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INCIDENCE OF HYPERHOMOCYSTEINEMIA AND MTHFR C677T POLYMORPHISM AMONG YOUNG PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

INCIDENCA HIPERHOMOCISTEINEMIJE I POLIMORFIZAM MTHFR C677T KOD MLADIH BOLESNIKA SA AKUTNIM INFARKTOM MIOKARDA

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Summary: Hyperhomocysteinemia is considered an independent risk factor for premature cardiovascular disease. Mutation MTHFR C677T reduces the activity of methylenetetrahydrofolatereductase and may cause hyperhomocysteinemia. Incidence of hyperhomocysteinemia (homocysteine above 12 μmol/L), homocysteine level, and distribution of MTHFR C677T genotypes (C/C, C/T and T/T) are compared between young patients with acute myocardial infarction and healthy persons, matched by age. Study involved 86 patients younger than 45 years (77 men and 9 women) and 35 controls. Homocysteine was measured by an HPLC method and the MTHFR C677T genotype determined using PCR amplification and digestion with Hinf I. Statistical analyses included chi-square and Mann-Whitney U tests. Hyperhomocysteinemia was present in 32.6% patients and 14.3% controls, revealing a significant difference (P= 0.038). Median homocysteine levels in patients (10.4 μmol/L) and controls (9.6 μmol/L) were significantly different (P=0.035). Among patients, 50.0% had C/C, 41.9% C/T and 8.1% T/T genotype, and the genotype had no influence on hyperhomocysteinemia incidence and homocysteine level. Genotype distribution in patients was not significantly different from that observed in controls. The conclusion is that young patients with acute myocardial infarction have higher incidence of hyperhomocysteinemia and higher homocysteine levels than healthy young adults, while there is no significant difference in the distribution of MTHFR C677T genotypes.

Keywords: hyperhomocysteinemia, MTHFR, myocardial infarction, young adults

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Kratak sadržaj: Hiperhomocisteinemija se smatra nezavisnim faktorom rizika za preuranjeni razvoj kardiovaskularnih bolesti. Mutacija MTHFR C677T snižava aktivnost metilentetrahidrofolatreduktaze i može dovesti do hiperhomocisteinemije. Incidenca hiperhomocisteinemije (homocisteinemija iznad 12 μmol/L), nivo homocisteina i raspodela MTHFR 677 genotipova (C/C,C/T,T/T) upoređeni su između mladih bolesnika sa akutnim infarktom miokarda i zdravih osoba iste dobi. Studija je obuhvatila 86 bolesnika mlađih od 45 godina (77 muškaraca i 9 žena) i kontrolnu grupu od 35 osoba. Homocistein je određivan metodom HPLC, a MTHFR 677 genotip PCR amplifikacijom i digestijom sa Hinf I. Podaci su statistički obrađeni pomoću Chi-square i Mann-Whitney U testa. Hiperhomocisteinemija je bila prisutna kod 32,6% bolesnika i 14,3% zdravih osoba, što predstavlja statistički značajnu razliku (P=0,038). Medijane homocisteinemija bolesnika (10,4 μmol/L) i zdravih osoba (9,6 μmol/L) bile su statistički značajno različite (P= 0,035). Raspodela MTHFR 677 genotipova kod bolesnika (50,0% C/C, 41,9% C/T i 8,1% T/T) nije se statistički značajno razlikovala od raspodele u kontrolnoj grupi. Genotip MTHFR 677 nije uticao na incidencu hiperhomocisteinemije i nivo homocisteina kod bolesnika. Može se zaključiti da mladi bolesnici sa akutnim infarktom miokarda imaju višu incidencu hiperhomocisteinemije i viši nivo homocisteina nego zdrave osobe iste starosti, pri čemu nema značajne razlike u raspodeli genotipova MTHFR.

Ključne reči: hiperhomocisteinemija, MTHFR, infarkt miokarda, mladi bolesnici

List of non-standard abbreviations
Hcy – homocysteine
MTHFR – methylenetetrahydrofolate reductase (NADPH)
MTR – methionine synthase
CBS – cystathionine β-synthase
HHcy – hyperhomocysteinemia
AMI – acute myocardial infarction
FFA – free fatty acids

Introduction

Homocysteine (Hcy) is a nonprotein sulfhydryl-containing amino acid, formed during the metabolic conversion of methionine. The Hcy degradation pathways can be directed towards transsulfuration to cystathionine and thence to cysteine, or remethylation to methionine. The term »total plasma Hcy« refers to the sum of free, protein-bound and disulfide forms of Hcy (1). According to a recent suggestion, which has become widely accepted, the cut-off value for mild hyperhomocysteinemia (HHcy) should be set at the Hcy concentration of 12 μmol/L (2). The etiology of HHcy is multifactorial and includes genetic, nutritional and lifestyle factors (3).

Functional polymorphism characterizes all key enzymes involved in Hcy metabolism: methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and cystathionine β-synthase (CBS). The polymorphism MTHFR C677T is held responsible for almost all genetic variations in Hcy concentration (4). The presence of T allele is associated with reduced catalytic activity, which is associated with an increase in Hcy concentration, especially under the condition of impaired folate status (5).

HHcy is related to premature atherothrombosis (2, 3, 6–8) and considered an independent risk factor for coronary artery disease (2, 8). Nevertheless, the controversal data from prospective studies dealing with the clinical benefit of Hcy lowering therapy, raise suspicion that HHcy is not an instigator, but merely an indicator of cardiovascular disease (9–11). The results of meta-analyses of the MTHFR C677T polymorphism and coronary heart disease (12, 13) confirmed a higher Hcy level in individuals carrying the TT genotype, but the evidence of higher risk for coronary heart disease in that population was not strong enough.

This aim of the study was to compare HHcy incidence, Hcy level and distribution of MTHFR C677T genotypes (C/C, C/T, T/T) between patients with acute myocardial infarction (AMI) younger than 45 years of age and healthy adults of eqiuvalent age.

Material and Methods

Study enrolled 86 patients (77 men and 9 women) with AMI younger than 45 years of age (mean age 40.1±3.5 years). No comorbidities which could influence the Hcy level (e.g. renal insufficiency, diabetes mellitus, malignancies) were present in the patients. The control group comprised 35 healthy persons (23 men and 12 women) with no history of coronary heart disease, diabetes mellitus and cerebrovascular disease. Their mean age was 36.3±4.5 years. Subjects from both groups received no dietary supplementation with vitamin B12, B6 and folate before the collection of blood for Hcy determination.

Blood samples were collected from the patients during the 48 h after admission, accoring to the data concerning the most appropriate moment to determine Hcy levels (14), and after 12 hours of overnight fasting from the controls. To minimize increases in the Hcy concentration from synthesis by erythrocytes, each specimen was placed on ice after collection, serum was separated during 45 minutes and kept at –20 °C prior to analysis. Total Hcy concentration was determined by the previously described HPLC method with fluorescent detection (15). Hcy concentrations above 12 μmol/L were considered as HHcy (2). For genotyping, blood was collected with sodium citrate as the anticoagulant, and the sample was stored at –20 °C, without prior centrifugation. The MTHFR C677T genotype was determined combining PCR amplification and digestion with restrictive endonuclease Hinf I (16).

The chi-square test was performed to evaluate the significance of difference in HHcy incidence, the accordance of distribution of MTHFR genotypes with Hardy-Weinberg equilibrium, the significance of difference in genotype frequencies between patients and controls and difference in HHcy incidence between MTHFR genotypes. The distribution of Hcy levels in our study did not follow the Gaussian model, so the results were expressed as medians (Me) and compared by Mann-Whitney U and Kruskal-Wallis tests. A P-value of less than 0.05 was considered to be significant. Statistical analyses were performed using the programme Statgraphics vers. 4.2 (17).

Results

HHcy was present in 32.6% of patients and 14.3% of controls, revealing a significant difference (P=0.038), as illustrated in *Figure 1*. The median Hcy values in patients (10.4 μmol/L) and controls (9.6 μmol/L) were significantly different (P=0.035). The distribution of MTHFR genotypes in patients was as follows: 50% C/C,

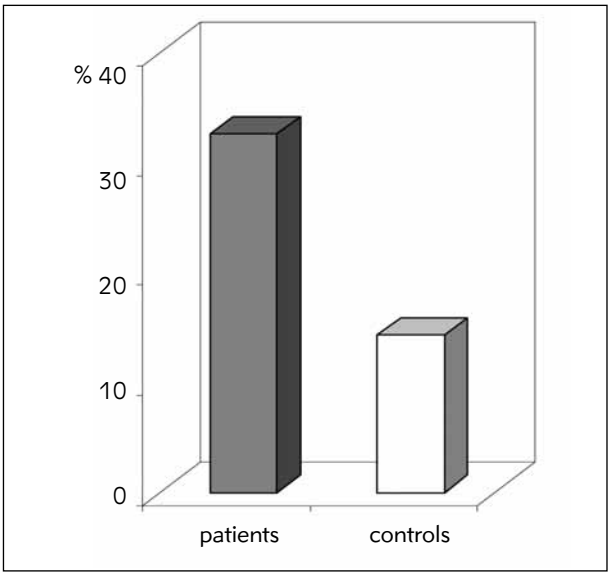

Figure 1 Comparison of HHcy incidence among patients and controls (statistically significant difference; P=0.038)

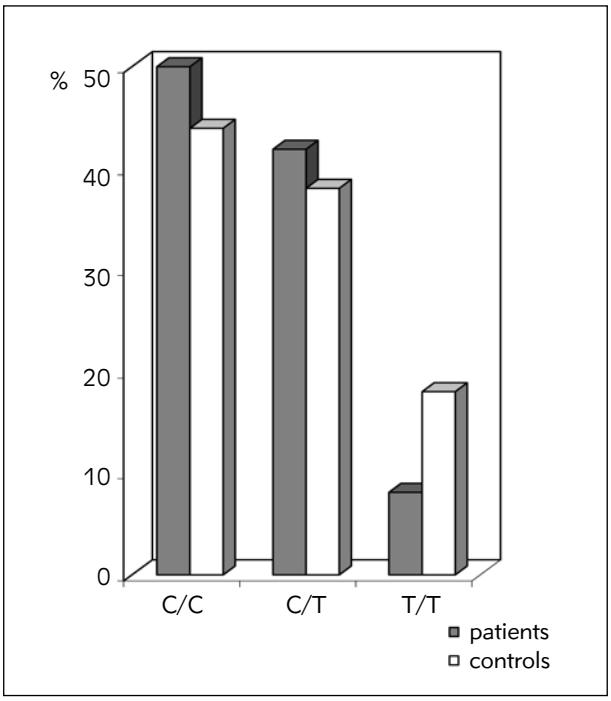

Figure 2 Comparison of distribution of MTHFR C677T genotypes between patients and controls (difference not significant; P=0.877)

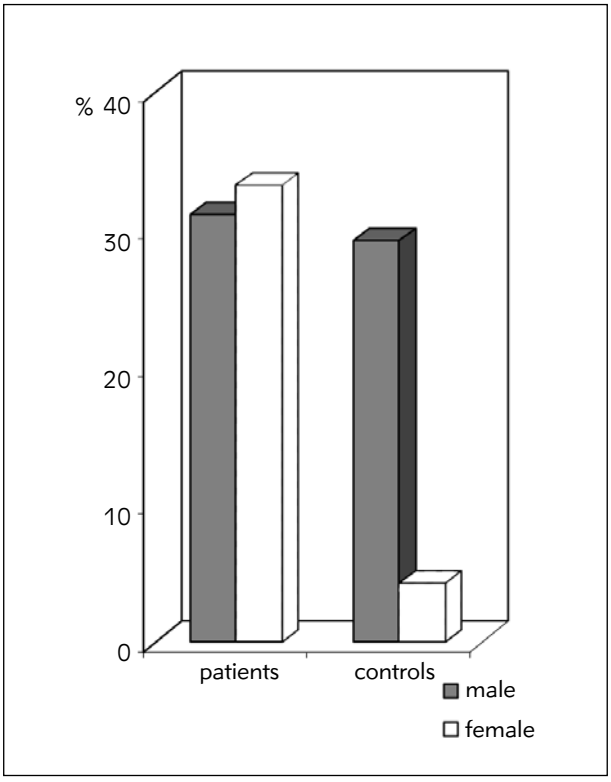

Figure 3 Difference in incidences of HHcy in patients and controls according to gender. Difference is statistically significant between healthy men and healthy women (P=0.033), while significance disappears when comparing male and female patients (P=0.890).

Table I The impact of MTHFR genotype on HHcy incidence and Hcy level in patients.

MTHFR C677T genotype	HHcy incidence (%)	Hcy level (μmol/L)
C/C	32.5	10.3
C/T	27.8	11.3
T/T	50.0	13.1
difference	not significant (P=0.415)	not significant (P=0.356)

Table II The impact of MTHFR genotype on HHcy incidence and Hcy level in controls.

MTHFR C677T genotype	HHcy incidence (%)	Hcy level (μmol/L)
C/C	5.9	9.9
C/T	25	11.1
T/T	25	14.5
difference	not significant (P=0.323)	significant (P=0.035)

41.9% C/T and 8.1% T/T, which is in agreement with Hardy-Weinberg equilibrium (P= 0.904). In the control group there were 44% C/C, 38% C/T and 18% T/T, following the Hardy-Weinberg equilibrium (P=0.826). No statistically significant difference was observed in the distribution of MTHFR C677T genotypes between patients and controls (P=0.877), as shown in *Figure 2*.

The incidence of HHcy in healthy men (29.2%) was significantly higher (P=0.033) than the incidence in healthy women (4.8%). The difference in the incidence of HHcy between male (31.1%) and female (33.3%) patients was not significant (P=0.890). *Figure 3* illustrates the difference in incidences of HHcy in patients and controls according to gender. A significant difference (P=0.004) was observed between the median homocysteine value in healthy men (10.5 μmol/L) and healthy women (8.8 μmol/L), while such an observation was not present after the comparison of Hcy levels in male (10.3 μmol/L) and female (9.95 μmol/L) patients (P=0.905).

Comparison of the HHcy incidence in women with AMI (33.3%) and in healthy women (4.8%) revealed a significant difference (P=0.001). Accordingly, the difference between medians of homocysteinemia in healthy women (8.8 μmol/L) and in female patients (9.95 μmol/L) was found to be significant (P=0.01). No significant differences were observed when incidences of HHcy (P=0.685) and medians of Hcy levels were compared between male patients and healthy young men (P=0.910). The impact of MTHFR genotype on HHcy incidence and Hcy levels is presented in *Table I* for patients and in *Table II* for controls.

Discussion

Acute myocardial infarction in young individuals represents a specific multifactorial disease occuring due to an interaction of numerous conventional and novel risk factors. Their determination is crucial because it offers a possibility for the recognition of individuals at high risk for AMI, prevention of recurrent events and progression of the atherothrombotic process in patients with an established disease. Among these novel factors, an important place belongs to disturbances in the Hcy metabolism, whose association with arteriosclerosis has been postulated on the basis of studies conducted on groups of young adults (1, 9, 18).

Results of our study point to a significantly higher incidence of HHcy in patients with AMI younger than 45 years than in healthy persons of equivalent age. Previously conducted studies revealed that 20–40% patients with coronary disease had HHcy (2) and some of them even marked HHcy as an independent predictive factor of mortality in myocardial infarction (13, 19–21). It would be of importance to highlight the concordance between ours and recently published results, postulating the relationship between HHcy and higher risk of cardiovascular adverse events in long-term follow up in young adults. The persistence of such an association after the adjustment according either to other moratlity predictor factors (e.g. age, sex, diabetes, coronary artery disease history, left ventricular systolic dysfunction) and protector covariables (beta-blockers treatment or coronary revascularization) additionally emphasizes the observed results (8).

The pathogenic mechanisms triggered by HHcy have not been elucidated completely. At this point, there are evidence to consider HHcy a prothrombotic factor, vasodilatation impairing agent, proinflammatory factor and endoplasmatic reticulum-stress inducer. An additional mechanism contributing to HHcy proatherogenic properties is incorporation of Hcy into proteins via disulfide or amide linkages, which consequently alter protein structure and function and may induce additional cellular toxicity and elicit the autoimmune response (22). Results from the recently finished studies on animals, which demonstrated the substrate switch from FFA to glucose due to impaired nitric oxide bioavailability through oxidative stress, suggest that the progression of coronary heart disease associated with HHcy should be evaluated in light of its impact on the alteration of cardiac metabolism. This suggestion indisputably needs verification in clinical studies, but draws attention to the fact that HHcy should be additionally considered a cardiac metabolic disease (23).

According to the results of our study, it can be stated that young patients with AMI have significantly higher Hcy levels when compared to healthy persons. Results of a large cohort study, conducted in Norway, demonstrated that an elevation in Hcy value of 4 μM increases by 1.4 times the relative risk for coronary heart disease (24). Our results, evaluated in sense of such findings, strengthen the hypothesis about a strong association between HHcy and the development of AMI in young adults.

In the control group, men had a higher incidence of HHcy and higher Hcy values than women, while such a gender-associated impact was absent from the group of patients. Data from literature state that, in healthy populations, Hcy concentrations are 21–25% higher in men than in women which is in accordance with the results in our control group (males had 16.2% higher Hcy concentrations than females). The discrepancy observed in patients needs careful interpretation. It can be treated as evidence that the importance of HHcy in etiopathogenesis of AMI is equal in both genders, but, taking into consideration the relatively small number of female patients, it is mandatory to evaluate it in a study which would include an equal number of participants of both genders.

Our study offers the finding that there is no significant difference in the distribution of MTHFR C677T genotypes between young patients with AMI and healthy people of equivalent age. The MTHFR C677T genotype had no influence on the incidence of HHcy, both in patients and controls. Furthermore, the statistically significant difference in Hcy levels between genotypes is evident only in the control group, while in patients such a difference can only be described as a trend without statistical significance. Our results are in an agreement with previously published findings, dealing with the importance of MTHFR C677T polymorphism in the development and prognosis of coronary artery disease (4–6, 12, 13, 25). Following such concordance, it is possible to draw the conclusion that the disease risk associated with mild to moderate HHcy is not an inherited risk, which could imply the possibility that an increase in the Hcy level is not a causal mechanism, but a marker for an environmental disease (e.g. vitamin deficiency) or even just a phenomenon associated with existing disease (4). Nevertheless, caution is recommended in reaching such a conclusion because there are data marking MTHFR C677T as a candidate gene variant for atherosclerotic cardiovascular disease (26). Taking these facts into account, it becomes obvious that an adequate evaluation of the role of MTHFR C677T in the pathogenesis of AMI in young adults needs additional verification by a larger study.

According to the mentioned results, we can conclude that hyperhomocysteinemia could be a very important factor during the development of acute myocardial infarction in young adults, while the importance of MTHFR C677T needs further clarification. Our conclusions, together with the controversial effectiveness of homocysteine-lowering therapy, direct the clinical follow-up of young adults with hyperhomocysteinemia towards the early detection of atherothrombotic signs and eliminating other factors, which additionally promote atherothrombotic effects of hyperhomocysteinemia.

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