

Role of Hyperhomocysteinemia and Methylenetetrahydrofolate Reductase C677T Polymorphism in Idiopathic Portal Vein Thrombosis

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Purpose: Portal vein thrombosis (PVT) is a rare and life-threatening vascular disorder characterized by obstruction or narrowing of the portal vein. Hyperhomocysteinemia and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism has been studied in PVT patients with conflicting results. In the present study the association of hyperhomocysteinemia and MTHFR C677T polymorphism with PVT risk was investigated in Iranians.

Materials and Methods: Our study population consisted of 10 idiopathic PVT patients and 80 healthy control subjects matched for age and sex. MTHFR C677T polymorphism was genotyped by the polymerase chain reaction technique combined with restriction enzyme fragment length polymorphism (PCR-RFLP) technique and plasma total homocysteine (tHcy) levels were determined by enzyme immunoassay method.

Results: Mean plasma tHcy levels were significantly higher in PVT patients (20.2 ± 6.8) than control subjects (10.9 ± 4.7) ($P=0.001$). Moreover, plasma tHcy levels were significantly higher in 677T allele carriers relative to 677C allele carriers in both PVT patients ($P=0.01$) and control subjects ($P=0.03$). Neither homozygote nor heterozygote genotypes of MTHFR C677T polymorphism correlated significantly with PVT risk ($P>0.05$). Moreover, MTHFR C677T polymorphism didn't increase the risk of PVT under dominant (CT+TT vs. CC) or recessive (TT vs. CC+CT) genetic models analyzed ($P>0.05$). The difference in frequency of minor 677T allele between PVT patients and control subjects was not statistically significant ($P>0.05$).

Conclusion: Based on the current study, we suggest that hyperhomocysteinemia constitutes a significant and common risk factor for PVT. Also, MTHFR C677T polymorphism is not a risk factor for PVT but is a contributing factor for elevated plasma tHcy levels.

Key Words: Homocysteine, Portal vein thrombosis, Genetic polymorphism, Methylenetetrahydrofolate reductase

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INTRODUCTION

Portal vein thrombosis (PVT) is a relatively rare disorder characterized by the complete or partial obstruction of the portal vein by thrombus formation [1]. PVT may be caused by a variety of local and systemic factors disturbing portal blood flow [2]. Abdominal pain is a common presentation of PVT. The etiology of PVT encompasses cirrhosis, malignancy, sepsis, inherited or acquired prothrombotic conditions such as factor V Leiden and G20210A prothrombin mutation, and certain hematologic diseases including paroxysmal nocturnal hemoglobinuria and myeloproliferative disorders [2,3]. However, despite extensive evaluations, no underlying cause can be identified in more than 25% of PVT cases, thus they are considered to be idiopathic [2]. Elevated plasma total homocysteine (tHcy) levels have been considered as an important risk factor for venous thrombotic disorders including PVT [4]. Hyperhomocysteinemia may be generated by acquired factors such as nutritional deficiencies of vitamin B12 and folic acid or by genetic alterations in enzymes involved in Hcy metabolism [5]. Methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the latter being involved in vitamin B12-dependent remethylation of homocysteine to methionine [6]. According to some studies, MTHFR C677T polymorphism has been linked with decreased MTHFR activity and elevated plasma tHcy levels [7,8]. It has been associated with different types of thrombosis including deep vein thrombosis, retinal vein thrombosis and cerebral vein thrombosis [8-10]. However, studies investigating the role of MTHFR C677T polymorphism in PVT are limited and to date no study has been reported from Iran. So, it was the purpose of this study to assess the risk of PVT associated with MTHFR C677T polymorphism and hyperhomocysteinemia in an Iranian population.

MATERIALS AND METHODS

1) Study population

Our study populations consisted of 10 patients (6 women and 4 men) with a diagnosis of idiopathic PVT. The idiopathic character was defined by the absence of identifiable cause at the time of diagnosis. The mean age of the patients was 51.2 ± 8.3 . The diagnosis of PVT was confirmed by means of laboratory investigations, ultrasonography and magnetic resonance imaging. The controls consisted of 80 subjects with a mean age of 49.1 ± 14.2 and matched for age and sex. The control

subjects had no history of thrombosis and/or use of vitamin B supplements and folate. Informed consent was obtained from all participants. The study was approved by the Iranian blood transfusion organization ethics committee with the assigned authentication number IBTO-P82-1000.

2) Plasma total homocysteine assay

Plasma tHcy levels were determined by an enzyme immunoassay based method using Axis Shield Homocysteine EIA Kit (Axis Shield Diagnostics Ltd. Dundee, United Kingdom) and according to manufacturer's instruction. Fasting blood samples were collected from all participants in ethylenediaminetetraacetic acid (EDTA) containing tubes (Pole Ideal Pars Ltd., Tehran, Iran) and immediately centrifuged at 4°C. Then, the separated plasma fraction was stored in a -20°C freezer till analysis of tHcy. The assay's detection range was 2.5-50.0 µM.

3) MTHFR C677T assay

Genomic DNA was extracted from blood leukocytes according to the method reported by Lahiri and Nurnberger [11]. The extracted DNA was stored at -20°C until analysis. To detect the C677T polymorphism, we selectively amplified a 198 bp region of the MTHFR gene by polymerase chain reaction (PCR) method as described previously [12]. The sequences of the primers for MTHFR C677T polymorphism were as follows: 5'-TGA AGG AGG TGT CTG CGG GA-3' and 5'-AGG ACG GTG CGG TGA GAG TG-3'. The reaction conditions were as follows: initial denaturation at 94°C for 2 minutes and 35 subsequent cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds and extension at 72°C for 30 seconds. These cycles were followed by a final extension cycle at 72°C for 7 minutes. Ten microliters of amplified products were digested with 5 units of Hinf I enzyme (Promega, Madison, WI, USA) at 37°C for 12 hours. After electrophoresis on a 3% agarose gel, CC homozygotes produced 1 fragment (198 bp) only. CT heterozygotes had 3 fragments (198, 175, and 23 bp) and TT homozygotes had 2 fragments (175 and 23 bp).

4) Statistical analysis

Plasma tHcy levels and ages were expressed as mean \pm standard deviation and were compared between PVT patients and control subjects using Student's t-test. Moreover, the correlation of mean plasma tHcy levels with different alleles of MTHFR C677T polymorphism was conducted by Student's t-test. Allele frequencies were calculated by gene counting in PVT patients and control

subjects. The genotypes and allele frequencies of MTHFR C677T variation were compared between PVT patients and control subjects using a chi-square test. Statistical analysis was performed by SPSS ver. 16.0 software (SPSS Inc, Chicago, IL, USA) with a statistical significance level of $P < 0.05$.

RESULTS

The study population consisted of 10 PVT patients and 80 healthy controls. As indicated in Table 1, the differences in sex distribution ($P=0.83$) and mean age ($P=0.26$) were not statistically significant between PVT patients and control subjects. Mean plasma tHcy levels were significantly higher in the PVT patients than in the control subjects (20.2 ± 6.8 vs. 10.9 ± 4.7 , $P=0.001$). Moreover, as shown in Fig. 1, MTHFR 677T allele carriers had significantly higher plasma tHcy levels than MTHFR 677C allele carriers in both PVT patient ($P=0.01$) and control subjects ($P=0.03$), indicating the effect of this polymorphism on plasma tHcy levels. Table 2 represents the genotype and allele distribution of MTHFR C677T polymorphism in PVT patients and control subjects. No significant correlation was observed between 677CT heterozygote genotype and 677TT homozygote genotype with PVT risk ($P=0.11$). Furthermore, the frequency of minor 677T allele in PVT

patients and control subjects didn't differ significantly (30.0% vs. 20.6%, $P=0.07$). Additionally, the associations of the MTHFR C677T polymorphism with PVT risk were evaluated under dominant and recessive genetic models. As shown in Table 3, the correlation between MTHFR C677T polymorphism and PVT risk was not significant in either dominant genetic model (odds ratio [OR]=2.14, 95% confidence interval [CI] 0.73-4.66, $P=0.12$) or recessive genetic model (OR=1.72, 95% CI 0.67-2.84, $P=0.23$) that were analyzed.

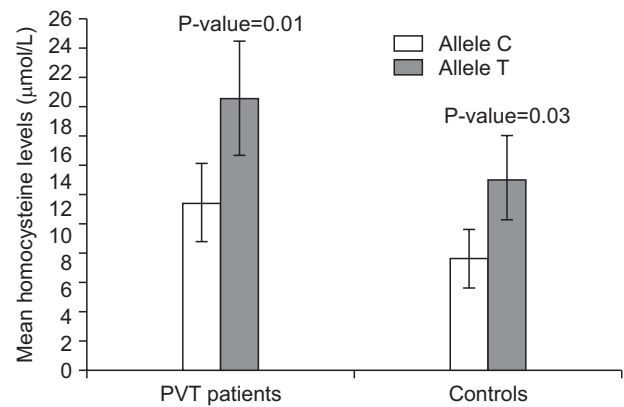


Fig. 1. The association between methylenetetrahydrofolate reductase (MTHFR) C677T alleles and plasma total homocysteine levels in portal vein thrombosis (PVT) patients and control subjects.

Table 1. Mean plasma homocysteine levels and other characteristics in PVT patients and controls

Study population	PVT patient (n=10)	Control (n=80)	P-value
Age (y)	51.2±8.3	49.1±14.2	0.26
Gender (male/female)	4/6	41/39	0.83
Plasma total homocysteine (µmol/L)	20.2±6.8	10.9±4.7	0.001

Values are presented as mean±standard deviation or number only. PVT, portal vein thrombosis.

Chi-square test was used to compare gender between the two groups, and mean values of age and homocysteine levels were analyzed by Student's t-test.

Table 2. Prevalence of MTHFR C677T allele and genotypes in portal vein thrombosis patients and controls

MTHFR C677T polymorphism	Genotype			Allele	
	CC	CT	TT	C	T
Control (n=80)	50 (62.5)	27 (33.8)	3 (3.8)	127 (79.4)	33 (20.6)
Patient (n=10)	5 (50.0)	4 (40.0)	1 (10.0)	14 (70.0)	6 (30.0)
P-value	0.11			0.07	

Values are presented as number (%).

MTHFR, methylenetetrahydrofolate reductase; CC, wild type; CT, heterozygote; TT, homozygote.

Table 3. Analysis of MTHFR C677T polymorphism in PVT patients and control subjects using dominant and recessive genetic models

Genetic model	Genotype	PVT patient (n=10)	Control (n=80)	OR (95% CI)	P-value
Dominant	CC	5 (50.0)	50 (62.5)	1	0.12
	CT+TT	5 (50.0)	30 (37.5)	2.14 (0.73-4.66)	
Recessive	CC+CT	9 (90.0)	77 (96.3)	1	0.23
	TT	1 (10.0)	3 (3.8)	1.72 (0.67-2.84)	

Values are presented as number (%).

MTHFR, methylenetetrahydrofolate reductase; PVT, portal vein thrombosis; OR, odds ratio; CI, confidence interval; CC, wild type; CT, heterozygote; TT, homozygote.

DISCUSSION

Idiopathic PVT is a relatively rare and life-threatening vascular disorder associated with serious outcomes. Identification of risk factors associated with PVT may have important application in diagnosis, treatment, management and prognosis of this disease.

Hyperhomocysteinemia has been proposed as a significant risk factor for thrombosis, and MTHFR C677T gene variant associated with increased homocysteine levels were also proposed as a candidate risk marker for PVT [13]. However, the contribution of hyperhomocysteinemia and MTHFR C677T polymorphism in the pathogenesis of PVT remains controversial [13-15] and to date no study has been reported from Iran regarding the role of this polymorphism in PVT.

Our study clearly showed that elevated plasma tHcy level is a significant risk marker for PVT highlighted by increased prevalence of hyperhomocysteinemia among PVT patients. This result is in accordance with the results of previous studies [13,15].

In the present study, the carrier of the MTHFR 677T allele had significantly higher plasma tHcy levels than the carrier of the MTHFR 677C allele. However, the association between the MTHFR C677T variant and PVT was not significant in our study. This conflicting result may be explained with the notion that interactions between both genetic and acquired factors are essentials in determining plasma tHcy levels. Therefore, MTHFR C677T polymorphism contributes indirectly to thrombosis via influencing plasma tHcy levels [16]. Differences in environmental factors such as vitamin B12 and folate levels in different populations can significantly alter the association of this polymorphism with PVT risk [5,17].

Studies investigating the association between MTHFR C677T polymorphism and PVT have yielded inconsistent results [13-15,18-21]. Our study showed that MTHFR C677T polymorphism didn't constitute a significant risk factor for PVT in either heterozygote or homozygote states and therefore it was in agreement with the results of Vayá et al. [14] and Mangia et al. [18] that demonstrated no significant

association between MTHFR C677T polymorphism and PVT. However, our study was inconsistent with the results of other studies reporting positive correlation between this MTHFR variant and PVT [13,15,19-21]. The reason for the inconsistency between the various studies remains to be determined. However, genetic background, variation in study design, gene environmental interaction, diet, sample size, and sample selection criteria may contribute to the variation in association study results [22].

Our study was inconsistent with a recent meta-analysis study that showed MTHFR C677T polymorphism as being a significant risk factor for PVT [4]. The possible reasons for these conflicting results may be related to the presence of publication bias in the meta-analysis study which could result in overestimation of the risk due to under representation of studies that found no association. Publication bias causes a potential problem for interpretation of the results in meta-analysis studies [23]. Another possible explanation for these inconsistent results may be due to the small sample size of our study. Some investigators have shown that hyperhomocysteinemia can be safely and successfully treated with vitamin supplementation [5]. Therefore, it is advisable to check plasma tHcy levels in PVT patients routinely and all cases with elevated plasma tHcy levels should be considered for vitamin supplementation therapy. Some of the limitations of our study were lack of data regarding the vitamin B12 and folate status in the study population and the small sample size which is inherent to the rarity of idiopathic PVT. As a consequence, data obtained from this study have to be considered preliminary. More studies with large sample sizes are required to confirm the current findings and explore the interaction between genetic polymorphism, homocysteine and PVT risk.

CONCLUSION

Our study demonstrated that elevated plasma tHcy level is a significant risk factor for PVT. Additionally, MTHFR C677T is not a significant risk factor for PVT but is a determinant of elevated plasma tHcy levels.

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