Original Research

Homocysteine Levels and MTHFR Polymorphisms in Young Patients with Acute Myocardial Infarction: A Case Control Study

Christos Eftychiou[1], Loizos Antoniades[1], Loukia Makri[2], Laura Koumas[3], Paul A. Costeas[3], Eleni Kyriakou[4], Evagoras Nicolaides[1], Dimitrios Papadogiannis[5]

[1]Cardiology Department, Nicosia General Hospital, [2]Statistical Service of Cyprus, [3]Molecular Hematology and Immunogenetics Center, Karaiskakio Foundation, Nicosia, [4]Chemistry Department, Nicosia General Hospital, Cyprus; [5]First Department of Propaedeutic Medicine, Laiko General Hospital, Athens University Medical School, Athens, Greece

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Introduction: Increased levels of homocysteine are known to be associated with coronary artery disease (CAD). The most common form of genetic hyperhomocysteinemia results from MTHFR polymorphisms. To examine the role of homocysteine levels and MTHFR polymorphisms in premature CAD and acute myocardial infarction (MI) in the Cypriot population, a case control study was performed in Nicosia General Hospital.
Methods: Sixty-three male patients less than 50 years old who presented with MI in Nicosia General Hospital were compared with 54 controls without CAD. Fasting homocysteine and lipids were tested within 24 hrs from admission, while MTHFR C677T and A1298C polymorphisms were also tested.
Results: Mean homocysteine levels were 14.5 μmol/L in patients and 12.3 μmol/L in controls (p=0.017). Mutant homozygous MTHFR C677T was present in 17.7% of the patients and 19.2% of the controls (p=0.838), while mutant homozygous MTHFR A1298C was found in 16.1% of patients and 13.5% of controls (p=0.690). Mean homocysteine levels were 12.6 μmol/L in patients with single-vessel CAD and 15.5 μmol/L in patients with multi-vessel CAD (p=0.025). Lower HDL appeared to be associated with higher levels of homocysteine with an odds ratio of 0.901, indicating that for each unit increase in HDL, the expected odds of having high homocysteine levels decreased by approximately 10%.
Conclusions: Higher levels of homocysteine are associated with acute MI and multi-vessel disease in Cypriot patients under the age of 50. The existence and extent of disease are not associated with MTHFR polymorphisms. Lower HDL is associated with higher levels of homocysteine.

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Address:
Christos Eftychiou

Cardiology Department, Nicosia General Hospital
215, Old Road Nicosia-Limassol
2029 Strovolos, Nicosia, Cyprus
e-mail: chiou6christos@yahoo.com

Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine and its elevated levels were first suggested as a risk factor for cardiovascular diseases in 1969.[1,2] It is now widely accepted that increased levels are associated with increased cardiovascular risk, independently of other atherosclerotic risk factors, and the risk is present even in the upper limits of the "normal" range of 5-15 μmol/L.[3,4] Evidence also suggests that increased homocysteine levels are a risk factor for premature coronary artery disease (CAD) and myocardial infarction (MI) in young patients.[5-8] Higher levels of homocysteine are associated with increased thrombogenicity, increased oxidative stress status, overactivation of redox-sensitive inflammatory pathways, impaired endothelial function and atherogenesis, while mild hyperhomocysteinemia is associated with decreased fibrinolytic activity in patients after ST-elevation MI.[9-11] Elevations in the

plasma homocysteine concentration can occur due to genetic defects in the enzymes involved in homocysteine metabolism, to nutritional deficiencies in vitamin cofactors, or to other factors, including some chronic medical conditions, smoking and drugs.[12-14] The most common form of genetic hyperhomocysteinemia results from the production of a thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity. However the role of the genetic factor alone has been controversial and evidence suggests that hyperhomocysteinemia is an additional precondition for the presence of CAD.[15-18] A meta-analysis also showed that the association of the MTHFR C677T polymorphism with CAD has a geographical variation between populations in Europe, North America, Asia and the Middle East.[19]

Therefore, in order to examine the role of homocysteine levels and the MTHFR polymorphisms in premature CAD and acute MI in the Cypriot population, a case control study was performed in Nicosia General Hospital.

Methods

Sixty-three male patients less than 50 years old who presented with acute MI in Nicosia General Hospital from June 2007 to September 2009 were compared with 54 controls without CAD. The diagnosis of acute MI was based on the presence of ischemic chest pain of at least 30 minutes duration and a typical increase in cardiac enzymes, accompanied by dynamic ST-segment elevation or depression of 1 mm or more in at least 2 adjacent leads. Premature acute MI was defined as having the above mentioned criteria at the age of 50 years old or younger. Patients with ST-elevation MI underwent thrombolysis and rescue percutaneous coronary intervention (PCI) was performed where needed. All patients underwent coronary angiography, and PCI or coronary artery bypass grafting when needed. Multi-vessel disease was defined by the presence of stenoses of >50% in ≥2 major epicardial coronary arteries.

Fasting homocysteine and lipid levels were tested within 24 hrs from admission, while MTHFR C677T and A1298C polymorphisms were tested by DNA isolation and PCR amplification. Characteristics such as age, risk factors for CAD, weight and height were also recorded.

Fifty-four controls were selected, aged over 50 years, without CAD or stroke. They were selected to have an age over 50 to exclude the possibility of presenting CAD before reaching this age. All control subjects underwent a coronary angiography with no CAD, or a negative stress test. They were also selected so as to be similar to the patient group regarding other CAD risk factors, such as diabetes mellitus, hypertension, cigarette smoking, hyperlipidemia, family history and body mass index (BMI). To avoid other variables that could influence the plasma concentration of homocysteine, we excluded patients and controls with a history of folic acid or vitamin B complex supply, a history of folic acid or vitamin B complex deficiency, and renal insufficiency (creatinine ≥1.5 mg/dL), as well those who had fasted for less than 4.5 hours before blood sampling.

The study was approved by the National Ethics Committee of Cyprus. Written informed consent was obtained from all patients and controls, according to the guidelines of the committee.

Statistical analysis

Data were collected and analyzed using the SPPS statistical software package. Chi-square tests and Fisher's exact test were conducted to identify possible associations between the existence of disease and certain categorical variables, while t-test analysis was used to test for differences in means of continuous variables between patients and controls. Multiple logistic regression analysis (stepwise) was used to predict the outcome of high homocysteine levels based on the levels of several predictors, such as hypertension, dyslipidemia, smoking status, age, family history, HDL and LDL. Odds ratio was used to evaluate the effect of significant factors associated with homocysteine. Statistical significance was defined as $p<0.05$.

Power analysis indicated that a minimum number of 54 subjects in each group would be sufficient to yield a power of 80% at the 5% level of significance.

Results

Sixty-three male patients less than 50 years old (range 21-50, mean 43.4 ± 6 years old) presented with acute MI in the Cardiology Department of Nicosia General Hospital. Fifty-five of them (87.3%) presented with STEMI and 8 (12.7%) with non-STEMI. The MI was anterior in 33 patients (52.3%), inferior in 27 (42.9%) and lateral in 3 (4.8%). Single-vessel disease was detected in 30 patients (47.6%) and multi-vessel disease in 28 (44.4%) patients. In 5 patients (8.0%) there was no significant coronary artery stenosis. In terms of

risk factors for CAD, patients and controls had no statistically significant differences in history of smoking, history of dyslipidemia, family history for CAD, diabetes mellitus or number of obese (BMI>30) patients (Table 1). Hypertension was more common in the control group than in the MI patients (42.6% vs. 20.6%, p=0.010). There were no statistically significant differences in the mean values of total cholesterol, LDL, HDL and triglycerides between patients and controls (Table 2). There was, however, a statistically significant difference in homocysteine levels, where the mean value was 14.5 ± 5.6 μmol/L in patients and 12.3 ± 4.1 μmol/L in controls (p=0.017).

No statistically significant differences were observed between patients and controls in MTHFR polymorphisms. Mutant homozygous MTHFR C677T was present in 11 (17.7%) of the patients and in 10 (19.2%) of the controls (p=0.838), while mutant homozygous MTHFR A1298C was present in 10 (16.1%) of the patients and in 7 (13.5%) of the controls (p=0.690). Any type of homozygosity (MTHFR C677T or MTHFR A1298C) was present in 21 (33.9%) patients and in 17 (32.7%) controls; the difference was again not statistically significant (p=0.894). There was no statistically significant difference in the values of homocysteine levels between any MTHFR homozygosity (mean 14.2 ± 5.9 μmol/L) or no homozygosity (mean 13.3 ± 4.5 μmol/L) in the patients and controls (p=0.378).

Mean homocysteine was 12.6 ± 3.9 μmol/L in patients with single-vessel CAD and 15.5 ± 5.5 μmol/L in patients with multi-vessel CAD (p=0.025). There were no significant differences between patients with single-vessel and multi-vessel CAD in regards of history of smoking, history of dyslipidemia, family history for CAD, or hypertension (Table 3). There was also no statistically significant difference in the mean values of total cholesterol, LDL and triglycerides. There were differences in the values of HDL and the number of obese patients. Mean HDL was 40.9 ± 6.9 mg/dl in patients with single-vessel CAD and 35.7 ± 6.2 mg/dl in patients with multi-vessel CAD (p=0.006; Table 4). There were 3 obese patients (BMI>30) with single-vessel CAD disease and 11 with multi-vessel CAD (p=0.007; Table 3).

No statistically significant differences were observed between MTHFR polymorphisms and the number of diseased vessels. Mutant homozygous MTHFR C677T was present in 7 (23.3%) patients with single-vessel CAD and in 4 (14.8%) patients with multi-vessel CAD (p=0.416), while mutant homozygous MTHFR A1298C was present in 4 (13.3%) and 4 (14.8%), respectively (p=1.000). Any type of homozygosity (MTHFR C677T or MTHFR A1298C) was present in 11 (36.7%) of the patients with single-vessel CAD and in 8 (29.6%) of the patients with multi-vessel CAD; the difference was again not statistically significant (p=0.574).

Finally, logistic regression analysis used the vari-

Table 1. Risk factors for coronary artery disease in patients and controls.

	Cases N (%)	Controls N (%)	p
Smoking	53 (84.1)	43 (79.6)	0.527
Dyslipidemia	46 (73.0)	37 (69.8)	0.703
Family history of CAD	24 (38.1)	14 (25.9)	0.161
Hypertension	13 (20.6)	23 (42.6)	0.010
Diabetes mellitus	2 (3.2)	5 (9.3)	0.246
Obesity (BMI>30)	15 (25.4)	20 (37.0)	0.182
Age	43.4 ± 6.0	58.0 ± 7.4	<0.001

CAD – coronary artery disease; BMI – body mass index.

Table 2. Homocysteine, total cholesterol, LDL, HDL and triglycerides in patients and controls

	Patients	Controls	p
Homocysteine (μmol/L)	14.5 ± 5.6	12.3 ± 4.1	0.017
Total cholesterol (mg/dl)	216.4 ± 43.6	210.7 ± 43.2	0.496
LDL (mg/dl)	147.0 ± 37.7	138.4 ± 40.5	0.312
HDL (mg/dl)	38.6 ± 7.4	41.5 ± 8.0	0.054
TG (mg/dl)	166.3 ± 90.8	163.3 ± 102.1	0.872

LDL – low density lipoproteins; HDL – high density lipoproteins; TG – triglycerides.

Table 3. Risk factors for coronary artery disease in patients with single-vessel and multi-vessel disease.

	Single-vessel CAD N (%)	Multi-vessel CAD N (%)	p
Total patients	30 (47.6)	28 (44.4)	
Smoking	26 (86.7)	23 (82.1)	0.726
Dyslipidemia	21 (70.0)	23 (82.1)	0.280
Family history of CAD	9 (30.0)	14 (50.0)	0.120
Hypertension	8 (26.7)	4 (14.3)	0.245
Obesity (BMI>30)	3 (10.3)	11 (42.3)	0.007

Abbreviations as in Table 1.

Table 4. Homocysteine, total cholesterol, LDL, HDL and triglycerides in patients with single-vessel and multi-vessel coronary artery disease.

	Single-vessel CAD	Multi-vessel CAD	p
Homocysteine (μmol/L)	12.6 ± 3.9	15.5 ± 5.5	0.025
Total cholesterol (mg/dl)	213.3 ± 41.3	219.9 ± 47.5	0.588
LDL (mg/dl)	143.7 ± 31.1	152.1 ± 43.9	0.423
HDL (mg/dl)	40.9 ± 6.9	35.7 ± 6.2	0.006
TG (mg/dl)	151.6 ± 80.8	179.2 ± 106.8	0.289

Abbreviations as in Tables 1 & 2.

ables of hypertension, dyslipidemia, smoking status, age, family history, HDL and LDL, in order to test whether they could predict high levels of homocysteine. HDL appeared to have a highly significant effect on the outcome under investigation ($p<0.001$). In particular, HDL had an odds ratio of 0.901, indicating that for a unit increase in HDL, the estimated odds of having high homocysteine levels decreased by approximately 10%. Additionally, a negative bivariate correlation coefficient between homocysteine and HDL appeared to be statistically significant ($r=-0.242$, $p=0.011$), indicating that with increasing HDL levels we should expect decreasing levels of homocysteine.

Discussion

Plasma homocysteine levels are recognized to be determined by both genetic and nutritional factors, but the level at which they are considered to be raised and deleterious remains uncertain. However, the mean value of 14.5 μmol/L in our patients is above the value of 13 μmol/L, which is considered as mild hyperhomocysteinemia.[20] On the other hand, MTHFR polymorphisms, which are a well known factor for hyperhomocysteinemia, were not shown in our study to be related either to acute MI or to the extent of the CAD; however, our study was limited by its small sample size. Even though there is a geographical variation in the association of the MTHFR C677T polymorphism and coronary heart disease among populations in Europe, North America, Asia and the Middle East,[19] our finding comes in agreement with a previous work of Panayiotou et al in the Cypriot population.[21] They investigated the relationship of homocysteine levels and MTHFR C677T genotype with subclinical atherosclerosis and concluded that increased homocysteine levels, but not the MTHFR C677T genotype, are associated with subclinical atherosclerosis and the presence of plaques in the carotid and femoral arteries. Our findings suggest that mild hyperhomocysteinemia is not only associated with CAD, but also with acute MI and multi-vessel CAD. In clinical studies, hyperhomocysteinemia was associated with activation of coagulation systems in patients with premature atherosclerotic arterial disease and elevated Factor VIIa, and with thrombin generation in patients presenting with an acute coronary syndrome.[22,23] These findings suggest an explanation for the prothrombotic effect of homocysteine in acute coronary syndromes. Additionally, in patients with acute MI, elevated homocysteine levels are associated with a higher risk of recurrent coronary events and death, independently of other risk factors and the extent of coronary artery disease.[24]

Despite the accumulated evidence for the association of hyperhomocysteinemia with CAD and acute MI, there is growing dispute concerning the causal

role of homocysteine and more support for the idea that the elevated plasma homocysteine concentration is an aftermath of CAD.[20] It has been demonstrated that plasma homocysteine levels increase after tissue damage. Elevated plasma levels of homocysteine promote endothelial damage and adhesion of leukocytes to the endothelial surface, and would therefore be an indicator of continuing tissue damage and an enhancer of inflammatory vascular wall thickening, rather than an initiator of atherosclerosis.[25]

Our study also showed that low HDL is a predictor of high homocysteine levels, and this supports the speculation that homocysteine levels are not the direct initiator of atherosclerosis.[26] Two studies by Liao et al and Mikael et al suggested that the mechanistic link may be a homocysteine-induced reduction in the concentration of HDL, by inhibiting the hepatic synthesis of apoA-I, the main HDL apolipoprotein.[27,28] The results of these two studies not only explain the documented inverse correlation between the plasma concentrations of HDL cholesterol and homocysteine, but also provide the mechanism linking homocysteine to the development of atherosclerosis.

The disappointing results of the NORVIT and HOPE-2 studies also cast doubt upon the direct role of homocysteine in CAD and the idea of intervening in its levels.[29,30] Recent meta-analyses and randomized placebo-controlled trials of folic acid supplementation in patients with CAD have not only failed to prove any benefit in terms of primary or secondary prevention, but also some of the evidence suggests that folic acid/vitamin B12 treatment might promote more rapid progression of the CAD.[31-37]

However, it is still widely accepted that, at a cellular level, homocysteine exerts a detrimental effect on vascular wall and endothelial cells, by decreasing NO bioavailability, increasing intracellular oxidative stress, and by triggering multiple pro-atherogenic mechanisms while epidemiological studies have clearly demonstrated that plasma homocysteine is an independent risk factor for atherosclerosis.[9] It is also accepted that hyperhomocysteinemia is associated with a worse clinical outcome and recurrent events.[24] Furthermore, the findings of our study suggest that mild hyperhomocysteinemia should not only be considered an additional factor for CAD, but also a risk factor for multi-vessel disease. These patients, however, would not benefit from homocysteine-lowering treatment, but they should be considered at higher risk and treated aggressively for their CAD risk factors.

Conclusion

Higher levels of homocysteine are associated with acute MI and multi-vessel CAD in Cypriot patients under the age of 50. The existence and extent of disease are not significantly associated with MTHFR C677T and MTHFR A1298C polymorphisms. Lower HDL is associated with higher levels of homocysteine. It is not clear that homocysteine has a causal role in atherosclerosis and current data do not support pharmacological lowering of homocysteine with folate. Patients with higher levels of homocysteine should be considered at higher risk for multi-vessel disease and a worse outcome and should be treated aggressively.

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