

## Survey of Thrombophilia Risk Factors and Markers in Patients with Deep Vein Thrombosis in Mazandaran Province, Northern Iran

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**Abstract:** Thrombophilia is an abnormality of blood coagulation that can cause serious problems such as deep-vein thrombosis (DVT). In this study, effect of hereditary factors on thrombophilia was evaluated. Total of 95 DVT patients underwent heparin therapy followed by warfarin for six months. Then warfarin was stopped, but heparin therapy was continued for two more weeks. In these patients, thrombophilia factors and other markers were determined. All data were analyzed with SPSS statistical software version 18.0 and descriptive statistics used by T-test and chi-square test. Number of male and (38.9 and 61.1%), respectively. About 18.94% of patients had increased levels of homocysteine. In addition, 10.52 % of patients had elevated levels of factor VIII. About 21.05% of patients were positive for factor V-Leiden. It was found that, DVT is most common among females. Companion of DVT with history of thrombosis, OCP and family history is more significant. Elevated levels of homocysteine, factor VIII and S protein deficiency are common.

**Key words:** Deep-vein thrombosis • Factor VIII • Homocysteine • Thrombophilia

### INTRODUCTION

Thrombophilia is a disorder of hemostasis in which there is a propensity for the occurrence of thrombosis [1-3]. Approximately 5-8% of the U.S. population has one of these clotting disorders. More than 60,000 Americans die each year from this disorder. The average annual incidence among whites is 108 per 100 000 person-years, with total incident about 250 000 cases occurring annually among US white race. The incidence appears to be similar or higher among blacks and lower among Asian and Native Americans [4, 5]. This disorder is multigenic and

multi-factorial. Many affected patients have more than one risk factor for thrombophilia (Table 1). Patients with more than one inherited risk factor are at a higher propensity for venous thrombosis, mortality and morbidity than patients with only one such risk factor. Nearly half of patients with deep-vein clots experience long-term health consequences that adversely affect their quality of life and there is no specific treatment for most of them. Recurrent miscarriage and cancer have also been associated with thrombophilia [6-9]. Understanding of the coagulation cascade in conjunction with molecular investigations has clarified some hypercoagulable states;

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particularly inherited disorders associated with thrombosis. Unfortunately; there are no epidemiologic data existing in Mazandaran, Iran. Current guidelines are not adequate for diagnosis of such disorder. This action required additional diagnostic measures and impose high casts to patients. According to this sort of autosomal resistance disorder and their hereditary states, recognition of prevalence is necessary. If one understands this hereditary disorder; then an appropriate diagnostic pattern must be designed. Hereinafter, with these appropriative designs, diagnostic measurements for thrombophilia was requested. Above issues propound that it is necessary to know prevalence of each risk factor of thrombophilia in Mazandaran-Iran. Since there are limited data available about prevalence thrombophilia and their adverse complications like pulmonary emboli created disorders; then present investigation was preformed. So, we studied the patients who admitted in Imam Khomeini hospital-Sari to evaluate these risk factors in patients with deep-vein thrombosis, in order to provide a proactive approach in future investigations.

## MATERIALS AND METHODS

**Patients:** After approval from Ethical committee from Mazandaran University of Medical Sciences, this study was preformed in Imam Khomeini hospital, Sari, Iran. Ninety-five patients were admitted in this hospital with a diagnosis of deep-vein thrombosis (DVT). The investigations were conducted during period of April 2009 to May 2010, in a cross-sectional study. Inclusions criteria include were:

1. Loss of surgical history that lead to hospitalization in two recent weeks.
2. Loss of known malignancy during last six months.
3. Loss of trauma history to vertebrae and limbs during four recent weeks.

In order of each positive below factors, patients were excluded from study:

1. CXR (For evaluating the Lung cancer).
2. PSA (For evaluating the Prostate cancer).
3.  $\beta$ -HCG (For evaluating the Testis cancer).
4. CA-125 (For evaluating the Breast, Ovary and Pancreas cancer).
5. Endoscopy and Colonoscopy (In patients with gastric symptoms for the roll outing the Gastro-esophageal cancer).

Admitted patients with DVT were treated by Heparin at first and six months after that with Warfarin. Warfarin was stopped and Heparin administered for second time for duration of two weeks. Needed laboratory exams requested after these therapeutic procedures. It should be mentioned that cutting of warfarin was needed to measuring of protein C & S serum levels.

**Measurement of Thrombophilia Markers:** Patients' blood samples sent to Iran's center of the blood bank in Tehran. All exams were duplicated for high accuracy. CBC (Platelet count) of citrated venous blood sample was analyzed by Cell counter machine. Peripheral blood smear (PBS) of venous blood smears on a slide that was read by laboratory science's expert. The APTT was measured by Synthasile kit and Sysmex CA<sub>1500</sub> with Thrombin kit and Sysmex CA<sub>1500</sub> anticoagulant by StacLOT-LA kit and Sysmex CA<sub>1500</sub> Factor VIII, IX, XI levels by Clotting kit of Technoclone Institute and IgG-Antiphospholipid IgM and IgM by ORGENTEC kit with ELISA on a clotted blood sample. The Level of Fibrinogen evaluated by Technoclone kit and ELISA method on a citrated blood sample. D-dimer was measured by Stago company D-dimer kit, FDP by Stago company FDP kit with Immunoassay Latex method on citrated blood sample, Homocysteine level by AXIS-SHIELD kit with ELISA method on citrated sample, Protein C,S activity Antithrombin III by Chromogenic kit with Chromatogenic method on citrated blood sample, Prothrombin G20210GA mutation by DNAEXT. F II<sub>20210</sub> kit with PCR method on citrated blood sample and Factor V Leiden was evaluated by DNAEXT.F V Leiden with APCR test on a citrated blood sample. In this study, data were collected through interview, observation and filling out a questionnaire.

**Statistical Analysis:** Collected data interred in SPSS Version 18.0. In this study, descriptive tests were used. T-test was used for quantitative variables and Chi-square for qualitative variables. In order to assess whether each risk factor was an independent predictor for the presence of thrombophilia marker, Pearson's regression analysis was performed adjusting for each risk factor independently. *P-Value* <0.05 was considered a significant difference.

## RESULTS

The study included 95 patients admitted to Imam Khomeini hospital with a diagnosis of deep-vein thrombosis (DVT) among tem, 37 patients (38.9%) were

Table 1: Frequency of normal and abnormal rates of platelets, PTT and TT of patients

Marker	Number of patients	Normal (percent)	Higher than normal (percent)	Lower than normal (percent)	Max	Min	Mean±SD	95% CI
PLT	95	79(83.15%)	5(5.26%)	11(11.57%)	713	37	289.58±114.91	266.17-312.99
PTT	95	75(78.94%)	17(17.89%)	3(3.15%)	82	17.30	34.93±9.67	32.96-36.90
TT	95	82(86.31%)	3(3.15%)	10(10.52%)	19.5	13	16.64±1.24	16.38-16.89

Table 2: Descriptive indexes of evaluated patients

Factors	Number of patients	Normal (Percent)	Higher than normal (Percent)	Lower than normal (Percent)	Mean ± SD	95% CI of the difference
Fibrinogen	95	90(94.73%)	1(1.05%)	4(4.21%)	337.47±88.657	319.41-355.53
D-dimer	95	71(74.73%)	6(6.31%)	18(18.94%)	1.180±5.772	0.004-2.356
Homocysteine	95	75(78.94%)	18(18.94%)	2(2.10%)	9.551±4.664	8.601-10.501
Protein-C	95	83(87.36%)	8(8.42%)	4(4.21%)	115.51±33.417	108.69- 122.31
Protein-S	95	83(87.36%)	2(2.10%)	10(10.52%)	99.13±26.273	93.77-104.48
Factor VIII	95	84(88.42%)	10(10.52%)	1(1.05%)	119.85±37.276	112.26-127.45
Factor IX	95	88(92.63%)	4(4.21%)	3(3.15%)	97.44±35.534	90.20-104.68
Anti-thrombin III	95	88(92.63%)	4(4.21%)	3(3.15%)	101.18±20.413	97.02-105.34

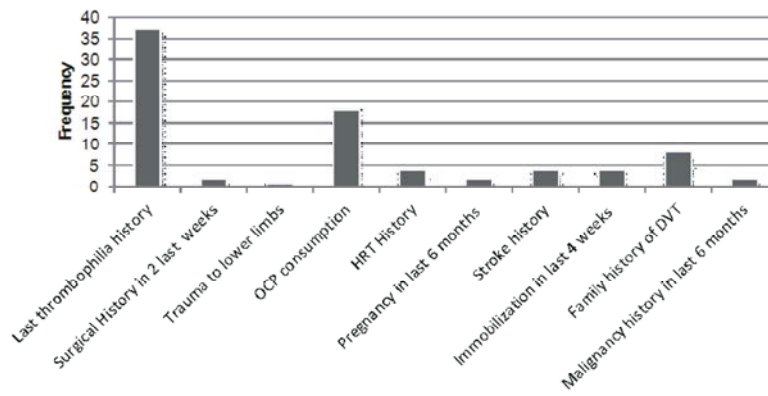


Fig. 1: Frequency of some of individual characteristics and clinical history of patients

male and 51 (61.1%) individuals were female. Mean age of them was 44.15 (95% CI, 40.99-47.30). All patients were normal as CXR, PSA, B-HCG and CA-125 and no sign of Para-neoplastic dysfunctions were observed. Figure 1, shows the frequency of some individual characteristics and clinical history of patients. Table 1 indicates the frequency of normal and abnormal rates of platelets (Physiological range =  $(150-400) \times 10^9$  per liter), PTT (Normal = 25 - 35 seconds) and TT (Normal = 14 to 16 seconds) of patients. Prevalence of IgM antibodies and anti-phospholipid was the same as a frequency. Thus in both cases most patients were negative and only one (1.05%) individual was positive. Patients were analyzed for antibody to IgM and IgG anti-cardiolipin. One patient (1.05%) was positive for antibody to IgM anti- cardiolipin, but all individuals were normal of IgG type. One case (1.05%) had elevated levels of fibrinogen (Normal range = 2.0 - 4.0 g/L). Level of FDP was normal in 64 patients (67.36) and 31 (32.63%) cases had an elevated level (Normal<10 mcg/mL). D-dimer was one of the measured factors (Normal < or =250 ng/mL). Results showed that

6.31 percent (6 cases) had elevated level of this factor (95% CI, 0.004-2.356). Patients were also assessed as levels of homocysteine that 18 cases (18.94%) had elevated and 75 (78.94%) had normal amounts (95% CI, 8.60-10.50) (Normal range in boys =  $3.48-24.2$  and Girls =  $3.9-29.0$  mmol/l). In our study, 8.42 percent of individuals had elevated rate of C-protein (Normal=70 - 140%)(95% CI, 108.70-122.31). Another investigation on protein was the S-protein (Normal range in male: 74 - 146%. female: 55 - 124%). As shown in Table 2, for 2 individuals (2.10%) of these 95 cases had higher rate of this protein than normal and 83 individuals (87.36%) were normal as level of this variable. Factor V Leiden was evaluated in our patients. About 20 patients (21.05%) were positive and 64 cases (67.36%) were negative. Other measured factor was protein VIII (Normal = 50 - 150 u/dl). Based on Table 2, the level of this factor was higher than normal in ten individuals (10.52%) (95% CI, 112.26-127.45). Changes in factor IX (Normal Range 50 - 150 u/dl), antithrombin III (Normal = 80% – 120 %) and prothrombin G20210GA mutation were also measured in our study.

At both assessments of factor IX, antithrombin III, 4 patients (4.21%) had levels higher than normal (Table 2). Total of 28 cases were tested for mutation of prothrombin G20210A, that twenty eight (96.6%) of cases had normal or wild form and 1 patient (3.4%) had heterozygote form. In our investigation, the history of endoscopy and colonoscopy was only seen in 2 patients (2.10%); either the positive result of beta-HCG test.

## **DISCUSSION**

Venous thromboembolic disease includes a wide spectrum of conditions with various severity and prognostic implications. DVT is an important cause of morbidity and mortality; as the awareness of this pathology has increased during the last 2 decades. Its etiologies are different and depend on several risk factors. Such risk factors can include inherited thrombophilic conditions or combinations of acquired and inherited risk factors. Recent studies have shown that algorithms combining simple diagnostic tests may provide a safe diagnostic approach to suspected DVT, with a 3-month thromboembolic risk similar to or lower than that reported after a normal phlebography, the accepted gold standard. For instance, Plasma D-Dimer measurements have been widely validated and allow ruling out the disease with an acceptable safety in selected patients [10-12]. Present study results showed that women with DVT had a clear advantage than men. In our study, mean age was about 44 years. In the study of Bezemer and colleagues median age of patients with pulmonary thromboembolism or DVT was 50 years [13]. In another study in Italy, median age of patients with upper limb DVT was calculated about 35 years [12]. In this study, the most common clinical history was related to thrombophilia which was about 37%. The second one was taking OCP with about 18% of patients. Laboratory factors in our study showed that V Leiden factor was impaired in 17% of cases. 3.4% of patients had heterozygous type of prothrombin G20210A. About 1.05% of cases were positive for antibodies to IgM anti-cardiolipin. Also 1.05% of cases had high levels of fibrinogen. Protein C has decreased in 4.21% of patients and protein S has decreased in 10.52% of them. Reduction of anti-thrombin III was observed in 3.15 percent of patients. About 1.05% was positive for anti-cardiolipin (IgM). Torres and colleagues [14] conducted a case-control study involving 100 consecutive patients with deep-vein thrombosis and 114 healthy controls from the hospital in Colombia. They reported that about 10% of patients carried the factor V Leiden mutation vs. 1/114

controls (OR=12.56). Activated protein C resistance was detected in 25/99 patients vs. 6/114 controls (OR = 6.08). Furthermore, mutation of prothrombin G20210A was in 4% of cases while none of the healthy cases have a mutation. Prothrombin mutation rate which was obtained in the study of Torres was almost the same as the rate of Prothrombin mutation in our study (3.4%). Martinelli and his colleagues [15] studied 115 primary upper-extremity DVT patients and 797 healthy controls. About 16 % of patients had family history of venous thrombosis. This rate was 18% in healthy cases. Heterozygosity for factor V Leiden and prothrombin G20210A in patient group were 9 and 10%, respectively. Both factors were seen about 3 % in control group (OR=6 & OR=5, respectively). Antithrombin, protein C, or protein S deficiency was 3% in patients group and about 0.8% in control group. Antiphospholipid antibody was seen in 7% patients. About 34% of patients were used oral contraceptive. History of thrombosis in Martinelli study is roughly similar to our study (20%). Furthermore, in Martinelli study mutation of factor V Leiden was about two times more than our study and the rate of prothrombin G20210A mutation was three times more in our study. The frequency of Antithrombin, protein C and protein S deficiency in this study were less than our study. Also history of OCP usage in study was about two times more than our study (34 vs. 18%). Bezemer and colleagues [13] investigated impact of various environmental and genetic factors in 1605 patients with pulmonary thromboembolism DVT and 2159 control subjects. They reported that all environmental risk factors in patient groups were clearly more compared to control group. In patients group with DVT or Pulmonary thromboembolism, 17.2% of cases had surgery history, 16.6% had Injury history and 30.9% with Immobilization, 4.2% with pregnancy, 6.2% with malignancy and 28.4% of them was taking OCP. Mutation of factor V Leiden in 15.3%, mutation of G20210A in 4.5% and protein C deficiency was seen in 2.2% of patients. In Bezemer's study the rate of past surgical history, immobilization and pregnancy were much higher than our study. And also history of OCP usage was two times more than our study. However, it is noteworthy that the cases used in this study will also include pulmonary thromboembolism.

## **CONCLUSION**

Overall, our study shows that in north of Iran, the prevalence of DVT is clearly higher among women than men and occurs in younger populations rather than other

countries. Among clinical histories, history of thrombosis, OCP and family history of DVT are more accompanied with venous thrombosis. In our survey, elevated levels homocysteine and factor VIII levels and protein S deficiency were the most frequent thrombophilic disorders. Finally, it is recommended that a multi-center study should be performed to evaluate the rate and risk factors of thrombophilia in Iran.

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