Indian J Med Res 142, July 2015, pp 46-52
DOI:10.4103/0971-5916.162096

A preliminary study of inherited thrombophilic risk factors in different clinical manifestations of venous thromboembolism in central Iran

Ali Karimi, Marziyeh Abolhasani*, Morteza Hashemzadeh-Chaleshtori & Batoul Pourgheysari**

*Cellular & Molecular Research Center, *Clinical Biochemistry Research Center & **Pathology & Hematology Department, Shahrekord University of Medical Sciences, Shahrekord, Iran*

Received July 11, 2013

***Background & objectives*: Inherited thrombophilia is known to be an important risk factor for developing venous thromboembolism. Whether such abnormalities may impact the development of deep vein thrombosis (DVT) and pulmonary embolism (PE) differently is not well defined. This preliminary study was undertaken to compare thrombophilic polymorphism in patients with DVT and PE.**

***Methods*: A total of 35 DVT, 23 DVT/PE, and 37 PE patients admitted to the Hajar Hospital, Shahrekord, Iran, between October 2009 and February 2011 were included in the study and 306 healthy volunteers matched by age and sex from the same geographical area with no history of venous or arterial diseases were included as control group. Factor V Leiden (*FV* 1691G/A, rs6025), prothrombin (*FII* 20210G/A), methylene tetrahydrofulate reductase (*MTHFR* 677C/T, rs1801133), and *PLA2* polymorphisms of platelet glycoprotein IIb/IIIa (*GpIIIa* 1565T/C, rs5918) were investigated by polymerase chain reaction-restriction fragment length polymorphism.**

***Results*: The number of patients with the investigated polymorphisms and homozygous carriers was significantly different among the groups ($P<0.05$). No significant difference was observed in the presence of *FV* 1691G/A and *FII* 20210G/A between any of the patients groups and the control group. *GpIIIa* 1565T/C and homozygous *MTHFR* 677C/T polymorphisms were higher in DVT patients compared with the control group (OR=6.65, 95% CI=3.09-14.30 and OR=4.08, 95% CI=1.35-12.38, respectively).**

***Interpretation & conclusions*: As none of the investigated polymorphisms were associated with PE, other thrombophilia polymorphisms may have a role in the pathogenesis of PE in these patients and should be investigated. Because of different prognostic risk factors among different types of patients, the treatment approach could be different.**

Key words Deep vein thrombosis - *GpIIIa* 1565T/C polymorphism - pulmonary embolism - thrombophilia - thromophilia risk factors - venous thromboembolism

Venous thromboembolism (VTE) remains a serious medical condition in patients with known or unknown risk factors causing significant morbidity and mortality[1]. VTE manifests as deep vein thrombosis (DVT) and pulmonary embolism (PE). Thrombophilia is defined as a predisposition to increased risk of VTE. The importance of genetic thrombophilia factors in developing VTE has been increasingly recognized. Inherited gene disorders related to the haemostatic system have been documented as risk factors in different studies[2-5].

Factor V Leiden (*FV* 1691G/A, rs6025), prothrombin gene mutation G20210A (*FII* 20210G/A), the variant 677C/T of methylene tetrahydrofolate reductase (*MTHFR* 677C/T, rs1801133) and polymorphism A2 (*PLA2*) in platelet glycoprotein GPIIb/IIIa (*GpIIIa* 1565T/C, rs5918) are among main inherited risk factors. The prevalence of these polymorphisms is different according to ethnicity in normal population and VTE patients[2-4]. Whether these abnormalities may impact differently PE and DVT is not well defined, and the available data are controversial. FV 1691G/A is a mutation which makes factor V less susceptible to cleavage by activated protein C, FII 20210G/A polymorphism is a variant that leads to increased plasma prothrombin level[5], 677C/T polymorphism of *MTHFR* leads to an elevation of homosysteine in plasma[6,7], and *GpIIIa* 1565T/C polymorphism of *GPIIb/IIIa* increases the affinity of IIb/IIIa receptor to fibrinogen and makes the platelet more susceptible to aggregation[8]. Several studies demonstrated increased incidence of DVT than PE in carriers of FV 1691G/A[9-11], but others could not find such increased risk[12]. Such different clinical manifestations have not been reported for *FII* 20210G/A[9-11].

As there are limited data to compare PE and DVT in the context of thrombophilia in non-Caucasians, this preliminary study was conducted to compare thrombophilia polymorphisms in patients with PE and DVT and to evaluate whether such abnormalities impact these two types of patients in central Iran differently.

Material & Methods

Patients and study design: The study population comprised 35 DVT, 23 DVT/PE, and 37 PE patients who were admitted to the Hajar hospital, Shahrekord, Iran. The patients consecutively enrolled between October 2009 and February 2011 were included in the study. DVT was diagnosed by compression ultrasonography, Doppler ultrasonography, and D-dimer. PE was diagnosed by clinical presentation, chest X-ray, ventilation perfusion lung scan, electrocardiogram, and laboratory findings (blood gases and D-dimer). Patients entered into the study if they had one or more episodes of PE or DVT. Control group included 306 healthy volunteers (faculty and staff of the same hospital, matched by age and sex) from the same geographical area without history of venous or arterial diseases. Ethical approval for the study was obtained from Shahrekord University of Medical Sciences' Ethics Committee. Written consent was obtained from all patients and control participants.

All patients and control participants underwent screening for investigated selected thrombophilia polymorphisms. Five ml of venous blood was collected from the anticubital vein without venous stasis. Genomic DNA was isolated from fresh blood or frozen samples. Polymerase chain reaction followed by restriction fragment length polymorphism was performed for the four polymorphisms (Thermocycler ACTEC, PC818, Japan). Primers and restriction enzymes (Tagc, Denmark and Fermentas, Russia) are shown in Table I.

The Mn*II* restriction enzyme digested the 267bp fragment of *FV* 1691G/A in to two fragments of 200bp

Table I. Restriction enzymes and primer sequences

Genetic polymorphism	Restriction enzyme	Primer sequences
FV 1691G/A	Mn*II*	F 5′ TGC CCA GTG CTT AAC AAG ACC A 3′ R 5′ TGT TAT CAC ACT GGT GCT AA 3′
FII 20210G/A	Hind*III*	F 5′ TCT AGA AAC AGT TGC CTG GC 3′ R 5′ ATA GCA CTG GGA GCA TTG AAG C 3′
PLA1/A2	Msp*I*	F 5′ TTC TGA TTG CTG GAC TTC TCT T 3′ R 5′ TCT CTC CCC ATG GCA AAG AGT 3′
MTHFR 677C/T	Hinf*I*	F 5′ TGA AGG AGA AGG TGT CTG CGG GA 3′ R 5′ AGG ACG GTG CGG TGA GAG TG 3′

FV, factor V; *FII*, prothrombin; *PLA1/A2*, platelet glycoprotein IIb/IIIa; *MTHFR*, methylene tetrahydrofolate reductase

and 67bp, whereas in wild type of *FV*, three fragments were produced (163, 67 and 37 bp). A 345bp fragment of prothrombin gene was amplified and digested with Hind*III* restriction enzyme for detection of *FII* 20210G/A polymorphism. There is no cleavage site in wild type whereas mutated allele produces two fragments of 322 and 23bp. A 264 bp fragment was amplified and digested to two fragments (222 and 42 bp) by Msp*I* restriction enzyme in *PLA1* polymorphism, whereas digestion of *GpIIIa* 1565T/C resulted in three fragments (173, 49, and 42bp). For identification of *MTHFR* 677C/T polymorphism a 198 bp fragment was amplified and digested by Hinf*I* restriction enzyme in to two fragments of 175 and 23bp.

Statistical analysis: Statistical analysis was performed using SPSS (SPSS Inc., Chicago, USA). Descriptive analysis was used to describe patients' characteristics. Odds ratio (OR) was used to describe the strength of association between genetic polymorphisms and PE or DVT. Fisher's exact test was used to make comparisons among the groups.

Results

A total of 95 patients (41 males and 54 females) with VTE entered in to the study. There was neither significant difference in the age among PE, DVT/PE, and DVT patients (53.78±18.69, 49.01±21.20, and 48.03±18.30 yr, respectively) nor between males and females. (35.1% of PE, 52.2% of DVT/PE, and 45.7% of DVT patients were male). Distal DVT was diagnosed in 31 patients and proximal DVT in 27. The most frequent transient risk factor prior to thrombosis in DVT was immobilization. In female patients with DVT, using contraceptive was the first acquired risk factor followed by immobilization. Surgery was the most frequent transient risk factor (47.4%) before PE episodes. No significant difference was seen in the family history among the three groups. Recurrent events were diagnosed in two (5.2%) of PE patients, six (26.1%) of PE/DVT, and 13 (37.1%) of DVT.

Investigated thrombophilia abnormalities: The presence of inherited thrombophilia abnormalities are shown in Table II. Overall, 22 polymorphisms were found in 37 PE patients and 15 polymorphisms in 23 DVT/PE patients compared to 35 polymorphisms in 35 DVT patients (1 per patient). The mean was 0.49 in the control group ($P<0.05$). Patients with DVT had more than four times of investigated genetic polymorphisms than control participants (OR: 4.52, 95% CI: 1.99-10.2). Such difference was not seen between the other two groups of patients and control group. There were more homozygous polymorphisms in DVT patients compared to PE and DVT/PE and the control group. Thirteen patients had coexistence of two polymorphisms.

Table III compares the presence of each polymorphism among the four groups. No significant difference was found in the occurrence of *FV*1691GA, *FII* 20210G/A, and *MTHFR* 677C/T among the four

Table II. Comparison of the total number of polymorphisms between patients and control participants

		Number of polymorphisms (%)				
		0	1	2	3	Mean polymorphism/person
Groups (number of cases)	PE (37)	18 (48.6)	16 (43.2)	3 (8.2)	0	0.59
	DVT/PE (23)	10 (43.5)	11 (47.8)	2 (8.7)	0	0.61
	DVT (35)	8 (22.9)	19 (54.2)	8 (22.9)	0	1.0
	Control (306)	174 (57.0)	114 (37.4)	17 (5.6)	1 (0.3)	0.49
		Number of homozygous polymorphisms (%)				
		0	1	2	3	Mean polymorphism/person
Groups (number of cases)	PE (37)	35 (94.6)	2 (5.4)	0	0	0.11
	DVT/PE (23)	20 (87.0)	3 (13.0)	0	0	0.17
	DVT (35)	24 (68.6)	10 (28.6)	1 (2.8)	0	0.34
	Control (306)	290 (95.1)	15 (4.9)	0	0	0.05

PE, pulmonary embolism; DVT, deep vein thrombosis
$P<0.05$ (multiple comparisons among the groups)

Table III. Presence of investigated thrombophilia polymorphisms in the study groups

	Polymorphisms Number of affected cases (%)			
Groups (number of cases)	*FV* 1691G/A	*FII* 20210G/A	*MTHFR* 677C/T	*GpIIIa* 1565T/C
PE (37)	2 (5.4)	1 (2.7)	14 (37.8)	5 (13.5)
DVT/PE (23)	1 (4.3)	0	11 (47.8)	3 (13.0)
DVT (35)	2 (5.7)	0	18 (51.4)	15 (42.9)
Control (306)	7 (2.3)	3 (1.0)	110 (35.8)	31 (10.2)
P value	0.21	0.66	0.24	<0.001

FV 1691G/A, factor V Leiden; *FII*, prothrombin; *MTHFR*, methylene tetrahydrofolate reductase; *GpIIIa*, platelet glycoprotein GPIIIa; PE, pulmonary embolism; DVT, deep vein thrombosis; Fisher exact test (4×2 table for each polymorphism)

groups, but a significant difference was seen in GpIIIa 1565T/C (P<0.001). In comparison between patients, GpIIIa 1565T/C polymorphism was found to be more than four times higher in DVT patients compared to PE and DVT/PE (OR: 4.80, 95% CI: 1.51-15.25 and OR: 5.00, 95% CI: 1.250-19.99, respectively). Such difference was not seen in other polymorphisms between PE and DVT/PE patients, PE and DVT, and DVT/PE and DVT (data not shown in Table).

Table IV shows comparison of different polymorphisms individually between the three groups of patients and the control group. No significant difference was observed between any group of patients and the control group in the frequency of FV 1691G/A. Only one case of FII 20210G/A mutation was seen in PE patients, but not in DVT and DVT/PE, and three in the control group, with no significant difference. The frequency of *MTHFR* 677C/T polymorphism in patients subgroups was not significantly different from the control group. The prevalence of homozygosity of the polymorphism was significantly higher in DVT patients but not PE and DVT/PE, compared with the control group (P=0.02, 0.22, and 0.25, respectively). Of the 35 DVT patients, 15 carried *GpIIIa* 1565T/C polymorphism, significantly higher compared with the control group (P<0.001, OR: 6.65 and 95% CI; 3.09-14.30). No difference was seen in the frequency of this polymorphism between the other two groups and the control group.

Discussion

DVT and PE are usually considered to be similar in the context of thrombophilia. However, data on some of these polymorphisms are not consistent. In this study, we compared these two clinical manifestations of VTE and demonstrated some differences. Some of the investigated polymorphisms were associated with DVT but not with DVT/PE or PE alone. Our data demonstrated that the majority of patients showed single or multiple thrombophilic defects. Patients with DVT had significantly higher number of investigated thrombophilic polymorphisms compared with the control and the other two groups. This finding may support the hypothesis that thrombophilia leads to more stable clots which are less likely to detach and to make embolism. Previous data on the risk of PE or DVT associated with the presence of *FV*G1691A mutation are controversial. Rahimi *et al*[13] have reported the association between *FV*1691G/A mutation and DVT in Kurdish population in western Iran. Such divergence may be related to different backgrounds of Kurdish patients and ours. Gohil *et al*[14] reported a prevalence of 23.5 per cent in PE patients while Biswas *et al*[15] from India reported 10.3 per cent prevalence in DVT patients, both significantly higher compared to the control group. The homozygous *FV* 1691G/A has been found with more severe thrombotic phenotype, but the heterozygous with lower thrombotic risk[16]. Grifoni *et al*[17] found a higher prevalence in DVT compared to the control group, but not a significant difference between PE patients and the control group. The prevalence was significantly higher in DVT and PE patients in Turkey[18,19], but not in Chinese/Thai patients[14]. Although we found the frequency of the polymorphism two times higher in our DVT patients, it did not reach to a significant level, which could be related to the lower number of patients or different ethnic background. Our data did not support the hypothesis of *FV*1691G/A paradox[20] as we did not find any association between DVT and *FV* 1691G/A.

Table IV. Comparison of inherited thrombophilia polymorphisms between each group of patients and the control group

Inherited thrombophilia polymorphisms	PE patients (n=37)	DVT/PE patients (n=23)	DVT patients (n=35)	Control (n=306)
FV 1691G/A				
Number of cases (%)	2 (5.4)	1 (4.3)	2 (5.7)	7 (2.3)
OR (95% CI)	2.59 (0.52-12.98)	1.94 (0.23-16.50)	2.59 (0.52-12.98)	
FII 20210G/A				
Number of cases (%)	1 (2.7)	0	0	3 (1)
OR (95% CI)	2.8 (0.28-27.69)			
MTHFR 677C/T				
Number of cases (%)	14 (37.8)	11 (48.7)	18 (51.4)	110 (35.9)
OR (95% CI)	1.09 (0.54- 2.19)	1.63 (0.70-3.82)	1.89 (0.93-3.81)	
MTHFR 677C/T (homozygote)				
Number of cases (%)	3 (8.1)	2 (8.7)	5 (14.3)	12 (3.9)
OR (95% CI)	2.16 (0.58-8.04)	2.33 (0.49-11.2)	4.08 (1.35-12.38)	
GpIIIa 1565T/C				
Number of cases (%)	5 (13.5)	3 (13)	15 (42.9)	31 (10.1)
OR (95% CI)	1.37 (0.50-3.82)	1.33 (0.37-4.71)	6.65 (3.09-14.30)	

FV 1691G/A, factor V Leiden; *FII*, prothrombin; *MTHFR*, methylene tetrahydrofolate reductase; *GpIIIa*, platelet glycoprotein GPIIIa; PE, pulmonary embolism; DVT, deep vein thrombosis; OR, odds ratio; CI, confidence interval

Pertaining to *FII* 20210G/A and its incidence in patients with DVT or PE, the data are controversial and higher or similar prevalence has been reported compared to control group[13,14,21,22]. The increased risk of PE has been found with FII 20210G/A mutation, but decreased risk with *FV* 1691G/A in Italy[20]. No significantly difference was seen in the mutations in our patients and control groups and the frequency was lower compared with that previously reported in Caucasians and some other ethnics[5,10,14]. Our data were consistent with the studies which found no relationship between DVT or PE and this polymorphism[13,22]. The thrombophilia risk factors which were significantly associated with DVT but not PE or DVT/PE in our study were *GpIIIa* 1565T/C polymophism of platelet glycoprotein IIb/IIIa and homozygous *MTHFR* 677C/T. We have previously reported that patients with VTE have significantly higher prevalence of coinheritance of more than one polymorphism, which is reflected more clearly in *MTHFR* 677C/T/*GpIIIa* 1565T/C[23]. Here we found the highest frequency of *MTHFR* 677C/T and *GpIIIa* 1565T/C in our DVT patients. *GpIIIa* 1565T/C polymorphism increases the affinity of IIb/IIIa platelet receptor to fibrinogen and makes more aggregation as a baseline for thrombosis[8]. In this case the clots may be more stable and adherent to the vessel wall and are less likely to detach, resulting in PE. Our data were not in agreement with Ivanov *et al*[22] who found *GpIIIa* 1565T/C polymorphism higher in PE patients than in control group. Such discrepancy may be related to the ethnicity as well as risk factors and environment which equally influence the carriers and non-carriers. The association of polymorphism with arterial thrombosis has been reported in some studies[24,25].

The important finding of the present study was the prevalence of homozygous *MTHFR* 677C/T in DVT patients. The polymorphism results in a modest increase in homocysteine in plasma which may have a pathogenic significance in thrombosis[26,27]. Hyperhomocysteinemia may not be a direct cause of thrombosis but a marker of systemic or endothelial stress and platelet activation[28].

We only studied four thrombophilia polymorphisms which could be considered as a limitation of our study. The other thrombophilia polymorphisms may have a role in PE pathogenesis. Inherited additional unknown or known risk factors may contribute to thrombosis in PE patients. In addition, limited number of patients was another limitation of our study.

In conclusion, our data indicated that *FV* 1691G/A and *FII* 20210G/A polymorphisms were not associated with PE, DVT/PE, and DVT in Central Iran. The *GpIIIa* 1565T/C polymorphism of *GP IIb/IIIa* and homozygous *MTHFR* 677C/T were associated with increased risk of DVT, but not PE and DVT/PE. Other thrombophilia risk factors may have a role in PE pathogenesis and should be investigated in this population. Because of different prognostic risk factors in PE and DVT, the treatment approach could be different.

Acknowledgment

This work was supported by grant no. 884 by Deputy for Research and Technology of Shahrekord University of Medical Sciences, Shahrekord, Iran.

References

1. Beckman MG, Hooper WC, Critchley SE, Ortel TL. Venous thromboembolism: a public health concern. *Am J Prev Med* 2010; *38* (Suppl 4): S495-501.
2. Dalen JE. Should patients with venous thromboembolism be screened for thrombophilia? *Am J Med* 2008; *121* : 458-63.
3. Hooper WC, De Staercke C. The relationship between FV Leiden and pulmonary embolism. *Respir Res* 2002; *3* : 8.
4. Turkstra F, Karemaker R, Kuijer PM, Prins MH, Buller HR. Is the prevalence of the factor V Leiden mutation in patients with pulmonary embolism and deep vein thrombosis really different? *Thromb Haemost* 1999; *81* : 345-8.
5. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; *88* : 3698-703.
6. Fermo I, Vigano D'Angelo S, Paroni R, Mazzola G, Calori G, D'Angelo A. Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease. *Ann Intern Med* 1995; *123* : 747-53.
7. Bezemer ID, Doggen CJ, Vos HL, Rosendaal FR. No association between the common *MTHFR* 677C -> T polymorphism and venous thrombosis - results from the MEGA study. *Arch Intern Med* 2007; *167* : 497-501.
8. Feng D, Lindpaintner K, Larson MG, Rao VS, O'Donnell CJ, Lipinska I, *et al*. Increased platelet aggregability associated with platelet GPIII alpha Pl(A2) polymorphism - The Framingham Offspring Study. *Arterioscler Thromb Vasc Biol* 1999; *19* : 1142-7.
9. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T, *et al*. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism - pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. (Study Group for Polled - Analysis in Venous Thromboembolism). *Thromb Haemost* 2001; *86* : 809-16.
10. Martinelli I, Battaglioli T, Razzari C, Mannucci PM. Type and location of venous thromboembolism in patients with factor V Leiden or prothrombin G20210A and in those with no thrombophilia. *J Thromb Haemost* 2007; *5* : 98-101.
11. De Moerloose P, Reber G, Perrier A, Perneger T, Bounameaux H. Prevalence of factor V Leiden and prothrombin G20210A mutations in unselected patients with venous thromboembolism. *Brt J Haematol* 2000; *110* : 125-9.
12. Juul K, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. Factor V Leiden and the risk for venous thromboembolism in the adult Danish population. *Ann Intern Med* 2004; *140* : 330-7.
13. Rahimi Z, Mozafari H, Shahriari-Ahmadi A, Alimogaddam K, Ghavamzadeh A, Aznab M, *et al*. Deep venous thrombosis and thrombophilic mutations in western Iran: association with factor V Leiden. *Blood Coagul Fibrinolysis* 2010; *21* : 385-8.
14. Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism A meta-analysis involving approximately 120,000 cases and 180,000 controls. *Thromb Haemost* 2009; *102* : 360-70.
15. Biswas A, Bajaj J, Ranjan R, Meena A, Akhter MS, Yadav BK, *et al*. Factor V Leiden: Is it the chief contributor to activated protein C resistance in Asian-Indian patients with deep vein thrombosis? *Clin Chim Acta* 2008; *392* : 21-4.
16. Coppola A, Tufano A, Cerbone AM, Di Minno G. Inherited thrombophilia: Implications for prevention and treatment of venous thromboembolism. *Semin Thromb Hemost* 2009; *35* : 683-94.
17. Grifoni E, Marcucci R, Ciuti G, Cenci C, Poli D, Mannini L, *et al*. The thrombophilic pattern of different clinical manifestations of venous thromboembolism. a survey of 443 cases of venous thromboembolism. *Semin Thromb Hemost* 2012; *38* : 230-4.
18. Celik M, Altintas A, Celik Y, Karabulut A, Ayyildiz O. Thrombophilia in young patients with acute myocardial infarction. *Saudi Med J* 2008; *29* : 48-54.
19. Kupeli E, Verdi H, Simsek A, Atac FB, Eyuboglu FO. Genetic mutations in Turkish population with pulmonary embolism and deep benous thrombosis. *Clin Appl Thromb-Hemost* 2011; *17* : E87-94.
20. Rossi E, Za T, Ciminello A, Leone G, De Stefano V. The risk of symptomatic pulmonary embolism due to proximal deep venous thrombosis differs in patients with different types of inherited thrombophilia. *Thromb Haemost* 2008; *99* : 1030-4.
21. Rosendaal FR, Reitsma PH. Genetics of venous thrombosis. *J Thromb Haemost* 2009; *7* (Suppl 1): 301-4.
22. Ivanov P, Komsa-Penkova R, Kovacheva K, Ivanov Y, Stoyanova A, Ivanov I, *et al*. Impact of thrombophilic genetic factors on pulmonary embolism: early onset and recurrent incidences. *Lung* 2008; *186* : 27-36.
23. Pourgheysari B, Boroujeni HR, Hasheminia AM, Drees F. *PLA2* polymorphism of platelet glycoprotein IIb/IIIa but not Factor V Leiden and prothrombin G20210A polymorphisms is associated with venous thromboembolism and more recurrent events in central Iran. *Blood Coagul Fibrinolysis* 2013; *24* : 471-6.

24. Saidi S, Mahjoub T, Slamia LB, Ammou SB, Al-Subaie AM, Almawi WY. Polymorphisms of the human platelet alloantigens HPA-1, HPA-2, HPA-3, and HPA-4 in ischemic stroke. *Am J Hematol* 2008; *83* : 570-3.

25. Kokturk N, Kanbay A, Aydogdu M, Ozyilmaz E, Bukan N, Ekim N. Hyperhomocysteinemia prevalence among patients with venous thromboembolism. *Clin Appl Thromb Hemost* 2011; *17* : 487-93.

26. Kamat GV, Metgud SC, Pattanshetti VM, Godhi AS. A cross-sectional study to detect the prevalence of hyperhomocysteinemia in cases of deep vein thrombosis. *Indian J Surg* 2010; *72* : 323-6.

27. Hoffman M. Hypothesis: hyperhomocysteinemia is an indicator of oxidant stress. *Med Hypotheses* 2011; *77* : 1088-93.

28. Said JM, Brennecke SP, Moses EK, Walker SP, Monagle PT, Campbell J, *et al*. The prevalence of inherited thrombophilic polymorphisms in an asymptomatic Australian antenatal population. *Aust N Z J Obstet Gynaecol* 2008; *48* : 536-41.

Reprint requests: Dr Batoul Pourgheysari, Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
e-mail: bat238@yahoo.com, pourgheysari@skums.ac.ir