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Investigating Inherited Causes in Patients Presenting with Thrombophilia

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ABSTRACT

Objective: To determine the frequency of different causes of inherited thrombophilia and evaluate clinical presentations in patients presenting with documented venous or arterial thrombosis.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology Rawalpindi, Pakistan from Jan to Jun 2022.
Methodology: One hundred and seven patients who fulfilled the selection criteria and gave informed consent in written form were enrolled. Clinical presentations were noted down and patients were assessed for inherited thrombophilia. Polymerase chain reaction was used for the detection of factor V Leiden mutation and prothrombin gene mutation. Pro C global clotting based screening test was used to determine the anticoagulatory capacity of protein C and S. Quantitative determination of the functional activity of antithrombin was performed on CS5100 automated coagulation analyzer.
Results: The mean age of the patients was 29.31±14.17 years. Inherited thrombophilia was present in 26(24.3%) patients. Factor V Leiden mutation was present in 14(53.8%), antithrombin deficiency in 6(23.1%), protein C deficiency in 3(11.5%), protein S deficiency in 2(7.7%) and prothrombin gene mutation in 1(3.9%) patient. Commonest clinical presentation was deep venous thrombosis in 10(38.5%) patients followed by pulmonary embolism in 5(19.2%), portal vein thrombosis in 3(11.5%), superficial venous thrombosis in 3(11.5%).
Conclusion: The commonest inherited cause of thrombophilia was factor V Leiden mutation and the commonest presenting complaint was deep venous thrombosis.

Keywords: Heritability, Thromboembolism, Thrombophilia.

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INTRODUCTION

Thrombophilia is a spectrum of abnormalities defined by a higher proclivity of blood to clot.[1] It could be the result of hereditary or acquired disorders1. As a result of the combination of numerous inherited and/or acquired predisposing variables, thrombophilia denotes a greater propensity to develop pathological intravascular venous or arterial thrombosis1. Thrombosis can occur in unusual locations such as the retinal, cerebral and splanchnic veins; however, the clinical manifestation of inherited thrombophilia is diverse.[2] Some people never develop thrombosis, while others may be asymptomatic until maturity, and still others get recurrent thromboembolism before the age of 30.2

Hereditary thrombophilia is a category of inherited illnesses defined by a malfunction or lack in natural anticoagulant processes, resulting in a higher tendency towards thromboembolism.[3] Activated protein C resistance (Factor V Leiden), a polymorphism in prothrombin that leads to an increased levels of prothrombin in the plasma, deficiencies of the anticoagulant factors, protein C, S and antithrombin III, an increased factor VIII levels and dysfibrinogenemia are all causes of hereditary thrombophilia.[3] Thrombotic disorders of hereditary nature except homocysteinemia, have an autosomal dominant inheritance and majority of such patients have a strong biological history.[4]

Various tests are available for detecting the causes of thrombophilia.[5] For eliminating confusion about the acute thrombotic effects or anticoagulant therapy on the results of the assay, laboratory investigations are carried out after several weeks of completing the course of oral anticoagulation in individuals with thrombosis.[6]

In Pakistan, estimates of the prevalence of inherited thrombophilia yielded variable results with one study revealing it to be 4%,[7] the other revealing it to be 2.48%,[8] and in another study, it was found to be inherited in 24.7% individuals.[9] Furthermore, estimates about different inherited causes of

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thrombophilia were also variable among different studies.[10]

In the absence of clinical symptoms, screening of the general population is not recommended due to the low prevalence of these illnesses. There is a need of conducting more prospective research to determine the prevalence of these illnesses and other causes of thrombosis. Therefore, the current study aimed to determine the frequency of different causes of inherited thrombophilia and to evaluate different clinical presentations in patients presenting with documented venous or arterial thrombosis. The study can help in guiding physicians about screening patients with inherited thrombophilia for its causes which if diagnosed earlier can help in early management of such patients and thus can improve rates of morbidity associated with the condition.

METHODOLOGY

This cross-sectional study was carried out at the Armed Forces Institute of Pathology Rawalpindi, Pakistan from January 2022 till June 2022, after taking approval from the Ethical review committee (IRB/22/850). The sample size of 107 patients was calculated keeping the expected prevalence of thrombophilia in patients presenting with thrombotic events as 7.52%,[11] with 5% margin of error and 95% confidence interval. Non-probability consecutive sampling technique was used.

Inclusion Criteria: Patients of both genders and aged 10-75 years who had a documented thrombotic event were included in the study.

Exclusion Criteria: Patients who were taking anticoagulants, had a history of either hypertension or diabetes, homocysteinemia or dysfibrinogenemia were excluded from the study.

Demographic details, clinical history and physical examination of all patients were carried out. Under aseptic measures, 10 ml of venous blood was withdrawn. 2ml was sent for the complete blood count. Polymerase chain reaction (PCR) was used for the detection of factor V Leiden mutation and prothrombin gene mutation. Pro C global clotting based screening test was used to determine the anticoagulatory capacity of protein C and S, on automated coagulation analyzer CS-5100 using Siemens ProC global kit. Ratios less than 0.8 warranted testing for protein C and S activity. If the ProC global screening test was positive, then chromogenic assay was done for functional protein C activity and clotting based assay was performed to determine protein S activity. Quantitative determination of the functional activity of antithrombin was performed on a CS5100 automated coagulation analyzer using Siemens Berichrom Antithrombin kit. Activated protein C resistance assay using Factor V deficient plasma was performed to screen for factor V Leiden mutation. Patients having ratios less than 0.8 were further confirmed by PCR for FVR506Q mutation. The Patient's sample was run in duplicate along with positive and negative controls. Zygosity was determined by conventional PCR followed by gel electrophoresis. Real time reverse transcriptase PCR assay was performed for prothrombin G20210A mutation on Cepheid GeneXpert. All findings were noted down on a predesigned proforma and were subjected to statistical analysis.

Data was analyzed using Statistical Package for the social sciences (SPSS) version 25.00. Quantitative data such as age of the patients was presented as mean and standard deviation. Qualitative data such as gender, inherited thrombophilia, clinical presentation, family history of thrombophilia and inherited causes were presented as frequency and percentages. Data was stratified for age, gender and family history. Chi square test was applied to see the association of the effect modifiers with inherited causes and a *p*-value of ≤0.05 was considered as significant.

RESULTS

The study enrolled 107 patients. The mean age of the patients was 29.31±14.17 years. With respect to the age, 16(15%) patients were of 10-15 years of age, 58(54.2%) were 16 to 30 years old, 22(20.6%) were 31 to 45 years old, 6(5.6%) were 46 to 60 years old and 5(4.7%) were >60 years old. There were 55(51.4%) males and 52(48.6%) females. Inherited thrombophilia was present in 26(24.3%) patients. With respect to clinical presentation of patients with inherited thrombophilia, 10(38.5%) presented with deep venous thrombosis (DVT), 5(19.2%) presented with pulmonary embolism, 3(11.5%) presented with portal vein thrombosis, 3(11.5%) presented with superficial venous thrombosis, 2(7.7%) presented with pregnancy loss, 1(3.8%) presented with arterial thrombosis, 1(3.8%) presented with dural venous sinus thrombosis and 1(3.8%) presented with mesenteric vein thrombosis. Among the patients with inherited thrombophilia, Factor V Leiden mutation was present in 14(53.8%) patients, antithrombin deficiency was

seen in 6(23.1%) patients, protein C deficiency was seen in 3(11.5%) patients, protein S deficiency was seen in 2(7.7%) patients and prothrombin gene mutation was seen in 1(3.9%) patients. Family history of thrombophilia was present in 33(30.8%) patients and among them 9(27.3%) had inherited thrombophilia. With respect to age, it was revealed that the majority of the patients who had inherited thrombophilia were of 16 to 30 years of age i.e. 13 out of 26(50%) patients and in terms of gender, inherited thrombophilia was more common in males i.e. in 15(57.7%) male patients compared to 11(42.3%) female patients (Table-I).

Table-I: Frequency of Qualitative Variables (n=107)

Variables	Frequency (Percentage)
Age Group	
10-15 years	16(15%)
16-30 years	58(54.2%)
31-45 years	22(20.6%)
46-60 years	6(5.6%)
61-75 years	5(4.7%)
Gender	
Male	55(51.4%)
Female	52(48.6%)
Presence of inherited thrombophilia	
Yes	26(24.3%)
No	81(75.7%)
Clinical presentation	
Deep Venous Thrombosis	10(38.5%)
Pulmonary embolism	5(19.2%)
Portal vein thrombosis	3(11.5%)
Superficial venous thrombosis	3(11.5%)
Pregnancy loss	2(7.7%)
Arterial thrombosis	1(3.8%)
Dural venous sinus thrombosis	1(3.8%)
Mesenteric vein thrombosis	1(3.8%)
Family History of thrombophilia	
Yes	33(30.8%)
No	74(69.2%)
Inherited thrombophilia in patients with a positive family history	
Yes	9(27.3%)
No	17(72.7%)
Inherited causes	
Factor V Leiden mutation	14(53.8%)
Antithrombin III deficiency	6(23.1%)
Protein C deficiency	3(11.5%)
Protein S deficiency	2(7.7%)
Prothrombin gene mutation	1(3.9%)
Inherited thrombophilia according to gender	
In males	15(57.7%)
In females	11(42.3%)
Inherited thrombophilia according to age groups	
10-15 years	3(11.5%)
16-30 years	13 (50%)
31-45 years	5(19.2%)
46-60 years	3(11.5%)
61-75 years	2(7.6%)

With respect to age, among the inherited causes of thrombophilia, factor V Leiden mutation was more common in patients of 10 to 15 years age group, antithrombin deficiency in 31 to 45 years patients, protein C deficiency in 16 to 30 years group, protein S deficiency in 16 to 30 years and 46 to 60 years patients and prothrombin gene mutation was more common in 16 to 30 years of patients. However, the association between the inherited causes of thrombophilia and age was not significant statistically i.e. $p>0.05$ (Table-II).

Table-II: Association Between age and Inherited Causes of Thrombophilia (n=26)

Inherited Causes	Age Group					*p*-value
	10-15 Years n=3	16-30 Years n=13	31-45 Years n=5	46-60 Years n=3	61-75 Years n=2	
Factor V Leiden Mutation	2 (14.3%)	7 (50.0%)	2 (14.3%)	2 (14.3%)	1 (7.1%)	0.602
Antithrombin III deficiency	0(0.0%)	2 (33.3%)	3 (50.0%)	0 (0.0%)	1 (16.7%)	0.168
Protein C deficiency	1 (33.3%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.784
Protein S deficiency	0 (0.0%)	1 (50.0%)	0 (0.0%)	1 (50%)	0 (0.0%)	0.092
Prothrombin gene mutation	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.931

In terms of gender, factor V Leiden mutation and protein S deficiency was equally encountered in both males and females, whereas, antithrombin deficiency, protein C deficiency and prothrombin gene mutation was more commonly seen in males. However, no significant association was observed between gender and inherited thrombophilia (Table-III).

Table-III: Association Between Gender and Inherited Causes of Thrombophilia (n=107)

Inherited Causes	Gender		*p*-value
	Male (n=55)	Female (n=52)	
Factor V Leiden Mutation (	7(50.0%)	7(50.0%)	0.910
Antithrombin III deficiency	4(66.7%)	2(33.3%)	0.441
Protein C deficiency	2(66.7%)	1(33.3%)	0.592
Protein S deficiency	1(50.0%)	1(50.0%)	0.968
Prothrombin gene mutation	1(100.0%)	0(0.0%)	0.329

Among the patients with inherited thrombophilia, family history was positive in 6 out of 9(66.7%) patients with Factor V mutation, in 1 out of 9 (11.1%) patients with antithrombin deficiency, in 1 out of 9 (11.1%) patients with protein C deficiency and in 1 out of 9 patients (11.1%) with protein S deficiency. However, this association between the inherited causes of thrombophilia and family history was statistically insignificant i.e. $p>0.05$ (Table-IV).

Table-IV: Association between family history of inherited thrombophilia and inherited causes of thrombophilia (n=107)

Inherited Causes	Family History Of Thrombophilia In Inherited Thrombophilia		*p*-value
	Positive (n=33)	Negative (n=74)	
Factor V Leiden Mutation	6(66.7%)	3(33.3%)	0.340
Antithrombin III deficiency	1(11.1%)	8(88.9%)	0.292
Protein C deficiency	1(11.1%)	8(88.9%)	0.960
Protein S deficiency	1(11.1%)	8(88.9%)	0.634
Prothrombin gene	0(0.0%)	9(100.0%)	0.458

DISCUSSION

The current study results revealed that inherited thrombophilia was present in 26(24.3%) patients and the commonest inherited cause of thrombophilia was factor V Leiden mutation that occurred in in 14(53.8%) patients followed by antithrombin III deficiency in 6(23.1%) patients. With respect to age, it was revealed that the majority of the patients who had inherited thrombophilia were of 16 to 30 years of age i.e. 13 out of 26 (50%) patients and in terms of gender, inherited thrombophilia was more common in males i.e. in 15(57.7%) male patients compared to 11(42.3%) female patients. There was no significant association between age, gender, family history of thrombophilia and inherited causes of thrombophilia.

Studies conducted internationally have revealed that the most frequent inherited form of thrombophilia is factor V Leiden thrombophilia.[11] The incidence of one copy of the factor V Leiden mutation in the general population in the United States and Europe is 3-8 percent; approximately 1:5000 people have two copies of the mutation.[12] Protein S deficiency occurs in 1 in 500 people,[13] about 1 in 500 people have moderate protein C deficiency,[14-15] and prothrombin-related thrombophilia is the second most common hereditary form of thrombophilia, affecting around 1.7-3 percent of the general population in Europe and the United States.[16] In the general population, the prevalence of hereditary antithrombin III deficiency ranges from 1:500 to 5000.[17] The findings of current study also supported the evidence of factor V Leiden mutation as being the commonest cause of inherited thrombophilia. Our study also similarly revealed higher frequencies of antithrombin III and protein C deficiency as other common causes.

Hereditary thrombophilia is commonly seen in young patients and it is very uncommon after 40 years of age.[18] This finding is consistent with current study findings too which revealed that inherited thrombophilia was more frequent in patients who were between 16 to 30 years of age. Therefore, studies have proposed that all individuals who are of young age and present with an arteriovenous thromboembolic event which is unprovoked, must be screened for inherited causes of thrombophilia.[19,20] This can help the treating physician in making decisions about the duration of anticoagulation to be in different clinical setups.[21]

LIMITATIONS OF STUDY

The current study had certain limitations. Firstly, the study was carried out at a single center and the sample size was small, so there is an issue of the generalizability of the results. Secondly, the cost-effectiveness was not assessed by the procedures.

CONCLUSION

The commonest inherited cause of thrombophilia in our study population was factor V Leiden mutation followed by antithrombin III and commonest presentation was DVT. In view of the results, it is recommended to screen all young patients for heritable causes of thrombophilia who present with recent history of thromboembolic phenomenon, so that prompt diagnosis and early intervention is provided to such patients in order to reduce the rates of morbidity as well as mortality in such patients.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

MS & MK: Conception, study design, drafting the manuscript, approval of the final version to be published.

AM & HSM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

RM & MSB: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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