ORIGINAL ARTICLE

Evaluation of Thrombophilia Profile in Northern Pakistan: Frequency and Presentation

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ABSTRACT

Objective: This study aims to ascertain the prevalence of thrombotic disorders and thrombophilia profile among patients in Northern Pakistan.

Study Design: Cross sectional descriptive study.

Place and Duration of Study: This study was conducted at the department of hematology, Shifa International Hospital, Islamabad, Pakistan from 1st June 2024 to 31st July 2025.

Materials and Methods: Ethical approval was obtained from the Institutional Review Board (IRB) prior to commencement of the study. The sample size was calculated using the WHO sample size calculator, considering a confidence level of 95% and anticipated prevalence of 2.5%. Non- probability consecutive sampling technique was employed. Data were collected using a structured proforma and analysed using SPSS version.

Results: Out of the 926 patients, there were 256 patients with portal vein thrombosis (PVT) (27.6%), 192 with stroke (20.7%), 160 with Deep vein thrombosis (DVT) (17.2%), mesenteric thrombosis 124 (13.3%) and 94 with Pulmonary embolism (PE) (10.1%) remaining 100 were presented with minor thrombotic episodes. Thrombophilia profiles revealed that 368 patients had protein S deficiency, 298 had anti-thrombin deficiency, 146 had protein C deficiency, and 114 patients had an activated protein C resistance. A total of 50 thrombotic disorders patients underwent molecular testing, out of which 10% were heterozygous for the Factor V Leiden mutation. 2% were homozygous for the Prothrombin (Factor II) mutation, and 88% did not exhibit any mutation.

Conclusion: According to this study, Protein S and anti-thrombin deficiencies are the most common deficiencies among thrombotic disorders patients in northern Pakistan. The prevalence of undetected site thrombosis, such as PVT, was higher than reported globally.

Key Words*: Anti-Thrombin, Factor V Leiden, Protein C Deficiency, Protein S Deficiency, Thrombotic Disorders.*

Introduction

Blockage of a vein caused by a thrombus (blood clot), is termed as Thrombotic disorders. It primarily presents as deep vein thrombosis (DVT), stroke, pulmonary embolism (PE), and portal vein thrombosis. Thrombotic disorders are acknowledged as a leading cause of morbidity and mortality on a global scale. It is placing a heavy strain on healthcare systems. In addition to contributing to acute patient care more difficult, the illness can have long-term effects such recurrent thromboembolism, persistent pulmonary hypertension and post-thrombotic syndrome.[1,7,9]

The pathogenesis of thromboembolism encompasses a complex interaction of three primary components, referred to as Virchow's triad: venous stasis, hypercoagulability, and endothelial damage. Under normal circumstances, naturally occurring inhibitors like anti-thrombin (AT), protein S (PS), and protein C (PC) effectively regulate haemostasis. These inhibitors have an essential role in preventing excessive thrombus formation by inhibiting the function of clotting factors. Together with protein S, activated protein C deactivates clotting factors VIII(a) and V(a), which inhibits the coagulation cascade[1,7,8]. Anti-thrombin also limits the pro-coagulant activity of thrombin by forming complexes with it and other serine proteases such factors IX(a), XI(a), and XII(a). Any genetic or acquired deficiency in these natural inhibitors exposes patients at risk for thrombophilia, a condition in which they are hypercoagulable[8,9,14].

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The prothrombin gene mutation, such as the G20210A variant, or Factor V Leiden (FVL) mutation is one of the most extensively studied genetic causes of thrombophilia. The mutation causes Factor V to become resistant to cleavage by activated protein C, extending its pro-coagulant activity. It involves the substitution of glutamine for arginine at position 506 (FVR506Q).[2,3,7,9,15] Similarly, insufficient inhibition of clotting factors and an increased risk of VTE are caused by deficits in PC, PS, or AT. The presence of lupus anticoagulant and mutations in the prothrombin gene are other noteworthy contributions. These inherited or acquired abnormalities disrupt the natural balance of coagulation, leading to a predisposition for thrombosis[7,9,16,17].

Depending on the location and degree of thrombosis, thromboembolism can manifest with a wide range of clinical symptoms. Pain, swelling, redness, and dilated superficial veins are the usual symptoms of deep vein thrombosis, which usually affects the major veins of the lower limbs. A potentially fatal consequence, pulmonary embolism presents as abrupt dyspnoea, chest discomfort, tachypnoea, and occasionally circulatory collapse. In contrast to portal vein thrombosis, which frequently results in stomach pain, ascites, or splenomegaly, cerebral venous thrombosis or stroke caused by thrombophilia can manifest as disorientation, speech abnormalities, or focal neurological disability. Clinical suspicion, laboratory testing, and radiographic confirmation are all necessary for an accurate and prompt diagnosis of various presentations.[3,7,9,15,17]

Geographical and ethnic factors affect the frequency of hereditary thrombophilia worldwide. Factor V Leiden mutations are reported to be 4–5% common in Western populations, although PC, PS, and AT deficits are quite uncommon, affecting less than 1% of the general population. Data is scant in South Asian nations, such as India and Pakistan, but recent studies point to regional differences in prevalence that may be caused by consanguinity, genetic origins, and a lack of knowledge about thrombophilia testing. Studies from Pakistan, show comparatively higher rates of FVL (4.9%) and AT deficiency (10.9%) than the global average. These results highlight how crucial population-specific research is for establishing treatment and diagnosis plans.[5,7,11,12]

Despite thrombophilia's clinical significance, there is limited research occurring in Pakistan. Only a handful of studies have attempted to correlate laboratory results with clinical manifestations, and even fewer have reported the prevalence of familial thrombophilia in VTE patients. Therefore, there is not enough information to support evidence-based practice regarding how long anticoagulant therapy should be administered, whether family members should be screened, or genetic counselling should be provided to high-risk groups. Regional epidemiological statistics are also essential for developing public health plans, enhancing provider awareness, and tailoring diagnostic procedures to fulfil local requirements.[6,9,15,18,19]

Long-term or even lifetime anticoagulation medication is frequently necessary for patients with thrombophilia, particularly those who have recurring episodes, were young when they first had the condition, have uncommon site thrombosis, or have a strong family history. However, determining underlying risk factors, especially genetic predispositions, is crucial in determining the duration of therapy. Lack of data makes it difficult to diagnose patients quickly and provide the best care possible, which results in unavoidable morbidity and mortality in areas like northern Pakistan where healthcare access and awareness are varied.[7,9,18,19]

Therefore, by assessing the prevalence of thrombophilia profile and clinical manifestations of thromboembolism in patients from northern Pakistan, this study seeks to fill this critically important gap. The goal of the study is to improve knowledge of hereditary thrombophilia in the local context by methodically evaluating genetic and laboratory risk factors in addition to clinical manifestations. In addition to adding to the body of scientific literature, the results will facilitate the development of regional guidelines, educate preventive measures, and enhance patient outcomes in this clinically significant but little-studied field.

Materials and Methods

This cross-sectional study was conducted in the Department of Haematology, Shifa International Hospital, Islamabad, from 1st June 2024 to 31st July 2025, following STROBE guidelines. Ethical approval

was obtained from the Institutional Review Board (IRB#0433-25, approval date 16-Nov-2023). A total of 926 patients with proven thrombotic disorders diagnosed through radiological imaging (Doppler ultrasonography, CT pulmonary angiography, MRI/MRV) and D-dimer testing, between the ages of 18-45, were included, as the prevalence of Thrombotic disorders are significantly higher in this age group. Patients who were already receiving anticoagulant medication or who had tested within two weeks of a thrombotic incident, to minimise the effect of laboratory variability on measured levels of protein C, protein S, and antithrombin, patients were excluded if testing was performed during an episode of acute thrombosis or while on anticoagulation therapy. The medical records of every patient whose blood sample was sent to the institute laboratory for thrombophilia screening were reviewed in order to collect demographic and clinical data.

To maintain the optimal anticoagulant-to-blood ratio, peripheral venous blood samples were collected in EDTA vacutainers for molecular studies and in 3.2% trisodium citrate tubes for serological assays, in accordance with standard laboratory protocols. To obtain platelet-poor plasma for serological testing, the samples were centrifuged for 15 minutes at 4000 rpm. This plasma was then used for thrombophilia screening, including protein S, protein C, anti-thrombin and Activated Protein C resistance (APCR). using a Sysmex 2500 automated coagulation analyser. A functional chromogenic assay based on the PROTAC activation method was used to evaluate protein C deficiency; 70–140% activity was accepted as normal. Using a clotting-based assay, protein S activity was assessed and reference values ranged from 60 to 130%. Using chromogenic functional test, anti-thrombin levels were assessed, with 75–125% being considered normal.

For Factor APCR, the patient's plasma was mixed with Factor V–deficient plasma in a 1:4 ratio and activated protein C resistance was assessed using the Pro C Global kit, with ratios of 0.86–1.10 regarded as normal.

Blood samples were subjected to molecular testing; an SNP Genotyping Assay (a Multiplex end-point Allelic Discrimination Assay) was used to evaluate mutations in Factor V Leiden (FVL) and Prothrombin (PT). These tests are relatively expensive and not routinely covered in our resource-limited healthcare settings. Therefore, testing was restricted to patients who gave consent, has no affordability issues and with strong clinical suspicion of inherited thrombophilia, such as those with unprovoked or recurrent thrombotic events, young age at presentation, or positive family history. Only 50 patients out of 926 fulfilled the criteria and were successfully underwent molecular testing.

Strict internal and external quality controls were put in place to guarantee quality assurance. Internal QC with 2 levels per batch and external proficiency testing via CAP thrice yearly were employed. SPSS version 26 was used to analyse the data. While categorical variables (gender, clinical presentation, and thrombophilia profile) are expressed as frequencies and percentages.

Results

The study comprised 926 patients, of whom 432 (46.8%) were female and 494 (53.2%) were male. In terms of clinical presentation, 256 patients (27.6%) had portal vein thrombosis (PVT), 192 patients (20.7%) had stroke, 160 patients (17.2%) had DVT, 124 patients (13.4%) had mesenteric thrombosis, and 94 patients (10.1%) had PE. Minor occurrences of thrombosis were observed in the remaining 100 patients (10.8%).

Overall, DVT predominated in both genders, with a slightly higher frequency in males.

Figure 1 Illustrates the distribution of thrombotic disorders clinical presentations.

Among 926 patients, Protein S deficiency was found in 368 patients (39.7%), antithrombin deficiency in 298 (32.2%), protein C deficiency in 146 (15.8%), and activated protein C resistance in 114 (12.3%) from thrombophilia screening.

Figure 2 Demonstrates the prevalence of thrombophilia profiles.

Fifty patients with a known history of thrombotic disorders were subjected to molecular testing; of these, 88% had no detectable mutations in both gene, 10% were heterozygous for the Factor V Leiden mutation, and 2% were homozygous for the Prothrombin (Factor II, G20210A) mutation.

Overall, the study shows that this group has a significant prevalence of hereditary deficits, especially those related to Protein S and anti-

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thrombin. Higher than anticipated rates of unusual site thrombosis, particularly portal vein thrombosis and stroke, were noted in comparison to global statistics.

Table I: Primer Sequences Used for Detection of Factor V Leiden and Prothrombin Mutations

FVL F	GGG CTA ATA GGA CTA CTT CTA ATC TGT AAG A
FVL R	TTC TGA AAG GTT ACT TCA AGG AA
FVL	1691G/A
PMT F	TGT GTT TCT AAA ACT ATG GTT CCC AT
PMT R	CCA TGA ATA GCA CTG GGA GCA T

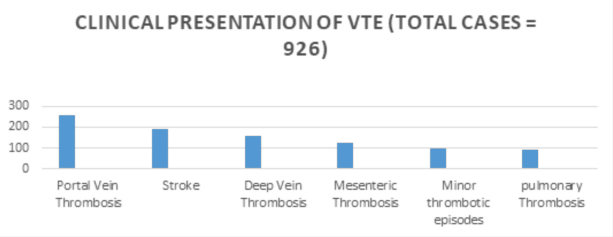

Figure 1: Illustrates the distribution of thrombotic disorders clinical presentations.

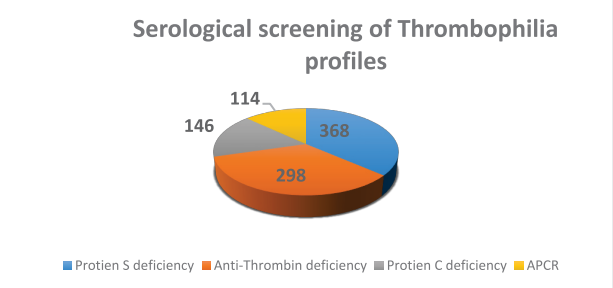

Figure 2: Demonstrates The Prevalence of Thrombophilia Profiles

Discussion

This study provides a current assessment of the clinical manifestations and thrombophilia profiles of patients with thrombotic disorders from northern Pakistan. In light of earlier research, the results are significant, especially the study by Khan et al. (2021), which found that in a smaller cohort, heritable thrombophilia rates were 3.2% for protein C deficiency, 2.5% for protein S deficiency, 10.9% for anti-thrombin deficiency, and 4.9% for Factor V Leiden mutation. According to the study's findings, thrombophilia-related abnormalities are quite common among thrombotic disorders patients in our area. Significant of our population (34.3%) had protein S deficit, which was closely followed by protein C deficiency (15.7%) and antithrombin deficiency (32.2%). 12.3% of cases had activated protein C resistance, which is significantly higher than the rates of ~4–5% found in previous Pakistani and Western-based investigations.[6]

Our thrombotic disorders subgroup's molecular test results confirmed the genetic component of thrombophilia. Among 50 tested patients, 10% were heterozygous for the Factor V Leiden (FVL) mutation and 2% homozygous for the Prothrombin (Factor II, G20210A) mutation; the remaining 88% had no detectable mutations. Although skewed by sample selection, these mutation rates are on par with or marginally greater than those observed in some recent studies conducted in Pakistan.[2,6,15,18]

These mutation rates are comparable to or slightly higher than those seen in some recent Pakistan-based studies, though skewed by sample selection. According to Maria Khan et al. (2021) in "Heritable Thrombophilia in Venous Thromboembolism in Northern Pakistan," of 182 VTE patients, ~4.9% had a Factor V Leiden mutation and ~10.9% had antithrombin deficiency[1,6,20].

Our cohort's clinical presentation also differed from several international findings. The prevalence of deep vein thrombosis (DVT) remained high (17.8%), consistent with global research showing that DVT is the most prevalent kind of thromboembolism. In contrast to previous local and Western cohorts, portal vein thrombosis (28.5%) and stroke (20.8%) were disproportionately common.[21] These results imply that inherited thrombophilia is more likely to cause "unusual site" thrombosis in our demographic, that individuals in our area may appear later in the course of the disease, or that referral patterns may favour more severe or uncommon presentations. Particularly for younger patients or those with recurrent thromboses, the significant prevalence of thrombophilia in this group supports the need for routine screening in northern Pakistani thromboembolic patients[5,11,12].

Decisions on prolonged or even lifetime anticoagulation therapy may be influenced by the early identification of deficiencies in protein C, protein S, or antithrombin as well as Factor V Leiden or Prothrombin mutations.[4,7,8,13,17] Furthermore, in high-risk circumstances like surgery, pregnancy, or extended immobility, bearers of mutations may benefit from genetic counselling and preventive measures. Our results highlight the necessity of raising awareness and increasing access to thrombophilia testing in Pakistan from a perspective

of public health. Before major problems arise, many patients remain undiagnosed.[10,21] Morbidity and mortality may be decreased by implementing cost-effective screening procedures, particularly in tertiary care settings, and perhaps by employing cutting-edge molecular tools for mutation identification. According to a recent article titled "Revisiting thrombophilia testing: leveraging next-generation sequencing for precision in VTE management" (Youssry & Ayad, 2025), patients with unprovoked or atypical VTE presentations should be given more opportunities to use broader genetic panels than just FVL and Prothrombin G20210A.[9,14,19] Finally, further research is needed to determine whether the unexpectedly high rates of protein deficiencies are caused by methodological factors, such as laboratory standards and cutoff values. It is also important to investigate the potential influence of demographic or environmental variables, including nutrition and illnesses.

Limitations: Some patients had incomplete clinical and molecular data, which may have influenced prevalence estimates. Functional levels of protein C, protein S and antithrombin may be influenced by acute thrombosis and inflammatory states; therefore, some deficiencies observed may represent transient reductions rather than hereditary thrombophilia.

Only 5.4% (50 out of 926 patients) of cohort underwent genetic testing due to cost constraints.

Despite these limitations, the study represents one of the largest cohorts reported from northern Pakistan evaluating comprehensive thrombophilia profiles. It provides valuable regional data, help to bridge an important knowledge gap and inform future diagnostic and management strategies.

Conclusion

According to this study, protein S and anti-thrombin deficiencies are the most prevalent among thrombotic disorders patients in northern Pakistan, highlighting a significant occurrence of hereditary thrombophilia within this group. Although the most frequent clinical manifestation noted was portal vein thrombosis which is abnormally high, while globally it is DVT. Therefore, our findings indicate a greater prevalence of thrombophilia in this region than previously reported.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.