR on different forms of MI among patients with STEMI and NSTEMI.

Methods

Study population

In this observational, retrospective study, 82 patients with MI were included, 62 (76%) men and 20 (24%) women, subjected to PTCA, and the bare metal stent implantation. The patients were admitted to the Clinic of Emergency Internal Medicine, Military Medical Academy in Belgrade, in the period from 2008 to 2010. Patients with acute and chronic autoimmune conditions, and the malignant diseases were excluded from the study.

Among all patients, 49 (60%) were presented with STEMI and 33 (40%) were presented with NSTEMI.

Patients with STEMI and NSTEMI were diagnosed and treated according to the criteria of the European Society of Cardiology/American College of Cardiology Foundation/World Heart Federation Task Force for the Universal Definition of Myocardial Infarction [19, 20].

The main risk factors (elevated cholesterol, hypertension, obesity, smoking, diabetes mellitus and family history of CAD) were documented for each patient. Diabetes melli-

tus was diagnosed as elevated fasting plasma glucose level of more than 11 mmol/L, or self-reported by patients who used insulin or oral hypoglycemic agents. Hypertension was documented when a blood pressure was more than 140/90 mmHg, or when patients use antihypertensive therapy. Total cholesterol values used in the study were obtained from the fasting plasma samples and included values of more than 4.64 mmol/L for men and 4.76 mmol/L for women. Positive family history was defined if there was a history of CAD in at least one first or second relative degree. Obesity was categorized as body mass index (BMI) of more than 25 kg/m^2. Levels of C-reactive protein (CRP) were determined (normal range up to 4 mg/L) at the hospital admission, and every day up to the end of hospital stay. Creatine kinase-MB fraction (CK-MB) (normal range from 0.00 to 25 U/L) was measured at the hospital admission, and after that every 6 h in the follow-up to 48 h (CK-MB-maximal value). Levels of triglycerides and total cholesterol were measured at the time of hospitalization. Information about current smoking status were documented, too. Thrombolysis in MI (TIMI) flow grade at baseline was determined by an angiography. All the patients gave written informed consents, and the study was approved by the Ethics Committee of the Military Medical Academy in Belgrade.

Polymorphism analysis

Blood samples with sodium citrate as an anticoagulant, were obtained from each patient by a peripheral venipuncture. DNA was extracted by the salting-out method [21]. Factor II G20210A (rs 1799963), factor V Leiden G1691A (rs 6025) and MTHFR C677T (rs 1801133) were genotyped using the AttomolQuicktype PCR kit (Germany), according to manufacturer's instructions. Both alleles of a gene of interest were specifically amplified. Examinations of the amplified products were performed by an agarose gel electrophoresis.

Statistical analysis

Results were presented as means ± standard deviation, for the numerical data with a normal distribution, or median, with 25% to 75% percentile for numerical, nonparametric data, and frequency distributions for categorical variables. For numerical variables, the statistical significance was determined by the Student *t*-test, or Mann-Whitney test for nonparametric numerical data. Statistical significance within the categorical variables, genotype frequencies, between patients with STEMI and NSTEMI was tested by the χ^2 test. Association of factor II G20210A, factor V Leiden G1691A and the MTHFR C677T genotypes with different forms of MI was determined by odds ratio (OR) and 95% confidence interval (CI); *p* values less than 0.05 were considered significant. Logistic regression analysis was used to determine independent predictors in patients with STEMI and NSTEMI.

We use the Statistical Package for Social Science (SPSS), version 13.0, for Windows.

Results

Clinical characteristics of patients

Characteristics of patients with STEMI and NSTEMI are presented in Table 1.

The analyzed groups of the patients had significantly different triglyceride levels (1.95 ± 0.98 mmol/L in STEMI *vs* 1.36 ± 0.53 mmol/L in NSTEMI, $p < 0.01$) and total cholesterol levels (5.59 ± 1.10 mmol/L in STEMI *vs* 4.91 ± 0.94 mmol/L in NSTEMI, $p < 0.01$). The median value of maximal CRP levels (CRP max) measured up to 72 h from the hospital admission was 39.20 (21.00–52.90) mg/L in the STEMI group and 20.10 (11.90–35.10) mg/L in the NSTEMI group ($p < 0.01$). Median CK-MB max, measured up to 48 h from the hospital admission was 262.00 (150.00–384.00) U/L in the patients with STEMI and 59.00 (46.00–123.00) U/L in the patients with NSTEMI ($p < 0.01$). TIMI flow grade 3 at baseline was present in 13 (26.53%) of the patients with STEMI and in 17 (51.51%) of the patients with NSTEMI ($p = 0.02$).

The usage of all medications [beta blockers, aspirin, clopidogrel, heparin, angiotensin converting enzyme (ACE) inhibitors, calcium blockers and statins] was not significantly different between the STEMI group and the NSTEMI group.

The frequency of risk factors for MI (smoking, hypertension, obesity, hypercholesterolemia, and positive family history) was not significantly different between patients with STEMI and NSTEMI.

Association between genotypes and disease characteristics

Distribution of factor II G20210A, factor V G1691A and MTHFR C677T genotypes in patients with STEMI and NSTEMI is presented in Table 2.

The frequency of MTHFR CT genotype ($p = 0.02$) and combined CT and TT genotypes of MTHFR C677T polymorphism was higher in patients with NSTEMI ($p = 0.05$).

In the analyzed group of 82 patients with MI, GG genotype of factor II G20210A was found in 79 (96%) of the patients, while three (4%) of the patients had GA genotype. Factor V G1691A GG genotype was present in 76 (93%) and GA genotype in six (7%) of the patients. MTHFR C677T CC genotype was found in 33 (40%) of the patients, while 36 (44%) and 13 (16%) of the patients had CT and TT genotypes, respectively.

Association between factor II G20210A, factor V G1691A and MTHFR C677T genotypes and clinical characteristics (male *vs* female, age ≤ 45 years *vs* > 45 years, univessel *vs* multivessel disease, STEMI *vs* NSTEMI) was not statistically significant.

We used traditional risk factors which were different among our patients with STEMI and NSTEMI, along with genetic factors, for prediction of NSTEMI development. Logistic regression analysis revealed that MTHFR C677CT genotype alone was predictor for NSTEMI development (OR 3.15; 95% CI 1.20–8.29; $p = 0.02$) (Table 3).

Table 1

Characteristic of patients with different forms of myocardial infarction

Characteristics	STEMI (n = 49)	NSTEMI (n = 33)	*p*-value
Age (years)*	57.84 ± 9.99	57.97 ± 9.74	0.48
Male/female†	40/9	22/11	0.12
CAD risk factors			
hypertension†	31 (63.26)	22 (66.67)	0.75
diabetes mellitus†	16 (32.65)	14 (42.42)	0.37
positive family history†	22 (44.89)	18 (54.55)	0.39
current smoker†	22 (44.89)	12 (36.37)	0.44
obesity (BMI > 25 kg/m^2)†	32 (65.31)	28 (84.85)	0.05
hypercholesterolemia*	34 (69.38)	16 (48.49)	0.06
Laboratory data			
trigyceride (mmol/L)*	1.95 ± 0.98	1.36 ± 0.53	< 0.01
CRP (mg/L)‡	39.20 (21.00–52.90)	20.10 (11.90–35.10)	< 0.01
fibrinogen (g/L)*	3.95 ± 1.37	3.61 ±0.90	0.100
total cholesterol (mmol/L)*	5.59 ± 1.10	4.91 ± 0.94	< 0.01
CK-MB at hospital admission (U/L)‡	23.00 (14.00–34.00)	27.00 (14.00–33.00)	0.71
CK-MB Max (U/L)‡	262.00 (150.00–384.00)	59.00 (46.00–123.00)	< 0.01
Coronary angiographic data			
multivessel coronary disease†	22 (44.90)	11 (33.33)	0.29
TIMI flow grade 3 at baseline†	13 (26.53)	17 (51.51)	0.02
Medications at the time of PTCA			
aspirin†	49 (100.00)	33 (100.00)	1.00
clopidogrel†	49 (100.00)	33 (100.00)	1.00
heparin/enoxaparin†	49 (100.00)	33 (100.00)	1.00
beta blockers†	24 (48.97)	15 (45.45)	0.75
ACE inhibitors†	10 (20.41)	9 (27.27)	0.44
calcium blokers†	12 (24.49)	13 (39.39)	0.15
statins†	39 (79.59)	25 (75.76)	0.68

Data are expressed as mean ± standard deviation, median (minimum–maximum) or number (percentage).
CAD – coronary artery disease; ACE – angiotensin-converting enzyme; BMI – body mass index; CKMB – creatine kinase-MB; CKMB Max – creatine kinase-MB form (maximal value up to 48 h from hospital admission); CRP – C-reactive protein maximal values measured up to 72 h from hospital admission; STEMI – ST elevation myocardial infarction; NSTEMI – non-ST elevation myocardial infarction; TIMI – thrombolysis in myocardial infarction; PTCA – percutaneous transluminal coronary angioplasty;* – statistical significance determined by the Student *t*-test, † – statistical significance determined by the χ^2 test; ‡ – statistical significance determined by the Mann-Whitney test.

Table 2

Frequencies of analyzed genotypes in patients with STelevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI)

Genotype	STEMI group n (%)	NSTEMI group n (%)	OR (95% CI)	*p*-value
Factor II G20210A				
GG	47 (95.92)	32 (96.97)	1.36	1.00
GA	2 (4.09)	1 (3.03)	(0.12–15.66)	
Factor V G1691A				
GG	44 (89.80)	32 (96.96)	3.64	0.39
GA	5 (10.20)	1 (3.03)	(0.41–32.65)	
MTHFR C677T				
CC	24 (48.98)	9 (27.27)		
CT	16 (37. 21)	20 (60.61)	3.33 (1.22–9.15)	**0.02**
TT	9 (13.81)	4 (12.12)	0.84 (0.21–3.44)	1.00
CT+TT	25 (51.02)	24 (72.73)	2.56 (0.99–6.63)	**0.05**

MTHFR – methylemetetrahydrofolate reductase; OR – odds ratio; CI – confidence interval.
For testing null hypothesis by using the χ^2-test, GG genotypes were used as reference for Factor II G20210A and Factor V G1691A gene polymorphism, and CC genotype for MTHFR C677T gene polymorphism. There were no patients with AA genotype, for both Factor II and Factor V gene polymorphisms.

Table 3
Association of genetic and traditional risk factors with risk for NSTEMI development

Predictive factors for outcome	OR (95% CI)	*p*-value
Hypercholesterolemia	0.52 (0.11–1.42)	0.20
Factor II G20210A	1.01 (0.05–20.80)	1.00
Factor V G1691A	0.61 (0.06–6.14)	0.672
MTHFR C677T CT genotype	3.15 (1.20–8.29)	0.02
Trigyceride	0.47 (0.17–1.32)	0.15

NSTEMI – non-ST elevation myocardial infarction; OR – odds ratio; CI – confidence interval; MTHTR – methylenetetrahydrofolate reductase; MTHFR C677T CC+TT genotype and Factor II G20210A GG, factor V G1691A GG genotype were used as a reference for OR calculation in logistic regression model.

We also tried to use combined MTHFR C677T CT+TT genotypes, as a predictor for NSTEMI development. MTHFR C677T CC genotype was used as a reference for OR calculation in the logistic regression model. According to results of logistic regression analysis, combined genotypes CT and TT of MTHFR C677T polymorphism showed trend of increased risk for NSTEMI (OR 2.47; 95% CI 0.92– 6.62; $p = 0.07$), but without statistical significance.

Discussion

Patients from our study showed no difference according to the traditional risk factors (hypertension, diabetes mellitus, hypercholesterolemia, BMI, history of smoking and positive family history) for developing CAD and MI, in patients with STEMI and NSTEMI. Similar results were published in the study of Žaliaduonytė-Pekšienė et al. [21]. No difference according to the coronary risk factors among patients with STEMI and NSTEMI were obtained in the study of Miyachi et al. [22] too.

In our study, the patients with STEMI had mean total cholesterol and triglyceride levels higher than the patients with NSTEMI. In the previously mentioned study of Žaliaduonytė-Pekšienė et al. [21], there were no difference in mean total cholesterol and triglyceride levels in the two patients groups. In the study of Belle et al. [23], in France, patients with NSTEMI showed higher triglyceride levels than patients with NSTEMI. These differences can be explained by larger number of patients in the last study, which can increase a precision in statistics.

STEMI patients in our study had a higher absolute level of CRP, and maximal CK-MB levels, than the patients with NSTEMI. It may be due to a different inflammatory response to myocardial injury in these two MI groups, or a difference in some inflammation mediators which are included in pathogenesis of MI. Similar results were obtained in the study of Di Stefano et al. [24], where patients with STEMI had higher values of inflammatory markers at a hospital admission. Significant difference among STEMI and NSTEMI patients, according to the higher peak CRP levels, were demonstrated in the study of Habib et al. [25].

There are no many documented genetic data associated with MI that differentiate STEMI and NSTEMI. There are no genetic data which differentiate polymorphisms of coagulation factors II and V in STEMI and NSTEMI.

In our study, there were no differences in the frequency of polymorphism of factor II G20210A, and factor V G1691A between patients with STEMI and NSTEMI. In the study of Sode et al. [10], there was no association of factor V Leiden and prothrombin G20210A polymorphisms with the MI. In their study there were no differentiation of two categories of MI, STEMI and NSTEMI [27]. On the contrary, in the study of Ezzat et al. [26], in the Egyptian population, the prevalence of heterozygous factor V Leiden, and also recessive homozygous AA were higher in patients with MI than in the control group. In that study there was no differentiation between STEMI and NSTEMI patients. In one large GWAS study, factor V and factor II were documented as the risk factors for developing MI, but with no clear difference of an impact on STEMI and NSTEMI [27]. Among patients with STEMI, in our study, there were 5 of them (10.20%) who were heterozygous for factor V G1691A polymorphism, and only one (3.03%) patient was heterozygous among the NSTEMI cohort. According to the limited number of patients in our study, it is mandatory to include more patients to conclude about differences in factor V polymorphisms between STEMI and NSTEMI groups.

In our study, we found that there was a difference between patients with two forms of MI, STEMI and NSTEMI, in relation to genotypes of MTHFR C677T.

The frequency of CT genotype of MTHFR C677T was higher among the patients with NSTEMI in surveyed population, and lower in the STEMI patients.

When we used dominant model, namely CT+TT, in comparison with CC genotype, we found that these genotypes had increased risk for NSTEMI. There are no data about different genotypes of MTHFR in these two categories of MI, STEMI and NSTEMI. In the meta-analysis of Xuan et al. [28] by using the model TT versus CT for MTHFR gene, it was shown that there was significant risk for MI in Caucasian population. The results of our study are in accordance with the previously published study by Isordia-Salas et al. [13] in young Mexican patients (younger than 45 years). Polymorphism of MTHFR C677T was not associated with development of STEMI in young Mexican patients. It is known that MTHFR C677T genotypes have different ethnical and geographical distribution [29]. The TT genotype was common in Mexico (32%), southern Italy (26%), and northern China (20%). There was sort of a geographic increase in Europe (north to south) and China (north to south decrease). In the big meta-analysis of Alizadeh et al. [30], in the population of patients with MI, there were differences in MTHFR polymorphism according to the ethnical groups. Their results showed that T allele of C677T polymorphism is not associated with an increased risk for MI in the European, Asian and North American population, but is associated in the Af-

rican population. In that analysis, the CT genotype was associated with decreased risk of MI in the North American population and in elderly people. Again, this meta-analysis did not separate clinical forms of MI. Hyperhomocysteinemia is one of possible underlying mechanism for development of MI and CAD. Results of the study of Ho et al. [31] suggest that plasma homocysteine is important risk factor for CAD, and some other diseases, but it is also important to include factors, as MTHFR polymorphism, vitamin B12, triglycerides, total cholesterol, that can affect homocysteine metabolism.

The meta analysis of Kluijtmans et al. [32] has shown that all three MTHFR C677T genotypes confer different levels of atherothrombotic risks. CT heterozygotes have elevated risk in relation to CC homozygotes. The first explanation is that the CT genotype actively confers atherothrombotic risk. The second explanation proposed by these authors is that CC is a protective genotype for development of the atherothrombotic disease. We did not find studies which differentiated STEMI and NSTEMI according to polymorphism distribution of MTHFR C677T. Possible explanation for finding of no statistically significant difference between TT and CC MTHFR C677T genotypes is a small group of our patients, and, concequently, a small number of TT homozygous individuals. At the moment, it is not clear what underlines our finding of higher frequency of CT genotypes of MTHFR C677T in cohort of NSTEMI patients.

Discrepancies observed in all aforementioned studies demand better grouping of MI patients according to the age, gender, ethnic background, food intake, folic acid supplementation and, most important, different forms of the disease.

The limitation of our study was a small number of patients.

Conclusion

The MTHFR C677T CT genotype was significantly associated with development of NSTEMI among MI patients. As MI is a multifactorial disease in which combination of environmental factors and the genetic background both play role in its development, more studies are needed to determine clear association of MTHFR C677T gene polymorphism for development of NSTEMI.

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