Frequency of Hereditary Coagulation Risk Factors in Deep Vein Thrombosis Patients Referred to Iranian Blood Transfusion Organization, Kermanshah

Mehrdad Payandeh,[1] Hoshang Yousefi,[2] Mohammad Erfan Zare,[1,3] Atefeh Nasir Kansestani,[1,3] Zohreh Rahimi,[1,4] Dariush Pourmand,[5] Amir Hossein Hashemian,[6] Mahmood Aeinfar,[7] Mehrnoush Aeinfar,[1] Farhad Shaveisi Zadeh[8]

[1]Medical Biology Research Center, Kermanshah University of Medical Science, Kermanshah, Iran.
[2]Blood Transfusion Research Center, High Institue for Research and Education in Transfusion Medicine, Kermanshah, Iran.
[3]Student Research Committee, Kermanshah University of Medical Science, Kermanshah, Iran.
[4]Department of Biochemistry, School of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran.
[5]Department of Medical Lab Science, Paramedicine Faculty, Kermanshah University of Medical Science, Kermanshah, Iran.
[6]Department of Biostatistics, Faculty of Public Health, Kermanshah University of Medical Science, Kermanshah, Iran.
[7]Student Research Committee, Electronic Department, Faculty of Technology, Islamic Azad University,Kermanshah, Iran.
[8]Departments of Medical Genetics, Faculty of Medicine, Sahid Beheshti University of Medical Science, Tehran, Iran.

Corresponding Author: Mohammad Erfan Zare, BSC student of Medical Lab Science.
Medical Biology Research Center, P.O.BOX: 1568, Sorkheh Lizheh, Kermanshah University of Medical Science, Kermanshah, Iran.
E-mail: mezarelab@yahoo.com
Tel: +98 831 4276473
Fax: +98 831 4276471

Abstract

Introduction: The main inhibitors of coagulation pathway are antithrombin (AT), protein C and protein S. These inhibitors are necessary to prevent thromboembolism. Hereditary deficiency of inhibitors is the main cause of alteration in balance between the anti-clotting and the formation of thrombin. Patients with this abnormality are susceptible to venous thromboembolism (VTE). Two major clinical manifestation of VTE are deep vein thrombosis (DVT) and pulmonary embolism (PE). The aim of present study was to investigate the frequency of coagulation inhibitor proteins and resistance to activated protein C (APC-R) in DVT patients from Kermanshah province of Iran with Kurdish ethic background.

Materials and methods: We investigated all patients with thrombophilia who referred to Iranian Blood Transfusion Organization from May 2011 to March 2012. The levels of protein C, protein S and antithrombin were measured using STAGO kits, France (Diagnostica Stago) and the APC-R level was detected using Pefakit® kit.

Results: After excluding patients with confounding factors, 54 patients were remained. Our results showed that acquired risk factors are the most common causes of DVT in the present study. In our study protein C deficiency was found to be the most hereditary risk factor followed in frequency by APC-R. Also, in 16 patients (29.6%) there were combined hereditary risk factors with deficiency in 2 or 3 factors.

Conclusion: Our results showed protein C deficiency was the prevalent cause of DVT in our patients. Also, different pattern of hereditary risk factors in our patients compared to other regions of Iran could be attributed to different ethnic background of our patients.

Key words: Deep Vein Thrombosis, Coagulation, Coagulation Risk Factors, Hereditary

Introduction

An imbalance between the procoagulant systems and the regulatory mechanisms can lead to bleeding or thrombosis. An increase in procoagulant factors or a decrease in regulatory factors will tip the balance to excessive fibrin production, resulting in thrombus formation. These imbalances can be caused by genetic or acquired factors, or by a combination of the two. However, the process leading to excessive fibrin formation is not as simple as a single factor being disproportionate to the other factors. Rather, it consists of a combination of moderately imbalanced factors, which leads to excessive fibrin formation at the initial site of injury.(1) This, in turn, leads to vessel occlusion and fibrin clot growth.(2) The presence of multifactorial model, in which these factors interact in a cooperative manner, will lead to significant risk

for the development of thrombosis.(1) Therefore, a complete assessment of the recognized and established risk factors must be made to determine the overall risk potential for an individual. The main inhibitors of coagulation pathway are antithrombin (AT), protein C and protein S. These inhibitors are necessary to prevent Thromboembolism.(3, 4) Hereditary deficiency of these inhibitors is the main reason of change balance between the anti-clotting and the formation of thrombin.

Patients with this abnormality are susceptible to venous thromboembolism (VTE).(5) VTE is the third most common vascular disorder in the world after the ischemic heart failure and stroke.(6, 7) Two major clinical manifestation of VTE are deep vein thrombosis (DVT) and pulmonary embolism (PE). The cause of DVT could be environmental and/or genetics. Family and twin studies indicated that genetic factor accounts for about 60% of the risk for DVT.(8)

Antithrombin, protein C and S deficiencies and resistance to activated protein C (APC-R) that occur following to mutation in factor V gene, factor V Leiden (FVL), are the most important hereditary risk factors for DVT.(9)

The aim of present study was to investigate the frequency of coagulation inhibitor proteins and resistance to activated protein C in DVT patients from Kermanshah province of Iran with Kurdish ethic background.

Material and Methods

We investigated all patients with Thrombophilia who referred to Iranian blood organization from May 2011 to March 2012. The DVT diagnosis was made clinically and confirmed by color Doppler ultrasonography. Informed written consent was obtained from each individual before participation. Surgery (only when total anesthesia was administered), pregnancy, puerperium, oral contraceptive intake, plaster casts (excluding those of the upper extremities), trauma, immobilization in bed for >10 days, malignancy were considered as acquired risk factors for DVT. Patients with these risk factors were excluded from the study. Also, heparin, warfarine, vitamin K and antagonist drugs users were excluded. Blood samples were collected in tubes containing one-ninth volume of 0.109 M trisodium citrate as anticoagulant and were centrifuged at 3000 g for 15 minute to provide platelet free plasma and then frozen at -70ºC until performing experiments. Determination of protein C, protein S and antithrombin levels were performed using STAGO kits, France (Diagnostica Stago) on the STAGO, ST-4 semi automated coagulation analyzer according to instruction. Detection of APC-R level was performed using Pefakit® kit.(10) The SPSS software package version 16 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis.

Results

After excluding patients with confounding factors, 54 patients were remained. Most of the remaining patients were young with the mean age of 42.1±14.0 years including 36 (66.6%) female and 18 (33.3%) male. Venous thrombosis in leg was the most frequent clinical manifestation (n= 40, 74%). Our results indicated that acquired risk factors are the most common causes of DVT in the present study (61.7%). In our study, APC-R was determined the most single hereditary risk factors followed by protein C deficiency (Table- 1). Also, in 16 patients (29.6%) there were combined hereditary risk factors with deficiency in 2 or 3 factors (Table- 1). 4 patients (3 female and 1 male) had severe deficiency in protein C (<1%). In totally, protein C deficiency was observed as the most common hereditary risk factor in DVT patients that some patients had single and some of them had combined deficiency with other risk factors. Also, APC-R was the second risk factor -single or combined with other risk factors- in DVT patients, totally (Figure- 1 and Table- 1).

Discussion

Regarding the presence of acquired risk factors in 68% and 67% of DVT patients in our previous studies(8, 10) and around 62% in the present study, it seems that the most prevalent causes of DVT in our population to be the acquired risk factors.

Our previous study established the frequency of protein C and S deficiency in DVT patients from Kermanshah province.(10)

We indicated that the frequency of protein C deficiency was higher than the protein S deficiency in DVT patients. Similarly, in the present study protein C deficiency had the highest frequency in DVT patients.

Also, we observed a combined deficiency of protein C with other risk factors in 20.3% of patients. APC-R was the second frequent hereditary risk factor in our patients followed in frequency by protein S and antithrombin deficiency.

There are conflicting results in Asian countries related to the prevalence of hereditary risk factors in patients with thromboembolism.

Table 1. Prevalence of hereditary risk factors in male, female and total DVT patients

Sex	Risk factors											
	Protein C deficiency (%)	Protein S deficiency (%)	Antithrombin deficiency (%)	APC-R (%)	Protein C deficiency and APC-R (%)	Protein C and Antithrombin deficiency (%)	Protein S deficiency and APC-R (%)	Protein S and Antithrombin deficiency (%)	Protein C and S deficiency (%)	Protein C, S and Antithrombin deficiency (%)	Protein C, S deficiency and APC-R (%)	Antithrombin deficiency and APC-R (%)
Male (n=18)	3 (16.6)	3 (16.6)	3 (16.6)	3 (16.6)	0 (0)	3 (16.6)	0 (0)	0 (0)	2 (11.1)	1 (5.5)	0 (0)	0 (0)
Female (n=36)	7(19.4)	3 (8.3)	5 (13.8)	11 (30.5)	1 (2.7)	0 (0)	2 (5.5)	1 (2.7)	2 (5.5)	1 (2.7)	1 (2.7)	2 (5.5)
Total (n=54)	10 (18.5)	6 (11.1)	8 (14.8)	14 (25.9)	1 (1.8)	3 (5.5)	2 (3.7)	1 (1.8)	4 (7.4)	2 (3.7)	1 (1.8)	2 (3.7)

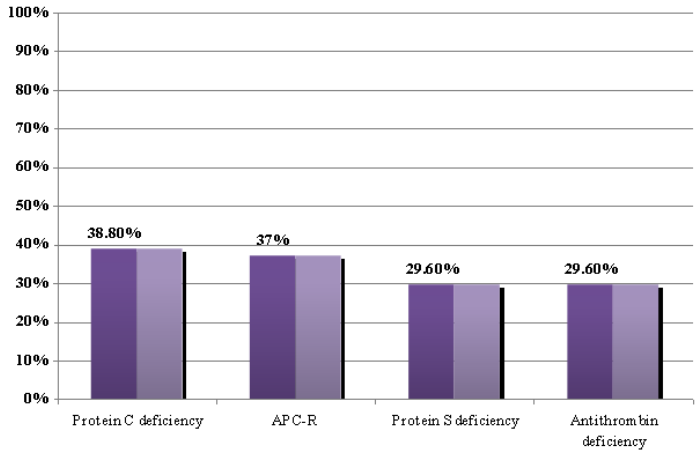

Figure- 1. Frequency of hereditary risk factors in DVT patients

A report from Tehran on DVT patients indicated that protein S deficiency had the highest frequency in DVT patients followed by APC-R, protein C and antithrombin deficiency.(11)

In Japan, most frequent risk factor for DVT was found to be protein S deficiency (17.7%) and protein C and antithrombin deficiency had lower frequency, respectively.(12) Further, in a study conducted in Taiwan, 85 consecutive and unrelated patients with otherwise unexplained thrombophilia were studied. A relatively higher prevalence of Anti thrombin III, Protein C and Protein S, but no factor V Leiden mutation was found in Chinese patients in Taiwan compared to that in western countries. In above mentioned study, protein S deficiency was the most frequent risk factor for VTE.(13) Another study from Kuwait demonstrated protein S deficiency had higher frequency than other hereditary risk factors in VTE patients.(14) Also, in a local study conducted by Armed Forces Institute of Pathology, Antithrombin III levels were studied in 32 patients with the median age of 29.1 years. Overall the frequency of Antithrombin III deficiency in young adults was 6.2% and was confined to patients with venous thromboembolic disease.(15) According to these data, Ahmadinejad et al,(11) was concluded that protein S deficiency is the major cause of DVT in Asian patients with thrombophilia.

In contrast, Kazemi et al,(16) studied hereditary risk factors in VTE patients from Tehran and indicated that the protein C deficiency had the highest frequency (25.6%) followed by protein S deficiency (13%). The findings of the present study are similar to their report. Further, a prospective study was conducted in Jordan on patients admitted or referred with thromboembolic disease to Jordan University Hospital and to the thrombosis/hemostasis laboratory at the University of Jordan. In this study the total number of studied patients was 217 and protein C, Protein S and Anti thrombin III deficiency were present in 17, 15 and 10 patients respectively. A positive family history was obtained in 65.3% of patients with thrombophilia. This study indicated that protein C deficiency was the most prevalent hereditary thrombophilic risk factor(17) that is in agreement with our results.

Our results demonstrated that APC-R had a high frequency among DVT patients. Previously, we investigated the prevalence of FVL mutation in 26 DVT patients with hereditary risk factors by PCR-based methods.(8) We the presence of factor V Leiden mutation in 26.9% of patients that is in accordance with the findings of present study. According to value of APC-R in plasma samples and kit guidelines, all of our patients had heterozygote mutation in FVL.

In contrast to Asian studies, American and European studies show very different pattern for hereditary risk factors of venous thrombosis. In these population, the frequency of coagulation inhibitors deficiency was low (1-5%) and other risk factors such as factor V Leiden and prothrombin

g.20210G>A were the major causes of venous thrombosis in their population.(18- 20) The different patterns of hereditary risk factors in various populations could be attributed to different ethnic background, sample size, and even demographic information such as age and sex of patients.

The results of this study could help clinicians in risk assessment and to manage their patients under diagnostic and prognostic points of view, as well as how long and how intensively to treat patients to enhance the outcome.

All the causes of hereditary thrombophilias can be diagnosed by relatively simple laboratory methods, however because of the low frequency of these disorders the screening of general population is not proposed in the absence of clinical symptoms. More prospective studies are required to define the occurrence of these risk factors and other causes of thrombosis in our region.

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