

Methylenetetrahydrofolate reductase polymorphisms in acute deep vein thrombosis

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ABSTRACT

Objectives: In this study, we aimed to investigate the effectiveness of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C gene polymorphisms in acute deep vein thrombosis (DVT).

Patients and methods: Between January 2017 and May 2018, a total of 64 patients (37 males, 27 females; mean age 35±18 years; range 19 to 59 years) who were followed with the diagnosis of acute DVT and 64 healthy controls (35 males, 29 females; mean age 43±14 years; range 23 to 57 years) without any vascular pathology were included. The MTHFR C677T and A1298C gene polymorphisms of the samples were identified using the real-time polymerase chain reaction.

Results: There was no statistically significant difference in the polymorphisms and allele frequencies of the MTHFR C677T and A1298C genes between the patient and control groups ($p>0.05$).

Conclusion: In this study, the MTHFR C677T and A1298C gene polymorphisms were not found to be risk factors for acute DVT.

Keywords: Acute deep vein thrombosis; gene polymorphism; methylenetetrahydrofolate reductase.

Deep venous thrombosis (DVT) continues to present a serious problem with pulmonary embolism (PE), venous coagulation, chronic venous insufficiency and post-thrombotic syndrome, despite the improved medical and surgical treatment options. It is not only associated with morbidity, but also with the complications, and it is also an important disease with high morbidity and mortality rates due to treatment-related complications.^[1] Deep vein thrombosis is a component of venous thromboembolism, a manifestation which may cause PE and covers both diseases. Venous thromboembolism is an important preventable health problem and DVT is seen at a rate of 160/100.000 per year in the North America and European countries.^[1,2] The incidence of venous thromboembolism ranges between 10 and 30% in patients admitted to the hospital due to acute medical conditions.^[3]

Within the folate cycle methylenetetrahydrofolate (MTHF) is converted into MTHF via the catalyst of the MTHF reductase (MTHFR) enzyme. This enzyme provides the balance between deoxyribonucleic acid (DNA) synthesis and methylation reactions. In addition, this enzyme also affects the plasma homocysteine levels which is a risk factor for thromboembolic cases.^[4,5] Most frequent polymorphisms of the MTHFR are the ones at 677th and 1298th regions. The 677 (C-T) polymorphism is located in the Exon 4 and causes the alanine on the 222nd codon to be converted into valine. The 1298 (A-C) polymorphism is located in the Exon 7. Glutamate in the 429th codon is converted into alanine.^[6] As a result, the MTHFR enzyme, which is involved in the conversion of homocysteine to methionine, purine-pyrimidine synthesis (DNA and ribonucleic acid [RNA] biosynthesis) and methylation reactions functions at an important junction.

Received: December 23, 2018 Accepted: January 19, 2019 Published online: February 07, 2019

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Citation:

Şenol S, Kargün K. Methylenetetrahydrofolate reductase polymorphisms in acute deep vein thrombosis. Turk J Vasc Surg 2019;28(2):69-72

Therefore, various metabolic problems arise as a result of decreased MTHFR enzyme activity. One of them is the homocysteine accumulation in the cell.^[7] However, most of these normal properties of endothelial cells may be impaired in the presence of increased levels of homocysteine. Hyperhomocysteinemia changes the vascular morphology, stimulates inflammation, activates endothelium and blood coagulation cascade, and prevents fibrinolysis.^[8]

In the present study, we aimed to investigate the effectiveness of MTHFR C677T and A1298C gene polymorphisms in acute DVT.

PATIENTS AND METHODS

Between January 2017 and May 2018, a total of 64 patients (37 males, 27 females; mean age 35±18 years; range 19 to 59 years) who were followed with the diagnosis of acute DVT and 64 healthy controls (35 males, 29 females; mean age 43±14 years; range 23 to 57 years) without any vascular pathology were included. Acute DVT diagnosis was made according to the patient's medical history, clinical findings, and venous Doppler ultrasound results. Control patients consisted of healthy individuals having normal venous Doppler ultrasound results. Those with kidney and liver function disorders or those having any known systemic diseases or malignancies, those younger than 18 years old and older than 60 years old, and those using warfarin were excluded from this study. A written informed consent was obtained from each

participant. The study protocol was approved by the Fethi Sekin Elazığ Training and Research Hospital Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Physical examination of all patients was performed and demographic characteristics were recorded. 2-3 mL blood samples were taken into the ethylenediaminetetraacetic acid (EDTA) tubes from the patient group and DNA isolations were performed using the DNA isolation kit (PureLink™ genomic DNA kits) at the Medical Genetic Laboratory. Isolated DNA samples were stored at -20°C, until analysis. Genotyping studies for MTHFR C677T and A1298C gene polymorphisms were performed by the ABI branded StepOne Plus model real-time polymerase chain reaction (PCR).

Statistical analysis

Statistical analysis was performed using the SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA). Differences in genotype distribution and consistency with Hardy-Weinberg equilibrium were tested by chi-square test. The test was also used to analyze the categorical variables. Intergroup comparisons were done with one-way analysis of variance (ANOVA). T test and Mann-Whitney U test were performed to compare intra-group variables. The results were analyzed with a confidence interval of 95% and a *p* value of <0.05 was considered statistically significant.

Table 1. Demographic and clinical characteristics of the groups

	Deep vein thrombosis group			Control group			<i>p</i>
	n	%	Mean±SD	n	%	Mean±SD	
Age (year)			35±18			43±14	0.09
Gender							
Male	37	57.8		35	54.6		
Female	27	42.1		29	45.3		
Smoking	14	21.8		11	17.1		0.42
Urea (mg/dL)			36.1±8.3			35.4±8.1	0.51
Creatinine (mg/dL)			0.82±0.13			0.81±0.17	0.91
Body Mass Index (kg/m ²)			27.8±4.9			25.9±2.6	0.78

SD: Standard deviation.

Table 2. Anatomical involvement locations and frequency

Area where thrombus is extended	n	%
Iliac vein	19	23.4
Femoral vein	46	56.8
Popliteal vein	16	19.7

RESULTS

The demographic and clinical characteristics of the study groups are given in Table 1. The ratio of males (57.8%) was slightly higher than female patients (42.1%) in the acute DVT group. The anatomical

Table 3. Genotype distributions of MTHFR gene polymorphisms

Polymorphisms	Genotypes	Deep vein thrombosis group (n=64)		Control group (n=64)		p
		n	%	n	%	
MTHFR C677T	C/C	40	62.5	36	56.2	0.68
	C/T	15	23.4	17	26.5	
	T/T	9	14	11	17.2	
MTHFR A1298C	A/A	32	50	28	54.4	0.86
	A/C	17	26.5	20	23.3	
	C/C	15	23.4	16	22.2	

MTHFR: Methylene tetrahydrofolate reductase; C/C: Cytosine/cytosine; C/T: Cytosine/thymine; T/T: Thymine/thymine; A/A: Adenine/adenine; A/C: Adenine/cytosine.

Table 4. Allel frequencies

Polymorphisms	Allele	Deep vein thrombosis group (n=64)		Control group (n=64)		p	OR
		n	%	n	%		
MTHFR C677T	C	95	74.2	89	69.5	0.90	1.02
	T	33	25.7	39	30.4		
MTHFR A1298C	A	81	63.2	76	59.3	0.66	0.92
	C	47	36.7	52	40.6		

OR: Odds ratio; C: Cytosine; T: Thymine; A: Adenine.

involvement locations and numbers in percentage of acute DVT cases are given in Table 2. The genotype distributions of the MTHFR gene polymorphisms in acute DVT and control groups are shown in Table 3.

For MTHFR C677T, 62.5% of the acute DVT and 56.2% of the control group were found to be homozygote (CC carriers). In addition, 23.4% of the acute DVT and 26.5% of control group were heterozygote (CT carriers). The mutant region (TT carriers) accounted for 14% of acute DVT patients and 17.2% of the control group, indicating no statistically significant difference ($p>0.05$).

For A1298C, that is another region of the MTHFR, 50% of acute DVT patients and 54.4% of the control group were found to be homozygotes (AA carriers). In addition, 26.5% of acute DVT patients and 23.3% of the control group were heterozygote (AC carriers). The mutant region (CC carriers) accounted for 23.4% of acute DVT patients and 22.2% of the control group, indicating no statistically significant difference ($p>0.05$).

There was no statistically significant difference in the allele frequencies for all polymorphisms ($p>0.05$) and the results are shown in Table 4.

DISCUSSION

Venous thromboembolism, which manifests as DVT or PE, is the third most common cardiovascular disorder after myocardial infarction and stroke.

Deep vein thrombosis causes thrombosis obstruction of the deep veins in the lower extremities and it is an important cause of mortality and morbidity. According to the studies, 1/1,000 elderly population is complicated with DVT.^[9-11]

Several studies have identified the MTHFR C677T polymorphism as an important risk factor for DVT.^[12] The genetically most frequently studied MTHFR, which encodes a key enzyme in thrombotic diseases, is shown as MTHFR C677T polymorphism.^[13,14] In a study, DVT risk increased by the MTHFR 677 (C-T) and PAI-1 4G/5G polymorphisms.^[15] In this study, there was no statistically significant difference in the MTHFR C677T gene polymorphisms and allele frequencies. In another study including 67 cases analyzed by the MTHFR 677 (C-T) and serum homocysteine levels, no direct correlation was observed between DVT and MTHFR 677 (C-T) polymorphisms and it was associated with the serum homocysteine levels.^[16] Also, a correlation between the serum homocysteine levels and DVT was found. Since the serum homocysteine levels were not examined in this study, it is not reasonable to establish a comment on indirect correlations. In another study, a significant correlation was found between DVT and MTHFR C677T and A1298C gene polymorphisms.^[17] In this study, there was no correlation between acute DVT and MTHFR C677T and A1298C gene polymorphisms and allele frequencies. The discrepancy among the studies may be due to the genetic differences in the populations,

and large-scale studies with homocysteine levels and scalar quantity may be more useful.

On the other hand, the small sample size is the main limitation to this study. We suggest that further large-scale studies may increase the statistical power of such studies.

In conclusion, the MTHFR C677T and A1298C gene polymorphisms were not associated with acute DVT. However, further studies on different polymorphisms of the MTHFR may be beneficial.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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