

CLINICAL STUDY

Plasma homocysteine level and 677C→T mutation on the MTHFR gene in patients with venous thromboembolism

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Abstract: *Background:* The aim of this study was to evaluate the role of plasma total homocysteine level and 677C→T mutation on the methylenetetrahydrofolate reductase (MTHFR) gene in the development of venous thromboembolism.

Methods: Thirty-six (18 male, 18 female and mean±SD; 48.3±15.5 years) patients with venous thromboembolism and 25 healthy adults (13 male, 12 female and mean±SD; 46.8±9.2 years) were included in the study. Fasting plasma total homocysteine level was determined by a high performance liquid chromatography. 677C→T mutation on the MTHFR gene in peripheral blood was detected by Real Time-PCR method.

Results: The level of plasma total homocysteine (18.5±10.6 µmol/L) was significantly higher in patients with venous thromboembolism than in the control group (11.0±4.7 µmol/L) (p=0.015). 677C→T mutation on the MTHFR gene heterozygosity was higher in the patient group than in the control group [13 (36.1 %) and 2 (8%) respectively] but this difference was not significant (p=0.07).

Conclusion: It is thought that a high plasma total homocysteine may cause venous thromboembolism (Tab. 2, Ref. 21). Full Text (Free, PDF) www.bmj.sk.

Key words: homocysteine, 677C→T mutation on the MTHFR gene, risk factor, venous thromboembolism.

Venous thromboembolism is a frequently seen disease with a high mortality. The acquired risk factors are age, obesity, smoking, drugs, immobilization and surgical operation. Congenital risk factors are deficiencies of protein C, protein S and antithrombin III and the presence of Factor V Leiden mutation (1).

An elevated plasma level of total homocysteine (tHcy) is an independent risk factor for arterial and venous thrombosis (2–5). The acquired factors such as advanced age and renal failure may cause an increased plasma tHcy levels. Mild or moderate hyperhomocysteinemia may result from a relative deficiency of folic acid and vitamin B12 and homozygosity for the common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene (677C T). Severe hyperhomocysteinemia (tHcy>100 µmol/L) is most often caused by the cystathionine β-synthase deficiency.

Homocysteine is converted to methionine in normal adults. Methyl group and B12 are the co-factors in this reaction and methylfolate is the methyl donor. Methylenetetrahydrofolate is converted to tetrahydrofolate by the enzyme MTHFR where methyl group is produced (3). In the mutation of the gene coding for MTHFR enzyme, cytosine changes with thymidine. This varia-

tion causes a decrease in the activity of the enzyme, so an increase in the homocysteine level is present. In the population, homozygote involvement (TT) is seen in 10 %, homozygote uninvolved (CC) in 47 % and heterozygote involvement (CT) in 43 % (2). In the study it was reported that C677T mutation was related to hyperhomocysteinemia but was not a risk factor for venous thromboembolism (6). The results of another study showed that the role of homocysteine in the intracellular metabolic pathways may be more important than its plasma concentration (3). There are conflicting results for the role of the 677C→T mutation on the MTHFR gene in the development of venous thromboembolism.

In this study, we aimed to investigate the role of plasma tHcy level and 677C→T mutation on the MTHFR gene in the development of venous thromboembolism.

Material and methods

For this study we included 36 patients diagnosed with venous thromboembolism in Cukurova University Faculty of Medicine, Department of Chest Diseases between 2003 and 2005 and 25 healthy adult with similar age and gender. Pulmonary thromboembolism was diagnosed by a moderate-high probability ventilation/perfusion scintigraphy or spiral computerized tomography of thorax done in case of clinical suspicion. Exclusion criteria were indefinite diagnosis, continuous vitamin supplementation and a disease that may cause an increased plasma tHcy level such as renal failure.

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Tab. 1. Demographic data, risk factors and diagnostic modalities in the study groups.

Characteristics	Patient group n=36	Control group n=25	p
Age	48.3±15.5	46.8±9.2	0.15
Male/Female	18/18	13/12	0.2
Cigarette smoking	16 (44.4%)	-	
Family history	3 (8.3%)	-	
Risk factors			
Oral contraception	2 (10.5%)	-	
Surgery	7 (36.8%)	-	
Malignity	5 (26.4%)	-	
Heart failure	2 (10.5%)	-	
Trauma	3 (15.8%)	-	
Diagnostic modality for pulmonary embolism*			
V/P scans	11 (36.7%)	-	
Spiral thorax CT	19 (63.3%)	-	

Data related to age, gender, height, weight, body mass index, smoking status, co-morbid disease, medication (such as oral contraceptive) and family history were recorded. Peripheral venous blood sample of 10 cc was drawn from the patient and the control group for determination of the presence of 677C→T mutation on the MTHFR gene and the levels of tHcy, blood urea nitrogen, creatinine, vitamin B12 and folate. The study was approved by the ethical committee of the faculty.

Assays

Total plasma homocysteine level was measured by the High Performance Liquid Chromatography (-HPLC-, Chromosystems, Switzerland) method. Serum homocysteine level was 5.5–17 µmol/L in men; 3.5–15 µmol/L in women as a reference value.

Detection of MTHFR gene mutation: 2 cc of peripheral venous blood was collected for DNA analysis. C677T were measured by PCR (7), Light Cycler (Roche, Germany).

Vitamin B12 and folic acid level were studied by electrochemiluminescence method (Roche Modular E-170 System, Germany). Reference ranges were 197–866 pg/ml and 3.1–17.5 ng/ml and measurement ranges were 30–2000 pg/ml and 0.6–20 ng/ml for vitamin B12 and folic acid respectively.

Blood urea nitrogen and creatinine level were studied by enzymatic colorimetric methods (Roche Modular DPP System, Germany).

Statistical analysis was done by the SPSS 14.0 for Windows. The independent samples T test was used in the analysis of continuous variables among the groups. Chi-square test was used in the analysis of categorical variables among the groups. $p < 0.05$ was considered as statistically significant.

Results

Demographic characteristics of the patient and control groups and determined risk factors are listed in the Table 1.

Tab. 2. Plasma tHcy level and the other laboratory findings in the study groups.

Characteristics	Patient group n=36	Control group n=25	p
tHcy (µmol/L)	18.5±10.6	11.0±4.7	0.015
Vitamin B12 (pg/ml)	329.1±272.5	299.6±102.2	0.7
Folic acid (ng/ml)	12.7±13.8	9.1±2.6	0.3
Blood urea nitrogen (mg/dl)	17.5±3.8	14.4±4.2	0.5
Creatinine (mg/dl)	1.0±0.3	0.8±0.1	0.06
MTHFR (n/%)			
Homozygotes (C/C)	23 (63.9)	23 (92)	0.07
Heterozygotes (C/T)	13 (36.1)	2 (8)	

There was no significant difference between the study groups with respect to age and gender. In the patient group, six patients were diagnosed to have only deep venous thrombosis (16.7%), 12 (33.3%) patients had only pulmonary embolism and 18 (50%) patients had both diseases. Considering the history of the patients, 19 (52.8%) patients had at least one risk factor for pulmonary embolism, whereas 17 (47.2%) had no risk factor.

In our study, plasma tHcy level of the patient group (18.5±10.6 µmol/L) was higher than the healthy control group (11.0±4.7 µmol/L) ($p=0.015$). However no statistically significant difference between the study groups with respect to the serum levels of creatinin, vitamin B12, folic acid, and blood urea nitrogen were observed.

Twenty-three (63.9%) of the patients with deep venous thrombosis and/or pulmonary embolism were MTHFR enzyme homozygote normal and 13 (36.1%) were MTHFR enzyme heterozygotes. MTHFR heterozygosity was higher in the patient group than the control group (23 (92.0%) and 2 (8.0%) respectively) but this difference was not significant ($p=0.07$) (Tab. 2).

In the subgroup analysis, the plasma tHcy level of the patients with a risk factor in their history (21.0±11.1) was higher than the ones without a risk factor in the history (16.2±5.5), whereas this difference was not statistically significant ($p=0.2$).

Discussion

In this study we found that plasma tHcy level was higher in patients with venous thromboembolism than in the control group. Hyperhomocysteinemia is known as a risk factor for vascular disease and atherosclerosis for a long time (8, 9). There are several studies suggesting that it may be an independent risk factor for venous thromboembolism (10–16). Suggested mechanisms explaining how hyperhomocysteinemia may lead to venous thrombosis are a) the toxic effect on the vascular endothelium reducing the activation of protein C, increasing the thrombotic tendency, b) abnormal methionine metabolism which affects the methylation of DNA and cell membranes (16).

In a recent study, Oger et al reported that a mild increase in tHcy was a risk factor for venous thromboembolism in the presence of low folic acid and vitamin B12. On the other hand, moderately high tHcy level was associated with an almost two fold

risk in the absence of surgery, trauma, or cancer (17). In our study, tHcy level in the patient group was found to be higher than in the control group, similar to the previous studies, but there was no difference with respect to the levels of vitamin B12 or folic acid.

Hainaut et al reported that tHcy level was higher in the group without a risk factor for venous thrombosis than in the group with a known risk factor (4). Although it was not statistically significant, tHcy level was higher in the group with a risk factor than the group without a risk factor in our study. Limited size of the study population may have an effect on our results, but also a mildly increased tHcy level may not be associated with the acquired risk factor such as trauma, surgery, and malignancy.

In a recent meta-analysis about the association between MTHFR C677T polymorphism and venous thromboembolism, it was concluded that the risk was 20 % higher in T677T genotype than C677C genotype (18). The C677T polymorphism of MTHFR gene resulted in elevated levels of serum homocysteine in homozygotes, especially in the presence of low folic acid levels (3). However some previous studies showed that MTHFR C677T genotype was not associated with an increased risk of venous thrombosis (3, 4, 17, 19, 20, 21). In the particular study it was shown that red blood cell folic acid level was more important than high plasma homocysteine level for the development of venous thrombosis (3). Results of the same study showed that methylfolate concentration in red-blood cells was an important risk factor in people with MTHFR C677C genotype, less important in heterozygotes, and not a risk in people who were homozygote for MTHFR T677T (3).

In our study, C677T MTHFR mutation was more common (but not statistically significant) in the patient group and tHcy level was higher in this group. And also plasma folic acid level was not different between the patient and the control groups. Our results may suggest that MTHFR enzyme gene mutation may be responsible for an increased plasma tHcy level, a finding not similar to other studies. But since intra cellular folic acid was not studied, the relationship between MTHFR C677T genotype and folic acid has not been explained.

Limitations of our study are a small size of the study group and other known genetic risk factors such as protein C, protein S, antitrombin III and Factor V Leiden mutation not being studied.

In conclusion, we suggest that a high plasma tHcy level might be a risk factor for development of venous thromboembolism, but may not be an independent risk factor. In addition, 677C→T mutation on the MTHFR gene may not be responsible for the development of venous thromboembolism.

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