ORIGINAL ARTICLE

Factor V Leiden, factor V Cambridge, factor II GA20210, and methylenetetrahydrofolate reductase in cerebral venous and sinus thrombosis: A case-control study

Mohammad Saadatnia, Mansour Salehi[1], Ahmad Movahedian[2], Seyed Ziaeddin Samsam Shariat[2], Mehri Salari, Marzieh Tajmirriahi, Elham Asadimobarakeh, Rasoul Salehi[1], Gilda Amini[1], Homa Ebrahimi[3], Ehsan Kheradmand

Department of Neurology, Isfahan Neurosciences Research Center, [1]Department of Genetic, Isfahan University of Medical Sciences, [2]Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences and Isfahan Pharmaceutical Sciences Research Center, [3]Department of Neurology, Islamic Azad University, Najafabad Branch, Najafabad, Isfahan, Iran

Background: Factor V G1691A (FV Leiden), FII GA20210, and methylenetetrahydrofolate reductase (MTHFR) C677T mutations are the most common genetic risk factors for thromboembolism in the Western countries. However, there is rare data in Iran about cerebral venous and sinus thrombosis (CVST) patients. The aim of this study was to evaluate the frequency of common genetic thrombophilic factors in CVST patients. **Materials and Methods:** Forty consequently CVST patients from two University Hospital in Isfahan University of Medical Sciences aged more than 15 years from January 2009 to January 2011 were recruited. In parallel, 51 healthy subjects with the same age and race from similar population selected as controls. FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene mutations by polymerase chain reaction technique were evaluated in case and control groups. **Results:** FV Leiden, FII GA20210, and FV Cambridge gene mutations had very low prevalence in both case (5%, 2%, 0%) and control (2.5%, 0%, 0%) and were not found any significant difference between groups. MTHFR C677T mutations was in 22 (55%) of patients in case group and 18 (35.5%) of control group (P = 0.09). **Conclusion:** This study showed that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low. Laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost benefit.

Key words: Cerebral venous and sinus thrombosis, factor II GA20210, factor V Cambridge, factor V Leiden, methylenetetrahydrofolate reductase

How to cite this article: Saadatnia M, Salehi M, Movahedian A, Samsam Shariat S, Salari M, Tajmirriahi M, Asadimobarakeh E, Salehi R, Amini G, Ebrahimi H, Kheradmand E. Factor V Leiden, factor V Cambridge, factor II GA20210, and methylenetetrahydrofolate reductase in cerebral venous and sinus thrombosis: A case-control study. J Res Med Sci 2015;20:554-62.

INTRODUCTION

Venous thrombosis is the third most common cardiovascular affliction after ischemic heart disease and stroke.[1] Cerebral venous and sinus thrombosis (CVST) is a rare presentation of venous thrombosis,[2] however, that was found to be the second common cause of stroke in young women,[3] and associated with substantial mortality and morbidity.[4] Various risk factors can be identified in 70-80% of patients, such as local infections (middle ear or facial skin infections), thrombophilic states (factor V [FV] Leiden [G1691A] gene mutation; methylenetetrahydrofolate reductase [MTHFR] C677T; deficit of antithrombin III, protein C, and protein S; FII GA20210 mutation; hyperhomocysteinemia with or without elevated factor VIII levels; and antiphospholipid syndrome), systemic inflammatory diseases (for example Behcet's disease), medication treatment (contraception), pregnancy, and the puerperium,[5-7] and in about 20% no risk factor is

Access this article online	
Quick Response Code:	Website: www.jmsjournal.net
	DOI: ****

Address for correspondence: Dr. Mohammad Saadatnia, Department of Neurology, Al Zahra Hospital, Sofeh Street, Isfahan, Iran.
E-mail: mosaadatnia@yahoo.com
Received: 23-03-2014; **Revised:** 24-12-2014; **Accepted:** 04-08-2015

identified.[2] FV G1691A (Leiden), FII GA20210, and MTHFR C677T mutations are the most common genetic risk factors for thromboembolism in Western countries[8,9] however, there is rare data about the association of these mutation and CVST in Iran. Therefore, the aim of this study is to evaluate the frequency of common genetic thrombophilic factors in CVST and to offer practical suggestion for doing laboratory investigations in CVST patients.

MATERIALS AND METHODS

Patients and control subjects

Forty CVST patients from two University Hospital in Isfahan University of Medical Sciences aged more than 15 years from January 2009 to January 2011 were recruited consequently. Patients were diagnosed according to accepted criteria with magnetic resonance imaging and venography by the absence of normal hypointense flow void in the involved sinuses and if needed angiography. In parallel 51 healthy subjects with age- and sex-matched from the same race (Iranian) selected as controls.

Blood sample collection and coagulation tests

After obtaining written informed consent for genetic analysis, 10 ml of peripheral blood was collected into ethylenediaminetetraacetic acid and deoxyribonucleic acid (DNA) analyzed for FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene mutations (Arg306Thr). Genetic test was done for all case and control, however, other following known etiologic factor was evaluated in CVST patients; antithrombin activity (amigdolytic assay); protein C activity; protein S antigen, anticardiolipin and antiphospholipid antibodies, and fasting homocysteine by enzyme-linked immunosorbent assay method and lupus anticoagulants by sensible partial thromboplastin time and Russell's viper venom time test. For antiphospholipid and anticardiolipin the positive result was higher than 10 GPL and for homocysteine the level higher than 14 was considered as hyperhomocysteinemia. For Antithrombin activity; protein C activity and protein S level, the blood sample was taken 6 months later than the acute phase and after finishing anticoagulant therapy.

Deoxyribonucleic acid extraction and analysis

Total genomic DNA was extracted from peripheral blood leucocytes by the salting-out procedure. DNA fragment of the FV Leiden was amplified and digested with Mnl I and Nla III (New England Biolabs). In the allele-specific polymerase chain reaction (PCR), the presence of the FV G1691A (Leiden) mutation was identified by using two primers: Sense primer FV1-TGC CCA GTG CTT AAC AGA CCA and antisense primer FV2A-TCT CTT GAA GGA AAT GCC CCA TTA, to prime for fragment 1 (F1); or FV2B-AAG GAC AAA AGT ACC TGT ATT CCA, to prime for F2. PCR was performed using 2U of Taq polymerase enzyme (Promega) and 1.5 mmol/L of magnesium chloride ($MgCl_2$), 500 ng of DNA, and 25 pmol of primers, respectively. The amplification conditions comprise of a denaturation step of 1 cycle at 95°C for 5 min followed by 1 cycle of denaturation (95°C, 1 min), annealing (60°C, 1 min), and 35 cycle of extension (72°C, 1 min). For optimized amplification of F2 dimethyl sulfoxide was added.

Products were resolved on 2% agarose gel and examined after staining with ethidium bromide. The same protocol was used for other mutation but primers and restriction enzyme used for FV Cambridge, MTHFR C677T, FII GA20210 were 5′ TGT CTT TCT GTC CTA AC 3′ and 5′ TCT TGA ACC TTT GCC CA 3′, BstNI; 5′ TGA AGG AGA AGG TGT CTG CGG GA 3′ and 5′ AGG ACG GTG CGG TGA GAG TG 3′, Hinf I; 5′ TCT AGA AAC AGT TGC CTG GC 3′ and 5′ ATA GCA CTG GGA GCA TTG AAG C 3′, HindIII, respectively. Also, we add 50 mmol/L of Tris-HCL (PH9), 50 mmol/L of KCL, and 1% Triton X-100 only for MTHFR amplification.

Review

Additionally, we performed a narrative review of all studies that evaluated FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene in CVST and venous thromboembolic disease. Key words were CVST, deep vein thrombosis, venous thromboembolic disease, and FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene mutations. We searched Medline-PubMed, ISI, Scopus, and Cochrane databases up to 2014. We searched references lists from articles identified by search as well as a key review article to identify additional articles. We identified case-control, case series, and case report.

Statistical analysis

The results were analyzed by SPSS for Windows version 18 (SPSS Inc., Chicago, IL, USA). The variables were compared between patients and control groups by Student's *t*-test or with Mann-Whitney U test or Fisher's exact test, as appropriate. We also calculated the crude odds ratio (OR) and 95% confidence intervals. $P < 0.05$ were considered as significant. The results were reported as the mean ± standard deviation (SD).

RESULTS

Table 1 shows clinical features of patients. The most frequent symptom was a headache (92.5%), and the most frequent sign was pupil edema. Mean age of CVST and controls was 33.45 (SD: 10.55) and 30.75 (SD: 9.01), respectively ($P = 0.19$); also, sex distributions between two groups have not any significant difference ($P = 0.085$). Venous infarction occurred in 28 (70%) patients and hemorrhagic infarct in

14 (35%). Frequency of etiologic factors showed in Table 2. The prevalence of hyperhomocysteinemia was significantly higher in patients than controls (OR: 2.732 [1.041-7.194]) (P = 0.033). FV G1691A (Leiden), FII GA20210, and FV Cambridge gene mutations (Arg306Thr) mutant had very low prevalence in both case and control and no significant difference found between two groups [Table 3]. MTHFR C677T mutation had not significant differences between groups (P = 0.09).

DISCUSSIONS

In this study we found low prevalence and nonsignificant differences of genomic mutations of FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene (Arg306Thr) in case and control groups in our population in central area of Iran. However, MTFHR mutations had more prevalent in CVST group with near significant differences, but other mutations had very low prevalence in both groups without significant differences.

The mutation in FV Leiden G1691A and the prothrombin gene G20210A are the two most prevalent identified risk factor of inherited thrombophilia. Table 4 showed the studies and the correlations of genomic mutations of FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene (Arg306Thr) with CVST in different regions of the world. The frequency of FV 1691A in patients with CVT varied from 3.7% to 25%, for prothrombin 20210A was found to be from 0% to 20% and for MTHFR 677TT frequency was reported from 0 to 36%.[13] However, two systematic meta-analysis showed that the summary OR for developing CVST was almost 2-3 in FV Leiden carrier, 5.5-9 in FII GA20210 carrier, and 2-4 in MTHFR C677T carrier.[69-71]

There are few studies investigating its association with CVST in the Middle East. Rahimi *et al.* study showed that in the Western population of Iran FV Leiden (16.7 %) but not FII GA20210 and MTHFR C677T mutation as a risk factor for CVST.[33] Otrock *et al.* study also showed 32.1% CVST patients in Lebanon had FV Leiden mutation.[43] The result of this study regarding to MTHFR C677T and FII GA20210 mutation was in accordance with Rahimi *et al.* study.[33] With regard to Table 4, it appears that in Iran the prevalence of FV G1691A (Leiden), FII GA20210, and FV Cambridge gene (Arg306Thr) much lower than the Western countries.

Race ethnic differences may account for the heterogeneous distribution of inherited thrombophilia. From the West to the East the frequency of FII G20120A mutation and FV Leiden mutation and correlation with CVST become less. In North America and Europe, this frequency and its correlation with CVST was high and in the Middle East is moderate and in Asia is low. Therefore, it appears higher frequency of FII G20120A and FV Leiden mutations in white Caucasian in the North and South America, moderate frequency in Caucasian in the Middle East and lower frequency in Asian and African (references).

Table 1: Clinical feature of the CVST patients at admission

Symptom and sign	CVST (*n* = 40) (%)
Headache	37 (92.5)
Pupil edema	33 (82.5)
Weakness	20 (50)
Loss of consciousness	18 (45)
Cognitive impairment	17 (42.5)
Seizure	9 (22.5)
Meningeal sign	5 (12.5)
Coma	2 (5)

CVST = Cerebral venous and sinus thrombosis

Table 2: Frequency of etiologic factors in CVST patients

Etiologic factors	*n* (%)
Hypercoagulable state	
Oral contraceptives	25 (62.5)
Anticardiolipin antibodies IgM	11 (27.5)
Anticardiolipin antibodies IgG	6 (15)
Antiphospholipid antibodies IgG	8 (20)
Antiphospholipid antibodies IgM	6 (15)
Nonanticardiolipin antibodies IgM	2 (5)
Nonanticardiolipin antibodies IgG	2 (5)
Lupus anticoagulants	0 (0)
ANA	1 (2.5)
ANCA	1 (2.5)
Hyperhomocysteinemia	10 (25)
Protein S deficiency	0 (0)
Protein C deficiency	0 (0)
Antithrombin III deficiency	0 (0)
Puerperium and pregnancy	4 (10)
Surgery	1 (2.5)
Infection disease	0 (0)
Trauma	0 (0)
Malignancy	0 (0)
Unknown etiology	10 (25)

CVST = Cerebral venous and sinus thrombosis; ANCA = Antineutrophil cytoplasmic antibodies; ANA = Antinuclear antibodies

Table 3: Frequency of factor V G1691A (FV Leiden), FII GA20210, MTHFR C677T and factor V Cambridge gene mutations in case and control groups

Mutation	Patient's *n* (%)	Controls *n* (%)	OR (95% CI)	*P*
Factor V Leiden G1691A	2 (5)	1 (2)	0.380 (0.33-4.384)	0.83
Factor II G20210A	1 (2.5)	0 (0)	0	0.903
MTHFR C677T	22 (55)	18(35.5)	0.446 (0.191-1.041)	0.096
Heterozygous	18 (56.3)	14 (43.8)		0.16
Homozygous	4 (50)	4 (50)		
Factor V Cambridge	0 (0)	0 (0)	0	

MTHFR = Methylenetetrahydrofolate reductase; OR = Odds ratio; CI = Confidence interval

Table 4: Summary of studies involving factor V Leiden, factor V Cambridge, factor II GA20210 and MTFHR in cerebral venous and sinus thrombosis

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study
					CVST	DVT	
Brasil	Gadelh, *et al.*, 2005[10]		26	217			Case-control
		G20210A	6 (23)	3 (1)	Yes		
		Factor V Leiden	2 (8)	3 (1)	No		
		MTHFR	1 (7)	15 (4)	No		
	Milano, *et al.*, 2003[11]		1				Case report
		G20210A			Yes		
	Rodrigues, *et al.*, 2004[12]		42	134			Case-control
		G20210A	7 (16.7)	1 (0.7)	Yes		
		Factor V Leiden	2 (4.8)	3 (2.2)	No		
	Voetsch, *et al.*, 2000[13]		14	225			Case-control
		G20210A	2 (14)	5 (2.5)	Yes		
		Factor V Leiden	1 (7.1)	13 (5.8)	No		
		MTHFR	0 (0)	45 (20)	No		
	Camargo, *et al.*, 2005[14]		50				Case series
		G20210A	8.70				
		Factor V Leiden	19.50				
Belgium	Simons, *et al.*, 2000[15]		2				Case report
		G20210A			Yes		
Canada	Eikelboom, *et al.*,1999[16]		1				Case report
		G20210A			Yes		
		Factor V Leiden			Yes		
China	Zheng, *et al.*, 2000[17]		145	122			Case-control
		G20210A	0 (0)	0 (0)	No	No	
		MTHFR	62 (53/9)	45 (36/9)	Yes	Yes	
	Yanqing, *et al.*, 2003[18]		364	140			Case-control
		Factor V Leiden	12 (3.2)	7 (5)	No	No	
	Zhang, *et al.*, 2010[19]		120	110			Case-control
		Cambridge II	0 (0)	0 (0)	No		
France	Zuber, *et al.*,1996[20]		19	57			Case-control
		Factor V Leiden	4 (21)	1 (2)	Yes		
	Biousse, *et al.*,1998[21]		35				Case series
		G20210A	2 (5.7)				
	Benbih, *et al.*, 2008[22]		1				Case report
		Factor V Leiden			Yes		
Germany	Weih, *et al.*, 1998[23]		12	187			Case-control
		Factor V Leiden	3 (25)	1 (0.53)			
	Lüdemann, *et al.*, 1998[24]		55	272			Case-control
		Factor V Leiden	8 (14.5)	17 (6.25)	Yes		
	Reuner, *et al.*, 1998[25]		45	354			Case-control
		G20210A	4 (8.9)	8 (2.3)	Yes		
	Weih, *et al.*, 1998[26]		1				Case report
		G20210A			Yes		
		Factor V Leiden			Yes		
	Weih, *et al.*, 2000[27]		33				Case series
		G20210A	4 (12)		Yes		
		Factor V Leiden	8 (24)		Yes		
	Heckmann, *et al.*, 2001[28]		3				Case report
		G20210A			Yes		
	Meenakshi-Sundaram, *et al.*, 2005[29]		1				Case report
		Factor Leiden			Yes		
	Lichy, *et al.*, 2006[30]		77	203			Case-control
		G20210A	8 (10.4)	5 (2.5)	Yes		
		Factor V Leiden	22 (28.6)	15 (7.4)	No		

(Continued)

Table 4: (Continued)

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study
					CVST	DVT	
Greece	Selvi, *et al.*, 2009[31]		1				Case report
		Factor V Leiden			Yes		
India	Biswas A, *et al.*, 2008[32]		155	120			Case-control
		Factor V Leiden	16 (10.3)	1(0.8)			
		Factor V Cambridge	0 (0)	0(0)	No		
Iran	Rahimi, *et al.*, 2010[33]		24	100			Case-control
		G20210A	0 (0)	1 (1)	No		
		Factor V Leiden	4 (16.7)	2 (2)	Yes		
		MTHFR	14 (58.3)	44 (44)	Yes		
Italy	Martinelli, *et al.*, 1996[34]		25	75			Case-control
		Factor V Leiden	5 (20)	2 (2.7)	Yes		
	Martinelli, *et al.*, 1998[35]		120	120			Case-control
		G20210A	22 (38)	3 (3)	Yes	Yes	
		Factor V Leiden	21 (34)	3 (3)	Yes	Yes	
	Madonna, *et al.*, 2000[36]		10	259			Case-control
		G20210A	5 (50)	16 (6.3)		Yes	
		Factor V Leiden	1 (10)	15 (5.8)			
		MTHFR	3 (33.3)	45 (17.4)			
	Rigamonti, *et al.*, 2002[37]		3				Case report
		MTHFR				Yes	
	Boncoraglio, *et al.*, 2004[38]		26	100			Case-control
		G20210A	3 (11/5)	3 (3)	No		
		Factor V Leiden	0 (0)	3 (3)	Yes		
		MTHFR	7 (27)	25 (25)	No		
		Homocystein	10 (38/5)	13 (13)	No		
	Tufano, *et al.*, 2014[39]		56	184			Original article
		Factor V Leiden	59 (5.4)	198 (8.2)	Yes		
		G20210A	71 (29.1)	193 (5.5)	Yes		
	Ventura, *et al.*, 200[40]		30	40			Case-control
		G20210A	9 (30)	1 (2.5)	Yes		
		Homocystein	13 (43/3)	4 (10)	Yes		
	Colaizzo, *et al.*, 2007[41]		184	286			Case-control
		G20210A	21 (11.4)	9 (3.1)	Yes	Yes	
		Factor V Leiden	15 (8.1)	10 (3.5)	No	Yes	
Lebanon	Uthman, *et al.*, 2004[42]		1				Case report
		Factor V Leiden			Yes		
		MTHFR			Yes		
	Otrock, *et al.*, 2008[43]		16				Case series
		G20210A	0 (0)		No		
		Factor V Leiden	5 (31.2)		Yes		
		MTHFR	8 (50)		Yes		
Mexico	Cantu, *et al.*, 2004[44]		45	90			Case-control
		MTHFR	10 (22)	9 (10)	Yes		
Poland	Kurkowska-Jastrzebska, *et al.*, 2003[45]		1				Case report
		G20210A Factor V Leiden			Yes		
Portugal	Verdelh, *et al.*, 2001[46]		1				Case report
		G20210A			Yes		
Spain	Sánchez Del Rio, *et al.*, 1996[47]		1				Case report
		Factor V Leiden			Yes		
	Alvarez, *et al.*, 1999[48]		64	103			Case-control
		G20210A	2 (2.7)	3 (3)	Yes	No	
		Factor V Leiden	9 (14/1)	1 (1)	Yes	Yes	

(Continued)

Table 4: (Continued)

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study
					CVST	DVT	
	Mira, *et al*., 2000[49]		36				Case series
		G20210A	6 (17)				
		Factor V Leiden	7 (19)				
	Mira, *et al*., 2002[50]		1				Case report
		Factor V Leiden			Yes		
	Ortín, *et al*., 2004[51]		1				Case report
		G20210A			Yes		
	Madroñero-Vuelta , *et al*., 2004[52]		1				Case report
		G20210A			Yes		
	Romero, *et al*., 2007[53]		15	15			Case-control
		G20210A	2 (13/3)	1 (6.6)	Yes		
		Factor V Leiden	4 (26.6)	8.30	Yes		
		MTHFR	6 (40)	8 (15)	No		
Sweden	Barba *et al*., 2008[54]	Cambridge II	1		Yes		Case report
Switzerland	Wilder-Smith, *et al*., 1997[55]		1				Case report
		Factor V Leiden			Yes		
Tunisia	Salem-Berrabah, *et al*., 2011[56]	Factor V Leiden	1		Yes		Case report
Turkey	Altinisik, *et al*., 2008[57]		50	25			Case-control
		G20210A	2 (4)	2 (8)	No	No	
		Factor V Leiden	3 (6)	0 (0)	No	No	
	Yakaryilmaz, *et al*., 2009[58]		1				Case report
		G20210A			Yes		
		Factor V Leiden			Yes		
UK USA	Ozkurt, *et al*., 2011[59]		1				Case report
		Factor V Leiden			Yes		
		MTHFR					
	Kellett, *et al*., 1998[60]		1				Case report
		G20210A			Yes		
	Hillier, *et al*., 1998[61]		15	300			Case-control
		G20210A	0 (0)	4 (1)			
		Factor V Leiden	2 (14)	24 (8)			
		MTHFR	4 (28)	136 (45)			
	Hourihane, *et al*., 1997[62]		1				Case report
		Factor V Leiden			Yes		
	Dulli, *et al*., 1996[63]		3				Case report
		Factor V Leiden			Yes		
	Liu, *et al*., 2000[64]		1				Case report
		G20210A			Yes		
		Factor V Leiden			Yes		
	Maag, *et al*., 2003[65]		1				Case report
		Factor V Leiden			Yes		
	Stephan, *et al*., 2003[66]		1				Case report
		Factor V Leiden			Yes		
	Porres-Aguilar, *et al*., 2007[67]		1				Case report
		G20210A			Yes		
	Kanaan, *et al*., 2008[68]		1				Case report
		MTHFR			Yes		
	Mahadeo, *et al*., 2010[69]		1				Case report

MTHFR = Methylenetetrahydrofolate reductase; CVST = Cerebral vein and sinus thrombosis; DVT = Deep vein thrombosis

Only one case report study in the Western countries showing correlation FV Cambridge and CVST,[54] however, in this study we did not find this mutation in our sample of Iranian population.

This study showed that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low in our case and control groups. Laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost benefit although the low sample size may be a limitation factor.

On the other hand with regard to high prevalence of CVST and venous thromboembolism in Iran, the Middle East and the East Asia[72-74] maybe other genomic thrombophilic risk factors is responsible for venous thrombosis, therefore it appears the role of other new mutations in the future should be investigated.

Most studies similar to this study showed a high prevalence of oral contraceptives pills (OCPs) as a risk factor in CVST.[70,75-79] However, the coexistence of OCPs and some prothrombotic states such as FV Leiden, GA20210, and hyperhomocysteinemia lead to magnify the risk of CVST in women.[79]

CONCLUSION

The findings of this study indicated that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low. So,laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost beneficial. Further studies with larger sample size and investigation of all thrombophilic risk factors is recommended.

Acknowledgments

The authors greatly appreciate all members of genetic department of Isfahan University of Medical Sciences.

Financial support and sponsorship

This study was supported by a grant from Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

MS conceived and designed the study, recruited samples and contributed to discussion and revision of the manuscript,MS analyzed the data and wrote the manuscript. All authors discussed the results and reviewed and edited the manuscript.

REFERENCES

1. Kniffin WD, Baron JA, Barret J, Birkmeyer JD, Anderson FA, Jr. The epidemiology of diagnosed pulmonary embolism and deep venous thrombosis in the elderly. Arch Intern Med 1994154861-866.866.
2. Koopman K, Uyttenboogaart M, Hendriks HG, Luijckx GJ, Cramwinckel IR, Vroomen PC, *et al.* Thromboelastography in patients with cerebral venous thrombosis. Thromb Res 2009;124:185-8.
3. Aaron S, Alexander M, Maya T, Mathew V, Goel M, Nair SC, *et al.* Underlying prothrombotic states in pregnancy associated cerebral venous thrombosis. Neurol India 2010; 58:555-9.
4. Kenet G, Kirkham F, Niederstadt T, Heinecke A, Saunders D, Stoll M, *et al.* Risk factors for recurrent venous thromboembolism in the European collaborative paediatric database on cerebral venous thrombosis: A multicentre cohort study. Lancet Neurol 2007;6:595-603.
5. Bousser MG, Ferro JM. Cerebral venous thrombosis: An update. Lancet Neurol 2007;6:162-70.
6. Kapessidou Y, Vokaer M, Laureys M, Bier JC, Boogaerts JG. Case report: Cerebral vein thrombosis after subarachnoid analgesia for labour. Can J Anaesth 2006;53:1015-9.
7. Procházka M, Procházka V, Lubušký M, Procházková J, Hrbáč T. Cerebral venous thrombosis in the users of hormonal contraceptives. Cesk Slov Neurol N 2007;70/103:678-84.
8. Dordevic V, Rakicevic L, Spasic M, Mikovic D, Kovac M, Radojkovic D. Factor V Leiden, FII G20210A, MTHFR C677T mutations as risk factors for venous thrombosis during pregnancy and puerperium. Vojnosanit Pregl 2005;62:201-5.
9. Iorio A, Barnes C, Vedovati MC, Favaloro EJ. Thrombophilia and cerebral vein thrombosis. Front Neurol Neurosci 2008;23:55-76.
10. Gadelha T, André C, Jucá AA, Nucci M. Prothrombin 20210A and oral contraceptive use as risk factors for cerebral venous thrombosis. Cerebrovasc Dis 2005;19:49-52.
11. Milano JB, Arruda WO, Nikosky JG, de Meneses MS, Ramina R. Cerebral and systemic venous thrombosis associated to prothrombin G20210 mutation: Case report. Arq Neuropsiquiatr 2003;61:1042-4.
12. Rodrigues CA, Rocha LK, Morelli VM, Franco RF, Lourenço DM. Prothrombin G20210A mutation, and not factor V Leiden mutation, is a risk factor for cerebral venous thrombosis in Brazilian patients. J Thromb Haemost 2004;2:1211-2.
13. Voetsch B, Damasceno BP, Camargo EC, Massaro A, Bacheschi LA, Scaff M, *et al.* Inherited thrombophilia as a risk factor for the development of ischemic stroke in young adults. Thromb Haemost 2000;83:229-33.
14. Camargo EC, Massaro AR, Bacheschi LA, D'Amico EA, Villaça PR, Bassitt RP, *et al.* Ethnic differences in cerebral venous thrombosis. Cerebrovasc Dis 2005;19:147-51.
15. Simons PJ, Vanhooren G, Longstreth WT Jr, Colven RM. Cerebral venous thrombosis and the G20210A mutation of factor II. Stroke 2000;31:543-4.
16. Eikelboom JW, Ivey L, Ivey J, Baker RI. Familial thrombophilia and the prothrombin 20210A mutation: Association with increased thrombin generation and unusual thrombosis. Blood Coagul Fibrinolysis 1999;10:1-5.
17. Zheng YZ, Tong J, Do XP, Pu XQ, Zhou BT. Prevalence of methylenetetrahydrofolate reductase C677T and its association with arterial and venous thrombosis in the Chinese population. Br J Haematol 2000;109:870-4.
18. Yanqing H, Fangping C, Qinzhi X, Zaifu J, Guangping W, Xiaoxia Z, *et al.* No association between thrombosis and factor V gene polymorphisms in Chinese Han population. Thromb Haemost 2003;89:446-51.
19. Zhang GS, Tang YM, Tang MQ, Qing ZJ, Shu C, Tang XQ, *et al.* Antithrombin Cambridge II(A384S) mutation frequency and antithrombin activity levels in 120 of deep venous thrombosis and

150 of cerebral infarction patients in a single center in Southern China. Blood Coagul Fibrinolysis 2010;21:588-91.
20. Zuber M, Toulon P, Marnet L, Mas JL. Factor V Leiden mutation in cerebral venous thrombosis. Stroke 1996;27:1721-3.
21. Biousse V, Conard J, Brouzes C, Horellou MH, Ameri A, Bousser MG. Frequency of the 20210 G-->A mutation in the 3'-untranslated region of the prothrombin gene in 35 cases of cerebral venous thrombosis. Stroke 1998;29:1398-400.
22. Benbih M, de Maistre E, Lecompte T, Mainard D, Laprevote MC, Alhenc-Gelas M, *et al.* Multiple osteonecroses and venous thrombosis: One case of patient with a novel mutation of protein C (N102S) and heterozygous for FV Leiden. Ann Biol Clin (Paris) 2008;66:437-40.
23. Weih M, Vetter B, Ziemer S, Mehraein S, Valdueza JM, Koscielny J, *et al.* Increased rate of factor V Leiden mutation in patients with cerebral venous thrombosis. J Neurol 1998;245:149-52.
24. Lüdemann P, Nabavi DG, Junker R, Wolff E, Papke K, Buchner H, *et al.* Factor V Leiden mutation is a risk factor for cerebral venous thrombosis: A case-control study of 55 patients. Stroke 1998;29:2507-10.
25. Reuner KH, Ruf A, Grau A, Rickmann H, Stolz E, Jüttler E, *et al.* Prothrombin gene G20210-->A transition is a risk factor for cerebral venous thrombosis. Stroke 1998;29:1765-9.
26. Weih M, Mehraein S, Valdueza JM, Einhäupl KM, Vetter B, Kulozik AE. Coincidence of factor V Leiden mutation and a mutation in the prothrombin gene at position 20210 in a patient with puerperal cerebral venous thrombosis. Stroke 1998;29:1739-40.
27. Weih M, Vetter B, Castell S, Ziemer S, Kulozik AE, Einhäupl KM. Hereditary thrombophilia in cerebral venous thrombosis. Cerebrovasc Dis 2000;10:161-2.
28. Heckmann JG, Tomandl B, Erbguth F, Neidhardt B, Zingsem H, Neundörfer B. Cerebral vein thrombosis and prothrombin gene (G20210A) mutation. Clin Neurol Neurosurg 2001;103:191-3.
29. Meenakshi-Sundaram S, Sridhar R, Jithendrian JJ, Durai RN, Arunkumar MJ, Sundar B. Recurrent venous thrombosis with factor V Leiden mutation. J Assoc Physicians India 2005;53:642-4.
30. Lichy C, Dong-Si T, Reuner K, Genius J, Rickmann H, Hampe T, *et al.* Risk of cerebral venous thrombosis and novel gene polymorphisms of the coagulation and fibrinolytic systems. J Neurol 2006;253:316-20.
31. Selvi A, Diakou M, Giannopoulos S, Zikou AK, Argyropoulou MI, Kyritsis AP. Cerebral venous thrombosis in a patient with sarcoidosis. Intern Med 2009;48:723-5.
32. 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.
33. Rahimi Z, Mozafari H, Bigvand AH, Doulabi RM, Vaisi-Raygani A, Afshari D, *et al.* Cerebral venous and sinus thrombosis and thrombophilic mutations in Western Iran: Association with factor V Leiden. Clin Appl Thromb Hemost 2010;16:430-4.
34. Martinelli I, Landi G, Merati G, Cella R, Tosetto A, Mannucci PM. Factor V gene mutation is a risk factor for cerebral venous thrombosis. Thromb Haemost 1996;75:393-4.
35. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. N Engl J Med 1998; 338:1793-7.
36. Madonna P, De Stefano V, Coppola A, Albisinni R, Cerbone AM. G20210A PRTH gene mutation and other trombophilic polymorphisms in patients with cerebral vein thrombosis. Stroke 2000;31:1787-8.
37. Rigamonti A, Carriero MR, Boncoraglio G, Leone M, Bussone G. Cerebral vein thrombosis and mild hyperhomocysteinemia: Three new cases. Neurol Sci 2002;23:225-7.
38. Boncoraglio G, Carriero MR, Chiapparini L, Ciceri E, Ciusani E, Erbetta A, *et al.* Hyperhomocysteinemia and other thrombophilic risk factors in 26 patients with cerebral venous thrombosis. Eur J Neurol 2004;11:405-9.
39. Tufano A, Guida A, Coppola A, Nardo A, Di Capua M, Quintavalle G, *et al.* Risk factors and recurrent thrombotic episodes in patients with cerebral venous thrombosis. Blood Transfus 2014;12 Suppl 1:s337-42.
40. Ventura P, Cobelli M, Marietta M, Panini R, Rosa MC, Salvioli G. Hyperhomocysteinemia and other newly recognized inherited coagulation disorders (factor V Leiden and prothrombin gene mutation) in patients with idiopathic cerebral vein thrombosis. Cerebrovasc Dis 2004;17:153-9.
41. Colaizzo D, Amitrano L, Iannaccone L, Vergura P, Cappucci F, Grandone E, *et al.* Gain-of-function gene mutations and venous thromboembolism: Distinct roles in different clinical settings. J Med Genet 2007;44:412-6.
42. Uthman I, Khalil I, Sawaya R, Taher A. Lupus anticoagulant, factor V Leiden, and methylenetetrahydrofolate reductase gene mutation in a lupus patient with cerebral venous thrombosis. Clin Rheumatol 2004;23:362-3.
43. Otrock ZK, Taher AT, Shamseddeen WA, Mahfouz RA. Thrombophilic risk factors among 16 Lebanese patients with cerebral venous and sinus thrombosis. J Thromb Thrombolysis 2008;26:41-3.
44. Cantu C, Alonso E, Jara A, Martínez L, Ríos C, Fernández Mde L, *et al.* Hyperhomocysteinemia, low folate and vitamin B12 concentrations, and methylene tetrahydrofolate reductase mutation in cerebral venous thrombosis. Stroke 2004;35:1790-4.
45. Kurkowska-Jastrzebska I, Wicha W, Dowzenko A, Vertun-Baranowska B, Pytlewski A, Boguslawska R, *et al.* Concomitant heterozygous factor V Leiden mutation and homozygous prothrombin gene variant (G20210A) in patient with cerebral venous thrombosis. Med Sci Monit 2003;9:CS41-5.
46. Verdelho A, Ferro JM, Palmeiro A, Antunes C. Cerebral venous thrombosis and familial prothrombin gene G20210A mutation. Rev Neurol 2001;33:335-8.
47. Sánchez Del Rio M, Gómez-Tortosa E, Outeriño J. Cerebral venous thrombosis and activated protein C resistance. Stroke 1996;27: 1440-1.
48. Alvarez A, Barroso A, Robledo M, Arranz E, Outeiriño J, Benítez J. Prevalence of factor V Leiden and the G20210A mutation of the prothrombin gene in a random group of patients with thrombotic episodes. Sangre (Barc) 1999;44:7-12.
49. Mira Y, Aznar J, Estellés A, Vayá A, Villa P, Ferrando F. Congenital and acquired thrombotic risk factors in women using oral contraceptives: Clinical aspects. Clin Appl Thromb Hemost 2000; 6:162-8.
50. Mira Y, Alfaro A, Estellés A, Vayá A, Ferrando F, Villa P. Cerebral venous thrombosis associated to homozygous factor V Leiden mutation in a female of Syrian origin. Haematologica 2002;87:ELT02.
51. Ortín X, Ugarriza A, Escoda L, Mesa R. Mutation of the prothrombin gene (G20210A) in a patient with cerebral venous sinus thrombosis and bilateral deep vein thrombosis. Med Clin (Barc) 2004;122:158.
52. Madroñero-Vuelta AB, Sanahuja-Montesinos J, Bergua-Llop M, Araguás-Arasanz C. Cerebral venous thrombosis associated to subacute De Quervain's thyroiditis in a carrier for the G20210A mutation of the prothrombin gene. Rev Neurol 2004;39:533-5.
53. Romero A, Marco P, Verdú J, Sánchez S, Castaño V. Genetic thrombophilia and cerebral venous thrombosis. Med Clin (Barc) 2007;128:655-6.
54. Barba P, Santamaría A, Tirado I, Martí E, Fontcuberta J. Antithrombin Cambridge II mutation as a risk factor to develop cerebral venous thrombosis. Thromb Haemost 2008;99:443-4.

55. Wilder-Smith E, Kothbauer-Margreiter I, Lämmle B, Sturzenegger M, Ozdoba C, Hauser SP. Dural puncture and activated protein C resistance: Risk factors for cerebral venous sinus thrombosis. J Neurol Neurosurg Psychiatry 1997;63:351-6.
56. Salem-Berrabah OB, Fekih-Mrissa N, Laayouni S, Gritli N, Mrissa R. Cerebral venous thrombosis associated with homozygous factor V Leiden mutation in a 15-year-old girl of Tunisian origin. Ann Saudi Med 2011;31:651-4.
57. Altinisik J, Ates O, Ulutin T, Cengiz M, Buyru N. Factor V Leiden, prothrombin G20210A, and protein C mutation frequency in Turkish venous thrombosis patients. Clin Appl Thromb Hemost 2008;14:415-20.
58. Yakaryilmaz F, Guliter S, Degertekin B, Tuncer C, Unal S. Cerebral sinus thrombosis in a patient with active ulcerative colitis and double heterozygosity for factor V Leiden and prothrombin gene mutations. Neurol India 2009;57:188-90.
59. Ozkurt S, Temiz G, Saylisoy S, Soydan M. Cerebral sinovenous thrombosis associated with factor V Leiden and methylenetetrahydrofolate reductase A1298C mutation in adult membranous glomerulonephritis. Ren Fail 2011;33:524-7.
60. Kellett MW, Martin PJ, Enevoldson TP, Brammer C, Toh CM. Cerebral venous sinus thrombosis associated with 20210A mutation of the prothrombin gene. J Neurol Neurosurg Psychiatry 1998;65:611-2.
61. Hillier CE, Collins PW, Bowen DJ, Bowley S, Wiles CM. Inherited prothrombotic risk factors and cerebral venous thrombosis. QJM 1998;91:677-80.
62. Hourihane JM, Deloughery TG, Clark WM. Homozygous hereditary resistance to activated protein C presenting as cerebral venous thrombosis. J Stroke Cerebrovasc Dis 1997;6:370-2.
63. Dulli DA, Luzzio CC, Williams EC, Schutta HS. Cerebral venous thrombosis and activated protein C resistance. Stroke 1996;27:1731-3.
64. Liu XY, Gabig TG, Bang NU. Combined heterozygosity of factor V leiden and the G20210A prothrombin gene mutation in a patient with cerebral cortical vein thrombosis. Am J Hematol 2000; 64:226-8.
65. Maag J, Prayson RA. Intracranial sinus thrombosis in a patient with Crohn disease and factor V Leiden mutation. Arch Pathol Lab Med 2003;127:1037-9.
66. Stephan CL, SantaCruz K, May C, Wilkinson SB, Cunningham MT. Fatal dural sinus thrombosis associated with heterozygous factor V Leiden and a short activated partial thromboplastin time. Arch Pathol Lab Med 2003;127:1359-61.
67. Porres-Aguilar M, Square JH, Storey R, Rodriguez-Dunn S, Mohamed-Aly MS. An unusual cause of cerebral venous sinus thrombosis: Prothrombin G20210A gene mutation. South Med J 2007;100:906-8.
68. Kanaan ZM, Mahfouz R, Taher A, Sawaya RA. Bilateral transverse sinus thrombosis secondary to a homozygous C677T MTHFR gene mutation. Genet Test 2008;12:363-5.
69. Mahadeo KM, Dhall G, Panigrahy A, Lastra C, Ettinger LJ. Subacute methotrexate neurotoxicity and cerebral venous sinus thrombosis in a 12-year-old with acute lymphoblastic leukemia and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Homocysteine-mediated methotrexate neurotoxicity via direct endothelial injury. Pediatr Hematol Oncol 2010;27:46-52.
70. Dentali F, Crowther M, Ageno W. Thrombophilic abnormalities, oral contraceptives, and risk of cerebral vein thrombosis: A meta-analysis. Blood 2006;107:2766-73.
71. Marjot T, Yadav S, Hasan N, Bentley P, Sharma P. Genes associated with adult cerebral venous thrombosis. Stroke 2011;42:913-8.
72. Janghorbani M, Zare M, Saadatnia M, Mousavi SA, Mojarrad M, Asgari E. Cerebral vein and dural sinus thrombosis in adults in Isfahan, Iran: Frequency and seasonal variation. Acta Neurol Scand 2008;117:117-21.
73. Saadatnia M, Mousavi SA, Haghighi S, Aminorroaya A. Cerebral vein and sinus thrombosis in Isfahan-Iran: A changing profile. Can J Neurol Sci 2004;31:474-7.
74. Saadatnia M, Naghavi N, Fatehi F, Zare M, Tajmirriahi M. Oral contraceptive misuse as a risk factor for cerebral venous and sinus thrombosis. J Res Med Sci 2012;17:344-7.
75. Shahsavarzadeh T, Javanmard SH, Saadatnia M. Impact of factor VIII and von Willebrand factor plasma levels on cerebral venous and sinus thrombosis: Are they independent risk factors? Eur Neurol 2011;66:243-6.
76. Saadatnia M, Fatehi F, Basiri K, Mousavi SA, Mehr GK. Cerebral venous sinus thrombosis risk factors. Int J Stroke 2009;4:111-23.
77. Saadatnia M, Tajmirriahi M. Hormonal contraceptives as a risk factor for cerebral venous and sinus thrombosis. Acta Neurol Scand 2007;115:295-300.
78. Walker ID. Factor V Leiden: Should all women be screened prior to commencing the contraceptive pill? Blood Rev 1999;13:8-13.
79. McBane RD 2nd, Tafur A, Wysokinski WE. Acquired and congenital risk factors associated with cerebral venous sinus thrombosis. Thromb Res 2010;126:81-7.