International Journal of Hematological Disorders, 2015, Vol. 2, No. 2, 47-53
Available online at http://pubs.sciepub.com/ijhd/2/2/5

DOI:10.12691/ijhd-2-2-5

A Study of the Natural Coagulating Inhibitors Polymorphism in Iranian Patients with the Vine Thrombosis

Mohsen Hamidpour[1,*], Hosien Jahangir[1], Mortaza Safi[2], Gholamreza Toogeh[3], Mehdi Allahbakhshian[1], Rafie Hamidpour[4], Ali Akbar Khadem mabodi[5]

[1]Department of Hematology & Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Cardio Vascular Research center, Shahid Modarres Hospital, Shahid Beheshti University of Medical Sciences
[3]Thrombosis Disease Research Center, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran-Iran
[4]Department of Herbal medicine, Pars Biosciences Research center, Kansas, USA
[5]Department of Biostatistical, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
*Corresponding author: mohsenhp@SBMU.ac.ir

Abstract **Introduction**: Anti thrombin III (AT-III), protein C (PC), protein S (PS) are named the natural coagulation inhibitors, quantitative and qualitative defects of these inhibitor proteins caused recurrent veins thrombosis. Despite there are many clinical and technical assays for improving diagnosis of this disease, but diagnosis and classification of this disease facing with some challenges. The diagnosis polymorphism and mutation of PC, PS, and Anti-III, recognizing the homozygote and heterozygote form in people who have any patient with vein thrombosis in their family. **Materials & Methods:** 42 patients with vascular thrombosis, who visited cardiovascular and thromboses clinic of Immam khomaini and Shahid Modarres General Hospitals have been studied in this research. After performing coagulation test and determining the activity of anti coagulation proteins (PC, PS, Anti-III), The DNA of patients samples were extracted and reproduce by PCR. Pieces of DNA upon reproduction sent to Biosciences Co in UK to determining its sequence. DNA sequences were comprised with wild type of proteins genome. **Results:** The activities of PS in 63.5%, PC in 29.2 and AT-III in 7.3% patients were reduced. There was a statistical relation between reducing plasma activity of PC and PS of patients and their familial thrombosis ($P< 0.05$). Results obtained from Chromas software showed that there are remarkable polymorphisms in some patients who are suffered from abnormal of related proteins. **Discussion & Conclusion:** Although, many studies are performed in development countries for recognizing this disease, there is not a lot of study which carried out in Iran about this issue. Therefore, it is required to perform more molecular tests on natural coagulation inhibitors and other effective proteins on creation of vascular thrombosis.

Keywords: *vascular thrombosis, polymorphism, natural coagulation inhibitors*

Cite This Article: Mohsen Hamidpour, Hosien Jahangir, Mortaza Safi, Gholamreza Toogeh, Mehdi Allahbakhshian, Rafie Hamidpour, and Ali Akbar Khadem mabodi, "A Study of the Natural Coagulating Inhibitors Polymorphism in Iranian Patients with the Vine Thrombosis." *International Journal of Hematological Disorders*, vol. 2, no. 2 (2015): 47-53. doi: 10.12691/ijhd-2-2-5.

1. Introduction

Thrombophilia is an inherited or acquired coagulapathy, that cause increased clotting tendency [1]. In 1856 Virchow concluded that at least one of the following factors may cause thrombosis: delays in blood flow (stasis), changes in blood composition, and damage to the vascular endothelium [2]. The vein thrombosis (VTE) encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE) [3]. Due to the nature of DVT (almost clinically is silent) it is difficult to estimate the exact prevalence of this disease. In recent epidemiological study estimated that annually about 80 patients per 100,000 is observed [4]. The risk of DVT prevalence in hospitalized patients who do not receiving prophylaxis drug are increased, the prevalence of the disorder in these patients are various from 10 % to 80 % [5]. Thrombus is also tend to accumulate within the family, so is known as the family thrombophilia [6]. Risk factors for vein thrombosis are classified into two categorize, genetic and environmental. Environmental risk factors are including; aging, cancer, trauma, surgery, lupus anticoagulant, pregnancy, postpartum, hormone replacement therapy and oral contraceptives and inactivity (for example, trips or are staying in bed too long) [7]. Genetic risk factors are including: quantitative or qualitative abnormalities of protein C, protein S, anti thrombin III, factor V Leiden (Arg506Gln), methylen tetra hydrofolate reductase C677T mutation, and prothrombin G20210A mutation [8,9,10,11]. The majority of Caucasian VTE patients have one of these

two thrombophilias: factor V Leiden (R506Q) and prothrombin G20210A [12,13,14]. The increase serum level of coagulation factors, such as fibrinogen , factor II, VIII, IX and XI, and some coagulable proteins such as: homocysteine thrombin activatable fibrinolysis (TAFI) are also the risk factors for vein Thrombosis [15,16,17]. Although the most common signs and symptoms of DVT in patients are clinically silent, but depending on the degree of obstruction and inflammation of the vessel, the symptoms are evident. These symptoms of DVT are including: sudden swelling of leg, redness or discoloration of the skin, warming the affected area of leg, low-grade fever, and pain when there is a physical exercise and Tachycardia. The Homan test is also one of the DVT symptoms, when the muscles of the legs (upper Knee) stretched with a truniked, if the knee move out the rapidly, a painful have seen in the muscles of leg. Pulmonary embolism is a condition that the main pulmonary artery embolus can block. Over 60 % of pulmonary emboli clinically are undetectable and death can occur in less than 30 minutes in these patients [18,19]. It has reported that 10% patients, who hospitalized in developed country PE is the most common cause of preventable death [6,7]. The recurrent able is one of the major problem of the thrombosis which is known as PTS (post thrombotic syndrome) [20,21]. More than 30 % of patients may experience this problem again 8 years after initial DVT [22,23]. The mutations that cause functional deficiency of natural anticoagulants inhibitors (protein C, protein S, and anti thrombin III) are one of the DVT cusses. Therfor we will be need to improve diagnosis. The aim of our study is to determine the level of natural coagulation inhibitors and characterized their polymorphism in Iranian patients with thrombosis.

2. Materials&Methods

Using Cross sectional method, 42 new case patients who were visited the cardiovascular and thrombosis clinic in two general hospitals (Imam Khomaini and Modarres) in Tehran during 2012-2013 were under study. The participants gave informed consent in accordance with the Deceleration of Helsinki. The clinical characteristic including: foot painful, redness, medical history, inflammation, infection and medication use, were collected by standard qeusioneree. The patients who were under anti coagulant therapy during past six mounts and the patients who had any infection and their sera inflammation assays positive (like CRP, RF), were excluded from study. For normal controls, 21 volunteer with normal clinical examination and normal coagulation tests were selected. They were matched with patients for age, sex and same job. 10 ml of anti coagulated blood was collected from patients and controls. Samples were centrifuged at 3000g for 10 min, and plasma was aliquted and immediately frozen at -70°C. Measurement of protein C, protein S activity was performed using clot formation methods by Protein C and S kits (stago Co. France). And anti-thrombin III measurement using colorimetric method (Stago Co. France)

DNA extraction

DNA samples were extracted from 3 ml of anti coagulated blood according to the company (Sinaclon) procedure and frozen in the micro-tubes at -20°C. DNA concentration was detected by spectrophotometer at 260°. DNA of Proteins C, S, AT-III reproduced by PCR method using master mix kit (Sinaclon) and their primers which detail in Table 1 to Table 3.

Table 1. The Protein S, Primers used for PCR

	Primer Sequence (5'...3')	Size
PS-F1	GGCTTCAGGATTTTTATTATAGTA	550
PS-R1	CTAACTGGGATTATTCTCACAT	
PS-F2	ACAATCATAATATTCCTCTGCC	298
PS-R2	TCTGTATTTTCCTGACTTAGC	
PS-F3	AGCTTTCTGTATTTCTTACTC	289
PS-R3	TACAGACTGCATCAAAGTGGG	
PS-F4	CCTATACTCATAATCGAGCC	348
PS-R4	TGGGCACACAGTAGATACTC	
PS-F5	ATCATTGAGAAAGGGAATGG	360
PS-R5	GTAAATACTGCTATGTATAC	
PS-F6	GCTTATATTGAATCTTTGCTCTG	406
PS-R6	AAATGTCGGTACTAGCCCCTAG	
PS-F7	CAAGATGCTAAAAGTCTTGG	415
PS-R7	GATAGCAAGAGAAGTAAGAATTTC	

Table 2. The AT III Primers used for PCR

Primer	Primer Sequence (5'...3')	Product length
PAT-F1	CCAGGTGGGCTGGAATCCTCTGCTTT	536
PAT-R1	CTTGGGCCTATGGAAGCCCCAAAGGT	
PAT-F2	AACTAGGCAGCCCACCAAACCC	310
PAT-R2	TGGAGAGGAAGAACTCGGAGGTC	
PAT-F3	GGATATGTCTTGTGTCAATAACTATCC	517
PAT-R3	CTTTTGGTCAGACTACCTTGCGGGTG	
PAT-F4	GAATTCCCATCTGTGGGTTGAAGCCA	231
PAT-R4	TGCATGCCTTAACACTGGAAACAGGC	
PAT-F5	AAGCATTTGAGGAATTGCTGTGTCTGT	326
PAT-R5	GGCTTCAGGATTTTTATTATAGTA	

Table 3. the Protein C, Primers used for PCR

Primer	Primer Sequence (5'...3')	Product length
PC-F1	GTGCTAGTGCCACTGTTTGT	220
PC-R1	ATCACCACCTAGCTCTCTTC	
PC-F2	AGCTCTGCTTCCTCAGACCC	637
PC-R2	CCCTGCTGGTTACCAGCTCG	
PC-F3	CTTGAACCCTGCACTGTGGC	355
PC-R3	CGCTTCCCTCTCGGTTTCTG	
PC-F4	CTAAGCCTATGCCCATATGA	349
PC-R4	AAGAAGCCTCTTGCTTAAGC	
PC-F5	GGCCTCAGGAAAGTGCCACT	745
PC-R5	AGAACAGCAGGCCGGTGTGC	

After PCR amplification, product was confirmed using 1% agarose gel.PCR product sent to Biosciences Co. (UK) for sequencing. The results were read by the aligner software chromas and compared with the normal sequence and the number of mutations and polymorphisms were observed.

Statistical analysis: The SPSS software, version 16.0, was used for data analysis. For descriptive purposes, we used mean (± SD) for quantitative variables and No (%) for qualitative variables. In addition, we utilized the T-test, ANOVA, and TUKEY to show the relationship between two variables under study.

3. Results

In this project, the goal of our study was determinate plasma Activity of the natural inhibitors protein and

diagnosis of any remarkable polymorphism or mutation in patients with VET. Forty six percent out of 42 patients were male and 54% were female, their age ranged from 16 to 70 years old. The result of the CBC, PT and PTT assays which characterized the performance of individuals' primary and secondary haemostatic showed often are various, and without any specific diagnosis, therefore we do not show the results. The clinical and laboratory characterization of thrombosis were variable. So we classified in three demographic groups: 1- patients associated with thrombosis familial history, 2- patients associated with other clinical and laboratory vascular characterization, 3- patients associated with coagulant proteins gene mutations. The demographic characterization summarized in Table 4 –Table 6.

Table 4. phenotype and clinical characterization of patients associated familial thrombosis

NO of Patients	Inhibitor activity %			Clinical sign of foot			gender	age
	PC	PS	AT-III	redness	inflammation	Painful		
1	74	15	28.9	+	+	+	M	42
2	39	60	114	+	+	+	F	52
3	64	31	125	-	+	+	F	32
4	17	28	112	-	-	-	M	16
5	38	110	127	-	-	-	M	24
6	125	26	131	+	+	+	M	46
7	74	17	120	-	-	-	M	32
8	68	21	125	+	+	+	F	70
9	92	22	112	-	+	+	M	48
10	72	40	95	-	+	+	F	42

* Normal Range activity: Protein C: 70-140%, Protein S: 65-140%, Anti thrombin III: 80-120%.

Table 5. phenotype and clinical characterization of patients associated with other clinical signs

No of patients	Inhibitor activity %			Clinical sign of foot			gender	age	Other condition
	PC	PS	AT-III	redness	inflammation	Painful			
1	94	56	108	-	+	+	M	38	Recurrent Thrombosis
2	65	89	115	+	+	-	M	30	hyperhemocysteinemia
3	86	114	109	+	+	+	M	33	hyperhemocysteinemia
4	120	51	109	+	+	+	F	30	hyperhemocysteinemia
5	82	29	95	-	-	-	F	29	Hypertension
6	157	70	104	+	+	+	F	53	Recurrent Thrombosis
7	50	32	93	+	+	+	M	61	Thrombosis After surgery
8	35	28	112	+	+	+	F	52	Thrombosis after surgery
9	82	34	132	-	-	-	M	28	Recurrent Thrombosis
10	123	6	93	+	+	+	M	24	Recurrent Thrombosis
11	13	31	128	-	-	-	F	41	Recurrent Thrombosis
12	57	62	98	+	+	+	M	42	Recurrent Thrombosis
13	45	105	99	-	+	+	F	64	Thrombosis after surgery
14	105	32	130	-	+	-	M	38	- Recurrent Thrombosis
15	92	88	29	+	+	+	F	33	hyperhemocysteinemia
16	92	56	102	-	-	-	F	32	Recurrent Abortion
17	132	87	85	-	-	-	F	29	Recurrent Abortion
18	144	47	95	+	+	-	F	42	-
19	102	44	93	-	+	-	F	33	Recurrent Abortion

* Normal Range activity: Protein C: 70-140%, Protein S: 65-140%, Anti thrombin III: 80-120%.

Table 6. Demographic of patients with clinical and laboratory data associated coagulant proteins mutation

No of patients	Inhibitor activity %			Clinical sign of body			gender	age	
	PC	PS	AT-III	redness	inflammation	Painful			
1	81	101	108	-	+	-	M	34	FXIII
2	106	116	99	-	-	-	M	35	FXIII
3	104	45	112	+	+	+	F	36	MTHFR
4	96	127	109	-	+	-	M	21	G20210
5	106	55	90	-	-	-	F	27	MTHFR
6	117	152	38	+	+	+	F	37	MTHFR
7	66	33	102	-	+	+	M	29	F V Leiden
8	102	151	81	-	-	-	M	27	G20210
9	110	77	92	-	-	-	M	33	MTHFR
10	92	103	150	-	-	-	F	39	F V Leiden
11	85	68	115	+	+	+	F	45	F V Leiden
12	103	145	116	+	+	+	M	42	F V Leiden
13	155	110	124	-	-	-	M	39	F V Leiden

* Normal Range activity:Protein C: 70-140%, Protein S: 65-140%, Anti thrombin III: 80-120%.

In ANOVA test, the mean activity level of AT-III were not deferent in three groups of patients with p value = 0. 744. There is a relative between the mean activity of Proteins S and C and clinical carecterazation of three groups (P < 0.001 and P< 0.039 in respect).

To detect and compromise the activity of three coagulant inhibitors between two groups of patients with thrombosis, we detail all results in Table 7.

Table 7. comparative of phenotype statistical between meal and female

Proteins	Statistic data of activity							
	Male (22)				Female (20)			
	Mean %	SD	Min	Max	Mean %	SD	Min	Max
AT- III	106.36	22.19	28.9	133	100.75	27.87	29	150
PC	86.4	3o.93	17	155	88.35	37.39	13	157
PS	68.3	46.4	6	151	60.55	32.74	21	152

The mean activity of Protein C and AT-III were not deferent between two groups with p value = 0. 394 and = 0.495 in respect. But the mean activity of Protein S was lower in female with P< 0.001.

DNA extraction: To confirm the purity of DNA extraction, OD of samples were read by the photometer. The results showed that the OD of DNA ratio (260/280 ratio) were under the 1.8. The PCR production of protein S with 15 exon and 7 primers, AT- III with 6 exon and 5 primers, protein C with 8 exons and 5 different primers were detedcted. All 42 patients' samples and control were characterised but, given that all 42 patients represent the same PCR products, characterization results from only one samples for each protein presented in 3 figures. Figure 1- Figure 3 show the PCR production of three proteins.

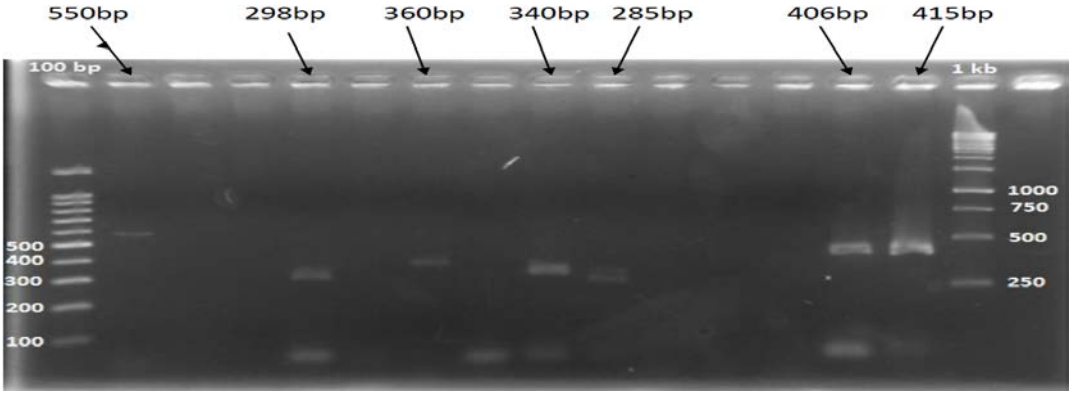

Figure 1. PCR product of protein S with seven Bands (550, 280,360, 340, 285, 406, 415 bp) in deferent Exons, two Molecular marks (ladder)

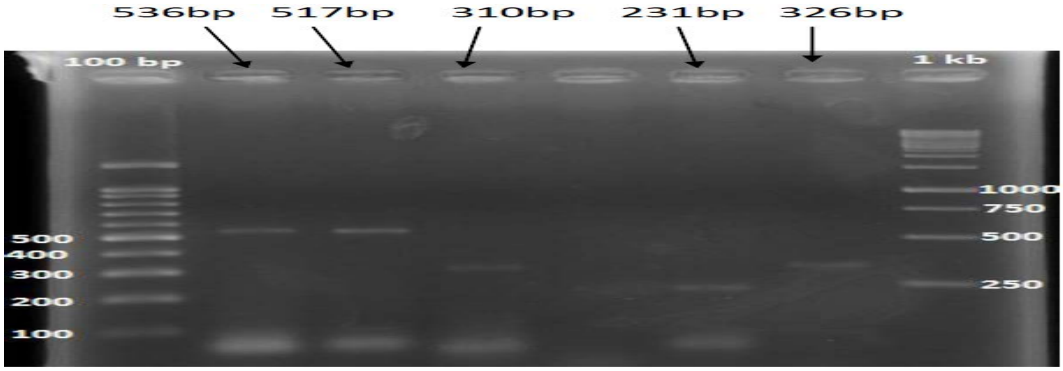

Figure 2. PCR product of protein AT-III with five Bands (536, 517,310, 231, and 326 bp) in deferent Exons, two Molecular marks (ladder)

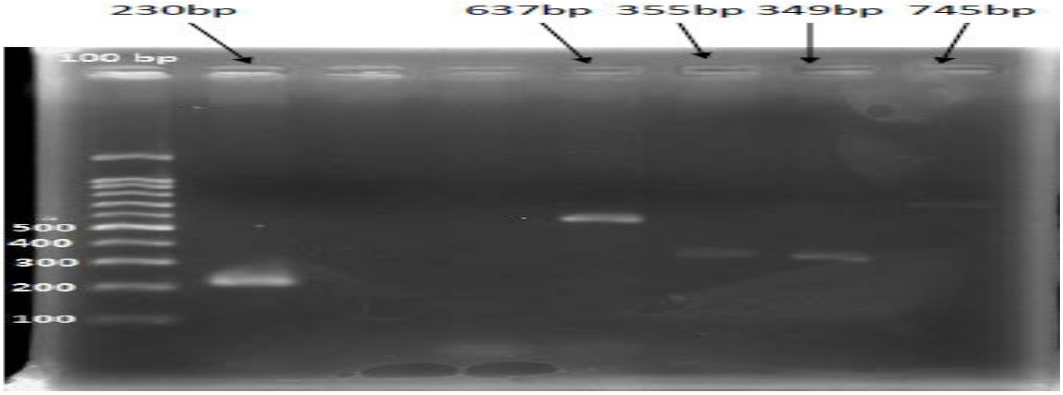

Figure 3. PCR product derived from protein C with five Bands (230, 637,355, 349, 745bp) in deferent Exons, two Molecular markers (ladder)

Sequencing of the amplified fragments: After amplification of DNA fragments for sequencing was sent to the Bio-Science CO, UK. The results were read by the aligner software chromas and compared with the normal sequence and the number of mutations and polymorphisms were observed. Table 8 showed the SNP variant in the Coagulant inhibitors proteins of the our patients with Vein Thrombosis

Results in Table 8 indicated that, the most nucleotide change that influence in the protein C , S and AT III anticoagulant of our patients who phonotypical showed low percentage of activity

Table 8. SNP variant in the Coagulant inhibitors proteins on Vein Thrombosis

Inhibitor coagulation factor	Gene	Nucleotide change	SNP	Risk allele	Ratio of patients
Protein C	PROC	c.565 C>T	rs146922335	T	3/42
Protein C	PROC	c.1000G>A	rs121918150	del	2/42
Protein C	PROC	c.1015G>A	rs121918158	A	2/42
Protein C	PROC	c.814C>T	rs121918154	T	2/42
Protein c	PROC	c.-21-121C>T	rs1799810	T	1/42
Protein S	PROS1	c.586A>G	rs121918474	G	4/42
Protein S	PROS1	c.1681C>G	rs121918476	G	3/42
Protein S	PROS1	c.2031A>T	rs267606981	T	4/42
Protein S	PROS1	c.835C>T	rs121918475	T	3/42
Protein S	PROS1	c.586A>G	rs121918474	G	1/42
Protein S	PROS1	c.1501T>C	rs121918472	C	3/42
AT-III	SERPINC1	c.981A>G	rs5877	G	1/42
AT-III	SERPINC1	c.1154-53G>A	rs2759328	G	1/42

4. Discussion

Thrombosis along with a family history and decreasing serum level or dysfunction of natural protein inhibitors are the major factor of thrombophilia in midel age. The average age of our patients is about 35 years, while E. Nizankowska-Mogilnicka In a study of 33 people with thrombosis has been reported that mean age of them were 43 years old [24]. The rate of patients with protein inhibitors in variant genetnic is deferent. Our finding in this survey indicate that, the activity of PS is decrease in 63.5 % of patients, PC deficiency in 29.2% and AT-III activity decreased just in 7.3 % of patients, 21.42% of patients decrease activity of both protein S and C, and just one patient had low Protein S and AT-III activity together, most of these patients had the familial thrombosis history. According to a study by Leonard Minuk, activity of PS and PC were decreased, respectively in 63 % and 40 % of cases [25]. In a survey in IBTO has been reported, the deficiency of PS was detected in 22.77%, PC deficiency in 6.68%, AT-III deficiency in 5.2% and compound deficiencies in 8.91% of patients who were under study by Iranian Blood Transfusion Organization (IBTO) [26]. Other study by Kinoshita, have reported that, out of 85 Japanese DVT patients, 25.8% patients had low protein S activity, 10.6% patients had low protein C activity, 7% patients had low AT-III activity and 21.2% of patients had both low protein S and C activities [27]. Liu et al, claimed that, 53% of 47 Chinese VTE patients have reduced activity of APC anticoagulant system, he reported that 10 patients had low protein S activity, 9 patients had low protein C activity, 5 patients had low AT-III activity and one of patients had both low protein S and C activities [28].

Using Tukey statistical method, we compared the patients with family history of thrombosis and patients associated with gene mutation, data showed that, PS and PC activity were significantly decreased (P <0.001) while the AT-III activity was normal. Several studies have been reported that, protein S deficiency is approximately 10 times more prevalent in Asians than in Caucasians [29]. In the other hand, the patients in our study like the samples who E. Nizankowska-Mogilnicka was studied, activity of PS in wemen significantly decreased .This is may be due to the presence of sex hormones in men [24]. There have been reported many mutation and polymorphism in the natural coagulant inhibitors (Protein S, C and AT-III), but a frequency of these variant genetic changes are remarkable which have related with phenotype of vein thrombosis.

At present, more than 200 mutations have been described in the protein S (PROS1), and large deletions/duplications can also be identified as causes of protein S deficiency [29]. The most common PROS1 mutation is a p.Lys196Glu mutation (rs121918474, c.586A>G, protein S Tokushima, p.Lys155Glu in the mature protein numbering, which accounts for 9–30 % of protein S molecule abnormalities in people of Japanese decent [30]. We have found six remarkable mutation and polymorphism in protein S out of 42 samples (Table 8). Gene change variant with Rs 1219118474 was found in a patient (sample 9) with sever protein S deficiency (22%), and familial thrombosis history. At least 161 different protein C (PROC) mutations have been reported, and most of them are missenes mutations. The predominant genetic defects in the PROC may be different for different races [31]. We detected five remarkable mutations and poly morphism in 10 patients out of 42 samples. The p.Arg189Trp mutation of protein C (rs146922325, c.565C>T, p.Arg147Trp in the mature protein was reported by two independent studies in Chinese populations [32,33].

The heterozygous state of the p.Arg189Trp mutation is associated with decreased plasma functional activity and a relatively normal protein C antigen level, indicating type II protein C deficiency [34]. We recognized 2 Serpinc1

gene mutations in AT-III protein that could be related to antithrombine deficiency in our patients. In this study, the desired protein in patients with a family history were amplified and sequenced in a number of mutations and polymorphisms were observed. However, we were able to indicate several mutaion and polymorphism which related with natural coagulant inhibitors deficiency. Recent genome studies in European population, have found the genetic polymorphisms that are potentially related to VTE risk [35]. Usining Tukey test, statical analysis shwoed that there are relative for Protein C and S P< 0.031 and P< 0.001 in respectly. Finaly, an accumulating body of evidence strongly suggests that genetic studies should be carried out in ethnically diverse populations [36].

5. Sugestion

Identify new polymorphism and mutations in Iranian populatin recommended. The exact frequency of all disorders in the older population and the more time is need. It may help earlier diagnosis of arterial thrombosis in patients with low -risk individuals heterozygous protein.

6. Conclusion

We detected the serum activity of natural anticoagulant proteins (proC, proS, AT-III) were deficient in 50% of patient in different conditions. We also diagnosed 12 remarkable gene variations in patients. Whether the natural coagulant inhibitors' especially Protein S dysfunction detection in our study is an important, but the national surveys are needed to determine this problem.

Acknowledgements

The authors would like to thank the Cardio vascular research center of Shahid Modarres Hospital for supported this project.

References

[1] Prandoni P, Villalta S, Bagatella P, Rossi L, Marchiori A, Piccioli A, Bernardi E, Girolami B, Simioni P, Girolami A.The clinical course of deep-vein thrombosis. Prospective long-term follow-up of 528 symptomatic patients. Haematologic. 1997; 82(4):423-428.

[2] Virchow R. Phlogose und Thrombose im Gefässytem. Gesammelte Abhandlungen zur Wissenschaftlichen Medizin Frankfurt: Staatsdruckerei. 1856.

[3] Heit JA, O'Fallon WM, Pett erson TM, Lohse CM, Silverstein MD, Mohr DN, Melton III L Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-basedyduts. Arch Intern Med. 2002; 162: 1245-1248.

[4] White R. The epidemiology of venous thromboembolism. Circulation 2003; 107:14-18.

[5] Geerts WH, Pineo GF, Heit JA. Prevention of venous thromboembolism: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy.Chest. 2004; 126(3Sup):38-40.

[6] Van Sluis GL, Sohne M, El Kheir DY, Tanck MW, Gerdes VE, Büller HR. Family history and inherited thrombophilia. 2006; 4:2182-2187.

[7] Roosendaal FR. Venous thrombosis: the role of environment, and behavior. Lecture in Hematology (Am Soc Hematol Educ Program). 2005; 1-12.

[8] Bertina RM. Genetic approach to thrombophilia. Thromb Haemost. 2001; 86:92-103.

[9] Poort SR, Roosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3' untranslated region of the prothrombin is associated with elevated plasma prothrombin level dna an increase in venous thrombosis. Blood. 1996; 88:3698-3703.

[10] Heit JA, Phelps MA, Ward SA, Slusser JP, Pett erson TM, De Andrade M. Familial segregation of venous thromboembolism. J Thromb Haemost. 2004; 2:731-736.

[11] Roosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watz ke HH,Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost. 1998; 79:706-708.

[12] Castoldi E and Rosing J. APC resistance: biological basis and acquired influences. J Thromb Haemost. 2010; 8:445-53.

[13] Franco RF and Reitsma PH. Genetic risk factors of venous thrombosis. Hum Genetic 2001; 109:369-84.

[14] Bounameaux H and Roosendaal FR. Venous thromboembolism: Why does ethnicity matter? Circulation. 2011; 123:2189-91.

[15] Nossent AY, Eikenboom JC, Bertina RM. Plasma coagulation factor levels in venous thrombosis. Semin Hematol. 2007; 44:77-84.

[16] Van Hylckama Vlieg A, Rosendaal FR. High levels of fibrinogen are associated with the risk of deep venous thrombosis mainly in the elderly. J Thromb Haemost. 2003; 1:2677-2678.

[17] Bovill EG, Hasstedt SJ, Leppert MF, Long GL. Hereditary thrombophilia as a model for multigenic disease. Thromb Haemost. 1999; 82:662-666.

[18] Tienan Zhu, Qiulan Ding, Xia Bai, Xiaoyan Wang, Florentia Kaguelidou Corinne Alberti, Xuqian Wei,Baolai Hua, Renchi Yang, Xuefeng Wang, Zhaoyue Wang,Changgeng Ruan, Nicole Schlegel, and Yongqiang Zhao. Normal ranges and genetic underlying variants of antithrombin, protein C and proteinS in the genral Chinese population. Haematologica 2011.

[19] Donaldson GA, Williams C, Scannell JG. A reappraisal of the application of the Trendelenburgnoitarepo to massive fatal embolism: report of a successful pulmonary-artery thrombectomy using acardiopulmonary bypass. N Engl J Med 1963;268: 171-174.

[20] Schulman S, Lindmarker P, Holmstrom M, Larfars G, Carlsson A, Nicol P, Svensson E, Ljungberg B, Viering S, Nordlander S, Leijd B, Jahed K, Hjorth M, Linder O, Beckman M. Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. J Thromb Haemost. 2006;4:734-742.

[21] SR, Hirsch A, Shrier I. Effect of postthrombotic syndrome on health-related quality of life after deep venous thrombosis. Arch Intern Med 2002; 162:1144-1148.

[22] Buller HR, Sohne M, Middeldorp S. Treatment of venous thromboembolism. J Thromb Haem2005 ; 3:1554-1560.

[23] Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007; 5:692-699.

[24] E. Nizankowska-Mogilnicka, L. Adamek, P. Grzanka, T.B. Domagala, M. Sanak, M. Krzanowski and A. Szczeklik. Genetic polymorphisms associated with acute pulmonary embolism and deep venous thrombosis. Eur. Respiratory .journal.2003; 1:25-30.

[25] L, Minuk, A, Lazo-Langner, J, Kovacs، M, Robbins, B, Morrow and M, Kovacs. Normal level of protein C and protein S tested in the acute phase of a venous thromboembolic event are not falsely detavele. Thrombosis Journal 2010, 8:10.

[26] Ahmadinezhad M., Rajabi A., Bashash D., Zoualfaghari S, Tabatabaei SMR., Atarodi K., Seyadmortaz L., Balouch S., Amini Kafiabad S., Abouaghasemi H. Hereditary thrombophilic deficiencies in Iranian patients patients with venous thromboembolis referred to special coagulantion laboratory of IBTO. Khoon. 2010, 1,7-15.

[27] Kinoshita S, Iida H, Inoue S, Watanabe K, Kurihara M, Wada Y. Protein S and protein C mutations in Japanese deep vein thrombosis patients. Clin Biochem 2005; 38:908-15.

[28] Liu HW, Kong YL, Bourke C, Lam CK, Lie AK, Wei D. High incidence of thrombophilia detected in Chinese patients with venous thrombosis. Thromb Haemost 1994; 71:416-9.

[29] Gandrille S, Borgel D, Sala N, Espinosa-Parrilla Y, Simmonds R, Rezende S, Lind B, Mannhalter C, Pabinger I, Reitsma PH, Formstone C, Cooper DN, Saito H, Suzuki K, Bernardi F, Aiach

M. Protein S deficiency: a database of mutations—summary of the first update. Thromb Haemost. 2000; 84(5):918.

[30] Yin T, Takeshita S, Sato Y, Sakata T, Shin Y, Honda S, Kawasaki T, Tsuji H, Kojima T, Madoiwa S, Sakata Y, Murata M, Ikeda Y, Miyata T. A large deletion of the PROS1 in a deep vein thrombosis patient with protein S deficiency. Thromb Haemost. 2007; 98(4):783-789.

[31] Hamasaki N, Kuma H, Tsuda H. Activated protein C anticoagulant system dysfunction and thrombophilia in Asia. Ann Lab Med 2013 33(1):8-13.

[32] Tong Yin, Toshiyuki Miyata. Dysfunction of protein C anticoagulant system, main genetic risk factor for venous thromboembolism in Northeast Asians. J Thromb Thrombolysis. 2014; 37:56-65.

[33] Naotaka Hamasaki, Hiroyuki Kuma, Hiroko Tsuda. Activated Protein C Anticoagulant system dysfunction and thrombophilia in Asia Ann Lab Med 2013;33:8-13.

[34] Tang L, Guo T, Yang R, Mei H, Wang H, Lu X, Yu J, Wang Q, Hu Y. Genetic background analysis of protein C deficiency demonstrates a recurrent mutation associated with venous thrombosis in Chinese population. PLoS ONE. 2012; 7(4):e35773.

[35] Bezemer ID, Bare LA, Doggen CJ, Arellano AR, Tong C, Rowland CM, Catanese J, Young BA, Reitsma PH, Devlin JJ, Rosendaal FR. Gene variants associated with deep vein thrombosis. JAMA .2008; 299(11):1306-14.

[36] Miyata T, Hamasaki N, Wada H, Kojima T. More on: racial differences in venous thromboembolism. J Thromb Haemost. 2012; 10(2):319-320.