Protein C and S Deficiency in Deep Vein Thrombosis Patients Referred to Iranian Blood Transfusion Organization, Kermanshah

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Abstract

Introduction: Normal homeostasis system has several inhibitor mechanisms in front of the amplifier's natural clotting enzyme to prevent fibrin clots in the vessels. The main inhibitors of coagulation pathway are antithrombin (AT), protein C and protein S. Patients with hereditary deficiency of coagulation inhibitors are susceptible to venous thromboembolism (VTE). One of the major clinical manifestations of VTE is deep vein thrombosis (DVT). The present study has investigated the frequency of protein C and S deficiency among DVT patients that by using of these results and results from our previous study; we determined the most important hereditary risk factors for DVT in the Kermanshah Province of Iran with the Kurdish ethnic background.

Materials and methods: We studied 150 patients from the Kermanshah Province of Iran with Kurdish ethnic background. Patients with hereditary risk factors were excluded from the study. Estimation of protein C and protein S were performed using kits from STAGO, France (Diagnostica Stago).

Results: After excluding patients with confounding factors, 50 patients were remained. We found 14 patients (28%) with protein C deficiency and 10 patients (20%) with protein S deficiency. Also, 2 patients (4%) had both protein C and S deficiency.

Conclusion: Comparing the results of this study with our previous study on DVT patients indicates that among inherited risk factors for DVT in our population the deficiency of protein C, S and FVL mutation are the most prevalent factors. Our results show that our population has different pattern for hereditary risk factors compared with other Asian pattern for DVT patients.

Key words: Protein C, Protein S, Factor V Leiden, DVT

Introduction

Normal homeostasis system using several inhibitor mechanisms to prevents fibrin clots in the vessels. In addition, fibrinolysis system has the main role in the lysis and solution of small amount of fibrin that has been made in bloodstream and therefore be considered as a first line of defense against thrombosis. The main inhibitors of coagulation pathway are antithrombin (AT), protein C and protein S. These inhibitors are necessary to prevent Thromboembolism.(1, 2) Hereditary deficiency of coagulation inhibitors lead to change balance between the anti- clotting and the formation of thrombin. These patients are susceptible to venous thromboembolism (VTE).(3) VTE is the third most common vascular disorder in the world after the ischemic heart failure and stroke.(4, 5) Two major clinical manifestations of VTE are deep vein thrombosis (DVT) and pulmonary embolism. The incidence of DVT is 1/1000 person-year in population. The cause of DVT could be environmental and/or genetics. Family and twin

studies indicated that genetic factors accounts for about 60% of the risk for DVT.(6) Antithrombin deficiency, protein C and protein S deficiency and resistance to activated protein C (APC-R) are the most important hereditary risk factors for DVT.(7) In large blood vessels, protein C binds to a specific receptor and the binding augments the activation of protein C by thrombin. Activated protein C, inactivates factors Va and VIIIa in the presence of free protein S and phospholipids, thereby inhibiting the generation of thrombin. Free protein S itself has anticoagulant effects: it inhibits the prothrombinase complex (factor Xa, factor Va, and phospholipids), which converts prothrombin to thrombin, and the tenase complex (factor IXa, factor VIIIa, and phospholipid), which converts factor X to factor Xa.(7, 8) Hereditary protein C deficiency is inherited as an autosomal dominant trait. Seventy percent of these people have spontaneously thrombosis and others, due to risk factors such as pregnancy, surgery and the use of oral contraceptives develop thrombosis. Protein S deficiency has similar clinical symptoms with protein C and antithrombin deficiency but thrombosis due to protein S deficiency mostly occur before age 35 and spontaneously thrombosis has fewer frequency.(1, 9- 10) Also, protein C and S are vitamin K-dependent plasma protein so, vitamin K deficiency lead to protein C and S deficiency.(7, 8) Further, two common genetic variants, factor V Leiden (FVL) and prothrombin g.20210G>A, have been consistently found to be associated with DVT.(11, 12) The presence of FVL mutation that has been found in 11–29% patients with VTE predisposes patients to DVT due to the resistance to activated protein C.(12-14) The c.677C>T polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene results in decreased enzyme activity and elevation of homocysteine levels. Because hyperhomocysteinemia is associated with venous thrombosis, MTHFR c.677C>T might contribute to the risk of thromboembolic diseases.(6)

The aim of present study was to investigate DVT patients for protein C and S deficiency that by using of these results and results from our previous study; we determine the most important hereditary risk factors for DVT in the Kermanshah Province of Iran with Kurdish ethnic background.

Materials and methods

150 patients were investigated from Kermanshah Province of Iran with Kurdish ethnic background. For all patients, we collected demographic information. The DVT diagnosis was made clinically and confirmed by color doppler ultrasonography. Informed written consent was obtained from each individual before participation. As acquired risk factors for DVT, we considered surgery (only when total anesthesia was administered), pregnancy, puerperium, oral contraceptives intake, plaster casts (excluding those of the upper extremities), trauma, immobilization in bed for >10 days, malignancy, heparin, warfarine, vitamin K and Antagonist drugs were collected and if each patient had these risk factors was excluded from this study. 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 minutes to provide platelet free plasma and then frozen at -70°C until tested. Estimation of protein C and protein S were performed using kits from STAGO, France (Diagnostica Stago) on the STAGO, ST-4 semi automated coagulation analyzer as the suggested by instructions. The SPSS software package version 11.5 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis.

Results

After excluding patients with confounding factors, 50 patients were remained. Most of our patients were young with a mean age of 40.07±13.0 years including 43 (80%) women and 12 men (20%). Venous thrombosis in legs was the most frequent clinical manifestation (*n*= 39, 78%). Our results showed that acquired risk factors are most common causes of DVT in our population (66.6% patients had acquired risk factors). In this study 14 patients (28%) had protein C deficiency and 10 patients (20%) had protein S deficiency. Also, 2 patients (4%) had both protein C and S deficiency. Frequency of protein C and S deficiency according to gender are presented in Table- 1.

Discussion

Regarding the presence the 68% of DVT patients with acquired risk factors in our pervious study(6) and around 67% in present study, it seems that the most prevalent causes of DVT in our population are acquired risk factors.

Table- 1. Frequency of protein C and S deficiency according to gender in DVT patients.

Sex	Cause of DVT		
	Protein C deficiencyn (%)	Protein S deficiencyn (%)	Protein C and S deficiency (%)
Female (n=40)	8 (20)	4 (10)	1 (2.5)
Male (n=10)	6 (60)	6 (60)	1 (10)
Total (n=50)	14 (28)	10 (20)	2 (4)

There is no report from frequency of hereditary risk factors among DVT patients from our area. Results of this study indicate that protein C and S deficiency have high frequency from hereditary risk factors in our DVT patients. Also, in our previous study(6) we investigate the prevalence of factor V Leiden c.1691G>A, prothrombin g.20210G>A and methylene tetrahydrofolate reductase (MTHFR) c.677C>T in 80 DVT patients (26 patients with hereditary risk factors of 80 DVT patients). In that study, we found 26.9% (from those 26 patients) with FVL mutation. The prevalence of prothrombin g.20210G>A variant in that 26 patients was 7.6%. Also, the MTHFR c.677C>T in patients was not statistically different from control group (P=0.12).

Results of this study indicate that protein C deficiency has high frequency than protein S deficiency in our population. An Iranian research that studied on patients from Tehran indicated that protein S deficiency was the most hereditary deficiency of coagulation inhibitors in DVT patients (19.3%) and protein C deficiency had lower frequency.(15) There are conflicting results in Asian countries that indicating higher prevalence of protein S deficiency in patients with venous thrombosis than other risk factors such as protein C deficiency. In Turkish study, protein S deficiency was determined as most frequent hereditary risk factor (43.5%) for Portal Venous Thrombosis (PVT).(16) In Japan, most frequent risk factor for DVT was determined to be protein S deficiency (17.7%) and protein C deficiency had lower frequency (7.9%).(17) A study on 85 DVT Taiwanese patients indicated that 50 patients had deficiency in coagulation inhibitors. In that study protein S deficiency was most frequent risk factor (32.9%) for VTE and protein C deficiency was the second frequent risk factor (18.8%) after protein S deficiency.(18) Furthere, a study from Kuwait indicated that protein S deficiency was most frequent hereditary risk factor for VTE.(19) Ahmadi nejad *et al*. (15) concluded that in Asia the most frequent hereditary risk factor for VTE was the protein S deficiency. In contrast, Kazemi *et al.*(20) determined that the most frequent hereditary risk factor for VTE was protein C deficiency (25.6%). Protein S deficiency was the second hereditary risk factor (13%) in patients from Tehran that is similar to our results.

In the present study we had two patients with both protein C and S deficiency (4%). In the study of Ahmadi nejad *et al*.(15) there was 2.5% of patients with both protein C and S deficiency. In contrast with our study, Kazemi *et al.*(20) detected 25.6% of DVT patients with both protein C and S deficiency. Furthermore, results of Japanese and Taiwanese studies indicated that 0.88% and 3.5% of DVT patients had both protein C and S deficiency respectively.(17, 18)

In contrast to Asian studies, American and European studies show very different patterns for hereditary risk factors of venous thrombosis. In these populations, deficiency of each of three coagulation inhibitors indicated low frequency (1-5%) and other factors such as FVL or prothrombin g.20210G>A are the major causes for venous thrombosis in their population.(21-24) Major causes for different patterns of hereditary risk factors seem to be difference between ethnic, region and even demographic information of patients. It's clear that protein C and S deficiency have high frequency in DVT patients from our population and they are one of the most important hereditary risk factors in our population.

In summary, comparing the results of this study with our previous study on DVT patients suggest that among inherited risk factors for DVT in our population deficiency of protein C, S and FVL mutation are the most prevalent factors. 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.

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