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RESEARCH ARTICLE

SPECTRUM OF THROMBOPHILIC DISORDERS IN TMH (MANY HEADS OF THE HYDRA) – A FIVE YEARS EXPERIENCE

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ABSTRACT

Background: Thrombophilia is characterised by increased propensity to vascular thrombosis, due to coagulation abnormalities leading to a procoagulant state. It accounts for 25% - 30% of cases of venous thromboembolism. The clinical features vary according to the site of the thrombus.
Aim: To study the presenting profile of thrombophilia in patients with spontaneous thrombosis, identify the thrombophilic risk factors and to study their response to anticoagulation.
Method: It was a prospective study, done in patients admitted in the medical wards of Tata Medical Hospital, Jamshedpur from September 2010 to December 2015.
Observations: Of the total of 18 patients of thrombophilia, 4 (22.2%) had acquired causes which included antiphospholipid antibody syndrome (APLA) in 2 patients (11.1%) and polycythemia vera in 2 patient (5.6%). The inherited 14 causes (77.8%) detected were low protein C activity (1 patient - 5.6%), low protein S activity (3 patients- 16.7%), combined protein C and S deficiency (4 patients – 22.2%), high level of factor VIII (1 patient – 5.6%), elevated serum fibrinogen level 2 (11.1%), high IX level (4 patients – 22.4%), combined high factor VIII and fibrinogen levels (2 patients -11.1%) and elevated factor VIII combined with factor IX level (1 patient – 5.6%). The clinical presentations included deep vein thrombosis (DVT) of the calf veins, sagittal vein thrombosis, extensive intestinal gangrene due to superior mesenteric vein thrombosis, pulmonary thromboembolism, digital gangrene of terminal phalanges, recurrent abortions, portal hypertension with ascites and Budd-Chiari syndrome. 2 of 18 (11.1%) patients developed haemorrhagic complications after starting anticoagulation while 2 (11.1%) expired after stopping anti-coagulation.
Conclusion: Thrombophilia screening should be done in patients presenting with thrombus in unusual sites, recurrent thrombosis and young patients without obvious risk factors. The risk of bleeding from anticoagulation should be judiciously balanced with the potential for recurrent thromboembolism due to under anticoagulation.

INTRODUCTION

Although there is no internationally accepted definition, the term thrombophilia is used to describe several heterogenous disorders with increased propensity for thrombosis, especially venous thrombosis[1]. It may be inherited or acquired. Of late, its role in the etiopathogenesis of thrombosis is being increasingly recognized. Inherited thrombophilia is indentified in approximately 25 – 30% of patients with thromboembolic disease [6]. The annual incidence of venous thromboembolism (VTE), which includes deep venous thrombosis and pulmonary embolism, is one or two per 1,000 persons [1,3,4].

Aim: To study the presentation profile of thrombophilia, identify the thrombophilic risk factors in patients with spontaneous vascular thrombosis and evaluate their response to anticoagulation.

Patients and methods: This was a prospective study carried out from September 2010 to December 2015 in the medical wards of Tata Main Hospital, Jamshedpur, with the approval of the ethics committee of the hospital.

Inclusion criteria: All patients with the clinical features of venous and or arterial thrombus without conventional risk factors for spontaneous thrombosis and had at least one thrombophilic risk factor.

Exclusion criteria: Patients with heart disease with or without atrial fibrillation, chronic liver disease and obvious risk factors for thrombosis including pregnancy and puerperium, prolonged bed rest, post operative period and use of oral anticoagulants within 3 months of presentation.

Assessment: Patients were assessed for the prothrombotic state by detailed history of symptoms related to venous thromboembolism (VTE) and arterial thromboembolism (ATE), clinical examination, history of previous episodes of thrombosis, risk factors for atherosclerosis, familial occurrence

of thrombosis and laboratory investigations. All patients had *de novo* (*in situ*) thrombosis.

Diagnosis of thromboembolism: ATE was diagnosed if patient had symptomatic myocardial infarction, ischemic stroke, transient ischemic attack (TIA), or peripheral artery disease and was objectively verified. Myocardial infarction was confirmed by typical ECG features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke was documented by computed tomography scanning or magnetic resonance imaging. Peripheral artery disease was considered thromboembolic in presence of acute signs and symptoms of ischemia and was documented by Doppler study or arteriography.

VTE was considered established if deep vein thrombosis was confirmed by compression ultrasonography or doppler venography, and pulmonary embolism was confirmed by ventilation-perfusion lung scanning, spiral computed tomography scanning, or pulmonary angiography. Portal vein thrombosis (PTV) was considered acute if there was abdominal pain and computerised tomography scan showed clot in the portal vein with surrounding contrast enhancement. PVT in absence of acute abdominal pain and associated with demonstrable portal cavernoma on imaging studies was labelled as chronic PVT.

Laboratory studies: included complete blood picture, platelet count, coagulation profile (Activated Partial Thromboplastin Time (APPT), Prothrombin Time (PT), International

Table 1 Clinical presentations and the site of thrombus associated with various thrombophilic states, and the duration of anticoagulation

Patient ID	Age & Gender	Previous /other thrombosis	Clinical Presentation	Site of thrombus	Thrombophilic condition	Duration of anticoagulation
Patient 1	43 yr / F	DVT right calf (1 yr back), two abortions	Dry gangrene of right index finger tip and auto-amputation	Digital arteries, Popliteal vein	APLA* syndrome	Long term
Patient 2	37 yr /F	Recurrent DVT (3 episodes), One abortion	Dry gangrene of left 2nd and 3rd toes	Digital arteries, Popliteal vein	APLA* syndrome	Long term
Patient 3	70 yr /M	DVT Rt leg MI (Coronary arteries)	Dry gangrene of finger tip	Calf veins, Coronaries and Digital arteries	Polycythemia rubra vera (JAK mutation negative) ς	Long term
Patient 4	43 yr /M	DVT right calf (3 yrs back)	Budd- Chiari Syndrome (ascites jaundice and pain abdomen)	Hepatic veins, Intrahepatic IVC and Renal veins	High Factor VIII 535 IU/dl and fibrinogen levels (506mg/dl)	Long term
Patient 5	41 yr /M	Nil	COPD like picture (Acute intermittent dyspnea)	Both main pulmonary arteries, left pulmonary artery and branches	High Factor VIII (325 IU/dl) and fibrinogen levels (535mg/dl)	Long term
Patient 6	21yr/M	Nil	DVT calf (Swelling of the left leg)	Extensive thrombus of popliteal vein and femoral vein	High VIII (450 mg/dl)	3 months
Patient 7	25yr/F	Nil	Recurrent seizures and altered sensorium	Superior Sagittal Sinus (SSS)	Protein S deficiency (38 .7 %)	Long term
Patient 8	41yr/F	Nil	Acute pain abdomen and upper GI bleed	Acute portal vein thrombosis	Protein S deficiency (20.2 %)	Long term
Patient 9	42yr/M	DVT left leg (2 yrs back)	Small bowel ischemia and gangrene (Acute abdomen)	Superior Mesenteric Vein (SMV)	Combined Protein C and Protein S deficiency (35.2 % and 38 % respectively)	Long term
Patient 10	45yr/F	Nil	Upper GI bleed (Portal hypertension)	Chronic portal vein thrombosis (portal cavernoma)	Combined Protein C and Protein S deficiency (37.2% and 21%)	Long term
Patient 11	41yr/M	Nil	Pain and swelling of left calf (DVT)	Popliteal vein	Protein S deficiency (18.%)	Long term
Patient 12	52yr/F	Nil	Pain & swelling of left calf followed by dyspnea (DVT and PTE)	Right popliteal vein, main pulmonary trunk, RPA and LPA	Protein C deficiency (20%), MTHFR resistance (heterozygous)	Long term
Patient 13	33yr/F	Sagittal sinus thrombosis (2yrs back)	Sudden onset of dyspnea (Acute PTE)	Right and left main pulmonary artery	Combined Protein C and Protein S deficiency (22.4 & 44 %)	Long term
Patient 14	42 yr/F	Nil	Pain in right leg, Exertional dyspnea (DVT and PTE)	Right femoral and popliteal vein, Right and Left main pulmonary artery	Combined Protein C and Protein S deficiency (32.2 % and 34 % respectively)	Long term
Patient 15	35 yr/F	Nil	Upper GI bleed (Portal hypertension)	Chronic Portal vein thrombosis (portal cavernoma)	Protein C deficiency (37.2%)	Long term
Patient 16	38yr/M	DVT right leg (5yrs back)	Acute anterolateral myocardial infarction	Coronary arteries (LAD and LCX)	High VIII (432 IU/dl) Factor IX (238 IU/dl)	Long term
Patient 17	58yr/F	Nil	Abdominal pain, distension	Splenic vein thrombosis	Polycythemia vera rubra (JAK2 mutation –Positive)	Long term
Patient 18	35 yr/M	Nil Positive family history of coronary artery disease	Acute ASMI (Anginal pain)	Coronary arteries Proximal LAD, OM1	MTHFR resistance (Heterozygous) Homocysteine level – Protein C deficiency (24.2%)	Long term

*LA – Strongly positive, APPT- prolonged aCL antibody –positive (38.5 Gpl) ς Hb -18.5gm%, Hct – 65%, RBC count – 6.1mill/cu mm, Platelet -6.4 L
Factor IX - normal range, 60-120%

Normalised Ratio (INR), Thrombin Time (TT), liver function tests (serum bilirubin, ALT, AST, ALP, serum albumin and globulin), renal function tests (blood urea, serum creatinine). In addition, haemogram, cell type, Ham's test, bone marrow aspiration and trephine biopsy was done wherever indicated.

Thrombophilia screen: Tests included measurement of Protein C and Protein S antigen by enzyme-linked immunosorbent assay (ELISA), functional assay (activity) of Protein C by clotting based assay and Anti-thrombin III measured by chromogenic substrate assays. Protein S deficiency type I was defined by lowered total protein S antigen levels (68IU/dL). Protein C deficiency types I and II were defined by reduced levels of protein C antigen (63IU/dL) and/or activity (64IU/dL). Anti-thrombin deficiency was defined by decreased levels of antithrombin activity (74IU/dL). Deficiencies were considered inherited if they were confirmed by measurement of a second sample that was collected 3 months later and were found in at least 1 family member (where possible), and acquired conditions were excluded. Factor VIII:C was measured by 1-stage clotting assay using VIII – deficient plasma and was considered increased at levels 150 IU/dL. Serum fibrinogen level was estimated using the Clauss method. Presence of lupus anticoagulant was defined by 3 methods - abnormal dilute Russel's viper venom test time, kaolin clotting time and activated partial thromboplastin time. Anticardiolipin antibodies IgG and IgM were quantified by immunosorbent assay. Serum homocysteine level was determined by fluorescence polarization immunoassay. Paroxysmal Nocturnal Haemoglobinuria (PNH) screening was done using acidified serum test (Ham's test) and sucrose lysis test (SLT). DNA was extracted from peripheral blood leukocytes from 5 ml venous blood sample collected in EDTA vials in all cases using phenol–chloroform extraction method followed by ethanol precipitation. DNA analysis was done by Polymerase chain reaction (PCR) to identify mutation in methylenetetrahydrofolate reductase (MTHFR) gene - C677T, prothrombin G20210A mutation and factor V leiden mutation (in case of resistance to activated protein C (APCR) and JAK2 mutation. Screening for thrombophilia was done at the time of admission, before starting anticoagulation and repeated at 3 months after the index episode after stopping anticoagulation for 2 weeks. 5 ml blood was drawn after an overnight fast, collected in plain and EDTA vials and sent for thrombophilia screening.

Treatment: Patients were commenced with subcutaneous heparin/Low Molecular Weight Heparin (depending upon the case). They were treated simultaneously with warfarin and its dose was titrated to obtain INR of 2 to 3. Once INR was in therapeutic range for 2 consecutive days, heparin was stopped (after 4 to 5 days) and warfarin was continued for at least 3 months or more as indicated by their underlying condition.

Follow up: Patients were kept under regular monthly follow up and were assessed for their response to therapy by clinical examination and imaging by CT, Doppler ultrasound or MR angiography as the case was, at 3 months, 6 months or more, of treatment as needed.

Observations: There was a total of 18 patients during the study period. Their age ranged from 24 to 70 years. Of these, 15 (83.3%) were less than 45 years and 3 (16.7%) were more than 45 years. The male to female sex ratio was 7:11. Of these 18 patients, 14 (77.8%) had inherited causes, while 4 (22.2%) had acquired causes. The inherited causes found were isolated protein C deficiency, isolated protein S deficiency, combined protein C and S deficiency, elevated factor VIII, elevated serum fibrinogen levels, elevated factor IX level and MTHFR gene resistance. The acquired causes identified were antiphospholipid antibody syndrome in 2 (11.1%) patients and polycythemia vera in 2 (11.1%) patients. Multiple risk factors were found in 8 (44.4%) patients. Of the 14 patients with inherited thrombophilia, 13 (92.9%) had venous thrombosis, while 1 (7.1%) patient had both arterial and venous thrombosis. The clinical presentations associated with thrombophilia are summarized in **table 1**.

Protein C, Protein S and Anithrombin lll levels: Of the 18 patients with thrombosis, 1 (5.6%) had isolated protein C, 3 (16.7%) had isolated protein S deficiency, none had antithrombin lll deficiency, 4 (22.2%) had combined protein C and protein S deficiency and 2 (11.2%) had protein C deficiency combined with heterogeneous MTHFR deficiency. The average low levels of protein C and protein S were 36.3% and 22.8% of the normal respectively while the lowest level of protein C and protein S respectively were 22.2% and 15.7% of the normal **(figures 1 and 2)**. The clinical manifestations related to protein C, protein S, combined protein C and protein S deficiency were as listed in the above table. The youngest age at the first clinical presentation was 25 years for protein S deficiency, 35 years for protein C deficiency and 33 years for combined protein C and protein S deficiency. Recurrent thrombosis up to the study period was seen in 2 out of the 10 cases (20%).

Figure 1 Gangrene of finger tip in apatient of Polycythemia vera

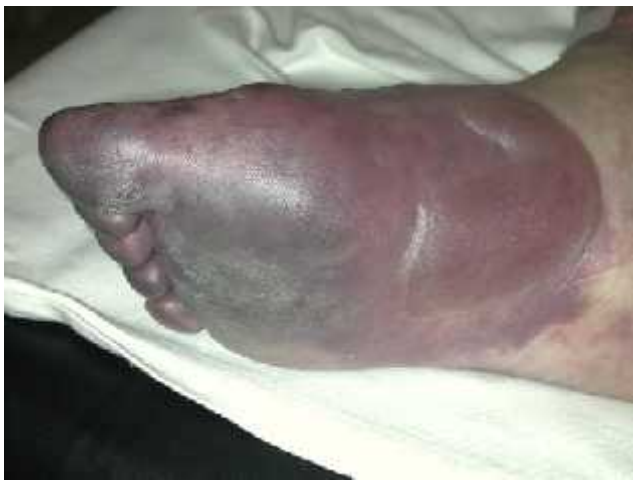


Figure 2 Phlegmesia cerulean dolens, (Factor VIII level – high)

Serum fibrinogen, Factor Vlll and Factor lX levels: Elevated levels of serum fibrinogen, factor VIII and factor IX were found in 2 (11.1%), 1 (5.6%) and 4 (22.2%) patients

respectively. Of these 7 patients, 3 (42.6%) had more than one thrombophilia risk factor. 2 (28.6%) had elevated serum fibrinogen combined with elevated factor VIII and 1 (14.3%) had elevated factor VIII combined with elevated factor IX level. The average high level of serum fibrinogen was 520.6 mg/dl and 436.5 mg/dl for factor VIII. The highest level of serum fibrinogen and factor VIII detected was 535 mg/dl and 536 mg/dl respectively **(figures 3 and 4)**. The youngest age at presentation was 21 years.

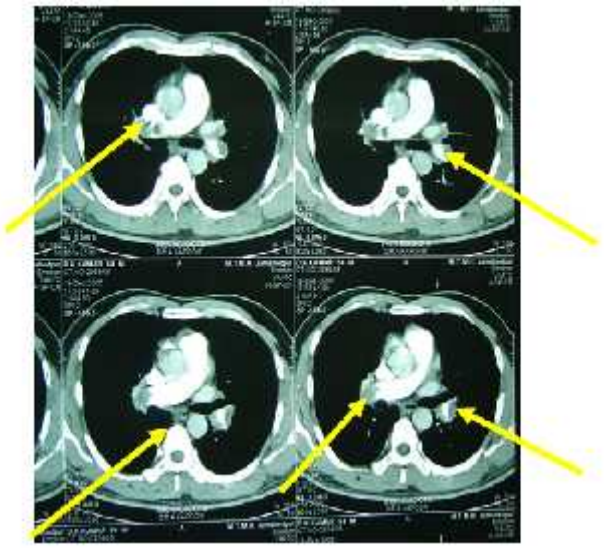


Figure 3 Thrombus in right and left main pulmonary arteries (Combined Protein C and S deficiency)

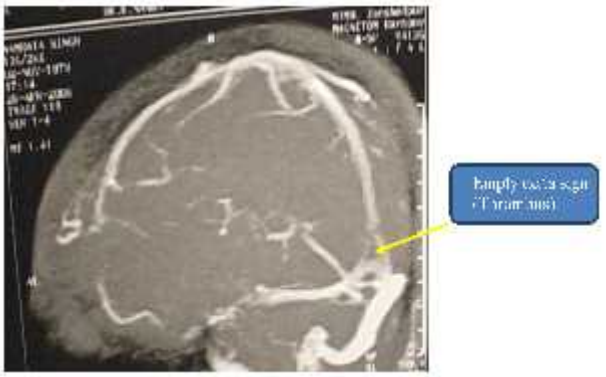


Figure 4 Cortical vein thrombosis (Protein S deficiency)

Life threatening thrombotic episodes were found in 10 (55.6%) out of 18 patients. Also recurrent thrombotic episodes were more common in patients with combined protein C and protein S deficiency and in those with more than one thrombophilia risk factor.

Patient's outcome*:* The shortest period of follow up was 140 days while the longest duration was 4.5 years. 5 (27.8%) patients were lost to follow-up while 2 (11.1%) expired. All 4(100%) patients who presented with acute pulmonary thromboembolism and 2 (100%) patients of myocardial infarction had near complete resolution of the thrombus with fibrinolytic therapy, while 1 patient of superior sagittal sinus (SSS) thrombosis and 3 patients with DVT had evidence of partial recanalization as was seen in MR Angiography at one year and Doppler study at 6 months respectively. One patient with acute superior mesenteric vein (SMV) thrombosis with intestinal gangrene underwent resection of the gangrenous bowel with anastomosis. He made an uneventful recovery. He put on anticoagulation as per the protocol and is well at follow up of two years.

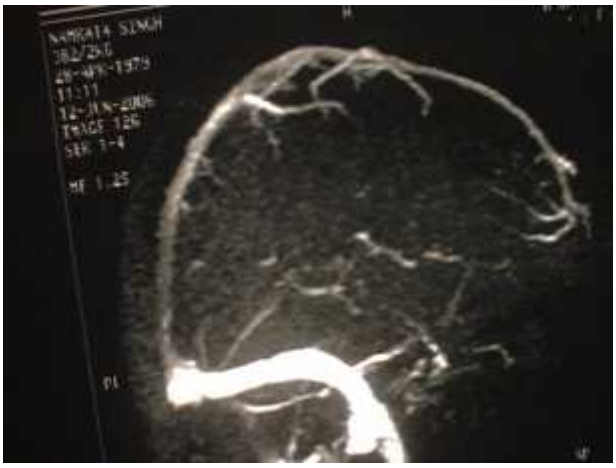
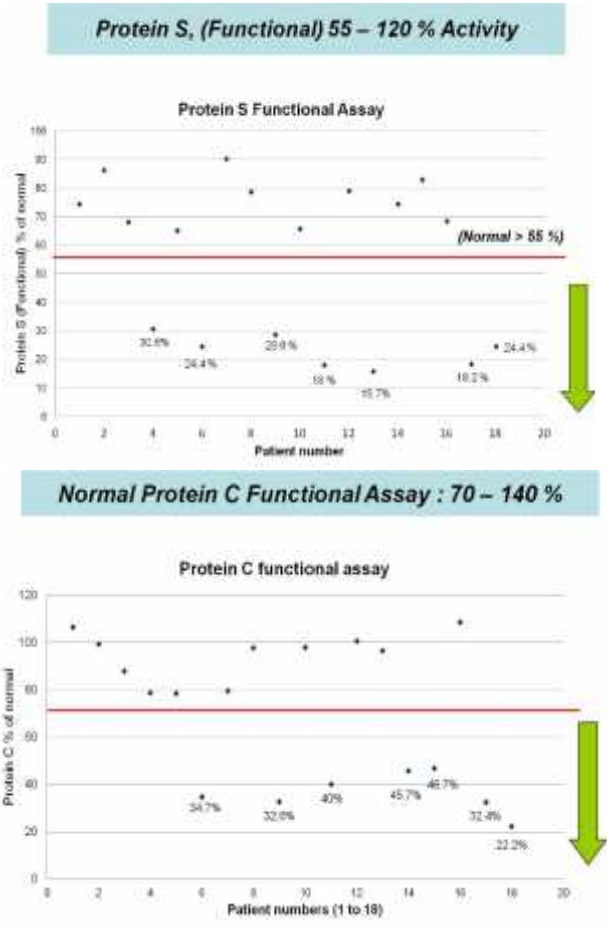
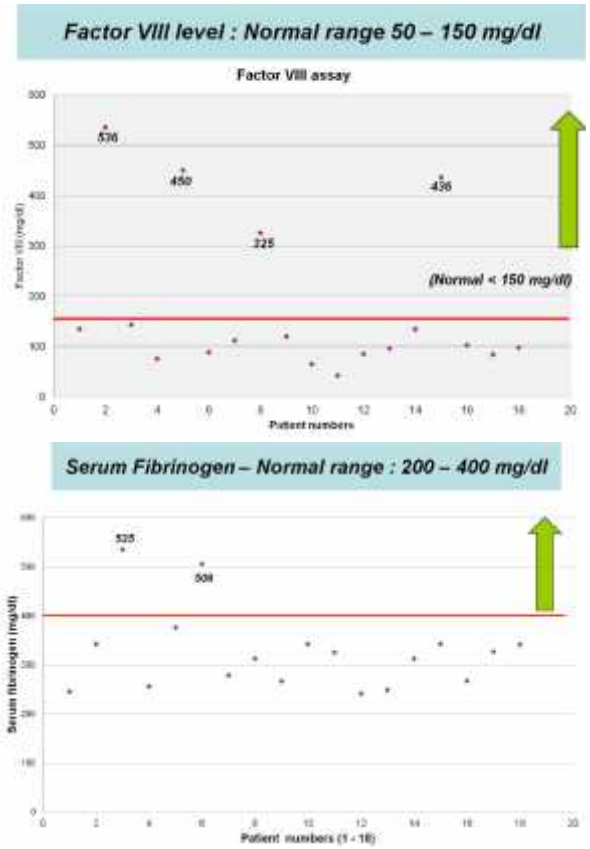


Figure 5 Resolution of the thrombus after anticagulation. (No filling defect)

Management dilemmas*:* 2 (11.1%) out of 18 patients had bleeding complications due to anticoagulation though their INR was in the therapeutic range. One patient of Budd-Chiari syndrome who had high factor VIII and fibrinogen levels, developed widespread ecchymotic patches and the other with polycythemia vera developed large haemarthosis of left knee joint following trivial trauma, due to which anticoagulation was stopped. 2 (11.1%) patients expired after stopping anticoagulation. Patient of polycythemia vera rubra developed acute myocardial infarction and died 15 days after anticoagulation was stopped while the patient with Budd-Chiari syndrome developed DVT and died of pulmonary thromboembolism during the period of stopping anticoagulation.





DISCUSSION

Thrombophilia is defined as a procoagulant state characterized by increased propensity for vascular thrombosis, especially venous thrombosis [1]. Such abnormalities represent one component of the triad (along with stasis and abnormalities of the vessel wall) put forward by Virchow in the 19th century to explain the mechanisms of thrombosis [2]. Hypercoagulability can be inherited or acquired although both genetic and environmental factors interact to provoke thrombotic events [1-4]. The inherited type, is also termed heritable or genetic thrombophilia. Inherited thrombophilic defects influence the risk of a first episode of venous thromboembolism (VTE). However, their influence on the risk of recurrent VTE is less certain [5].

The heritable factors predisposing to venous thrombosis include genetic deficiencies of the naturally occurring anticoagulants (antithrombin lll, protein C, and protein S), increased levels of factor Vlll, lX, fibrinogen, hyperhomocysteinemia and "gain of function" genetic polymorphisms (factor V Leiden, prothrombin gene mutation - G20210A and MTHFR gene mutation - C677T) [5]. Their prevalence varies widely between different ethnic groups, age, and geographic distributions. Among Caucasians, the factor V(FV) Leiden and the prothrombin G20210A mutations are the most common heritable risk factors predisposing to venous thromboembolism [5,6]. Acquired thrombophilic disorders are more common and include conditions such as the antiphospholipid syndrome, associated with lupus anticoagulant and anticardiolipin antibodies, polycythemia rubra vera, malignancies, paroxysmal nocturnal haemoglobinuria (PNH), nephrotic syndrome etc [2-5].

The present study is a compilation of 18 cases of thrombophilia who presented with various manifestations and their response to anticoagulation over a period of 6 years. Our study was designed to avoid false-positive results. As the thrombotic process itself may deplete Protein C, Protein S and Anti-thrombin levels, we repeated the thrombophilia tests after 2 weeks of stopping oral anticoagulation 3 months after the index episode.

Inherited thrombophilic factors found in our study were deficiency of clotting factors Protein C, Protein S and Anti-thrombin III. The prevalence of Protein C deficiency in Caucasians is estimated to be 0.2–0.5% and 3 to 6% in patients with thrombosis[2,7,8]. It is inherited in an autosomal dominant manner and is associated with familial venous thrombosis. Activated protein C (APC) inactivates coagulation factors Va and VIIIa, needed for efficient thrombin generation and factor X activation[2]. The inhibitory effect of APC is markedly enhanced by protein S, another vitamin K-dependent protein, thus their deficiencies increase the risk of thrombosis. Two subtypes of protein C deficiency (Type I and Type II) have been identified using immunologic and functional assays. Type I deficiency is characterized by reduced plasma protein C concentration at approximately 50% of normal. Individuals with the type II deficiency state have normal plasma protein C antigen levels with decreased functional activity[2,5]. Homozygous infants have a severe thrombotic tendency characterized as purpura fulminans.

Protein S deficiency itself, contributes to 2% of all venous thromboembolisms presenting to emergency[2]. A Scottish study conducted in healthy blood donors showed prevalence of hereditary protein S deficiency ranging from 0.03% to 0.13%[9]. Protein S also controls thrombin generation and fibrinolysis, in addition being cofactor for Protein C[10]. Thrombosis occurs in heterozygotes whose levels of functional protein S are in the range of 15–50% of normal. Protein S deficiency predisposes to a 5–11.5 times higher lifetime risk of venous thromboembolism than the general population[9]. Heterozygous protein S deficiency usually manifests in adulthood with a thromboembolic event and presents in neonates with purpura fulminans when present in the homozygous form[2]. Acquired protein C and S deficiency occurs during pregnancy, hepatic dysfunction, oral contraceptive usage and nephrotic syndrome[3,4].

Anti-thrombin (AT) deficiency is rare (0.02% of the population) and homozygous mutations are incompatible with life[11]. It is usually inherited in an autosomal dominant fashion, and is of 3 subtypes. Type I deficiency state results from reduced synthesis. Type II AT deficiency is produced by a molecular defect within the protein affecting the heparin binding site. Type III is characterized by normal anti-thrombin levels but impaired interaction between AT and heparin[2,5]. In a Spanish study of 2132 consecutive unselected patients with venous thromboembolism, 0.5% had anti-thrombin deficiency[2]. Thrombotic episodes are rare before puberty in AT- deficient individuals and the risk increases substantially with advancing age. In a prospective study from the Western part of India involving 432 young patients (< 45 years) of venous thrombosis for 6 years, protein C, protein S, and AT III deficiency was detected in 9.5%, 6.5%, and 2.6%, respectively[12].

Results from a large family cohort study by Bakhtawar K, Mahmoodi *et al* have suggested hereditary deficiency of protein C or protein S but not anti-thrombin III confers

increased risk of arterial thromboembolic events at a young age[13].

Factor V Leiden is found in about 2-7% of the Caucasians[6] and is responsible for 20 - 50 % cases of inherited thrombosis in Caucasian patients. Heterozygous individuals are at five times greater risk of thrombosis than the general population, while homozygotes are at a 10- fold greater risk[5]. The prevalence of heterozygosity for the factor V Leiden mutation in Europeans, Israeli, Arab, Canadian and Indian populations, ranges from 1 to 8.5%[2]. This condition was seen in only 3% of patients from western India in patients with venous thrombosis at various sites[14]. However, a high prevalence of this mutation has been reported in the Parsi population from Maharashtra[12]. This mutation makes activated factor V (Va) resistant to inactivation by activated protein C (APC)[2,4,5]. Factor Va cofactor, along with protein S, helps activated protein C (APC) in the degradation of factors VIIIa and Va, thus defective factor V gene increases risk for DVT with or without pulmonary embolism. Shah SR *et al* have reported acute SMV thrombosis in 2 patients who were heterozygous for factor V Leiden mutation in addition to having high serum fibrinogen and factor VIII levels[15]. This mutation is also known to be prevalent in patients with the Budd-Chiari syndrome[14]. In a study of 203 young patients of IS, Nedeltchev *et al* reported 0.9% incidence of Factor V Leiden mutation[16] suggesting its possible role in arterial thrombosis. This mutation was not detected in any of our patients.

The prothrombin G20210A gene mutation is associated with an elevated risk of deep vein thrombosis, although to a lesser degree than factor V Leiden[17]. It is identified in 1-2 % of the Caucasians[5]. The prothrombin gene mutation so far has been shown to be extremely rare in the non-white (black or Asian) population[2]. Studies from Western part of India by Ghosh *et al* suggest absence of this gene mutation, both in patients with thrombosis and controls[12]. Similar observations were noted by Mohanty D *et al* in a study from Western India (involving 86 patients and 242 healthy controls) of hereditary thrombophila as a cause of Budd-Chiari syndrome[14]. This mutation was also not found in our study. However, our sample size was small.

A case-control study of 185 Dutch individuals, by Kraaijenhagen *et al* demonstrated elevations in factor VIII levels to be an independent risk factor for venous thrombosis[19]. In Leiden Thrombophilia Study, levels above 150% have been shown to be associated with a fivefold increased risk of venous thrombosis[20,21]. Patients with a factor VIII level above the 90th percentile have been shown to have a 37% risk of recurrence at 2 years as compared with a 5% likelihood among patients with lower levels[22]. Some authors have also suggested an increased risk for arterial thrombosis, particularly myocardial infarction and ischaemic stroke (IS)[23]. In our study, one patient had extensive DVT (involving calf and thigh veins), 2 patients having combined factor VIII and serum fibrinogen excess had Budd-Chiari syndrome, pulmonary thromboembolism, while 1 patient having combined factor VIII and IX had coronary arterial thrombosis with myocardial infarction. Genetic factors such as increased von Willebrand factor levels and non–O blood groups while the acquired factors, such as hypertriglyceridemia, pregnancy, surgery, chronic inflammation, malignancy, liver disease, hyperthyroidism, and renal disease have been linked to its increased levels of factor VIII[18,20,23].

Increased level of factor IX was found in one of our patient in combination with excess factor VIII levels who presented with acute anterolateral MI. In a Dutch study by van Hylckama VA *et al*, elevated factor IX levels of more than 129 U/dL) as a cut off point for factor IX levels, increased the risk of thrombosis by 2 to 3 fold[2,24]. Factor IX is a circulating serine protease and along with factor VIII causes activation of factor X and hence, it has been postulated that elevated levels of factor IX can also increase the risk of thrombosis[2].

Two of our patients (11.1%) with high serum fibrinogen levels in association with high serum factor VIII levels had acute Budd-Chiai syndrome and acute pulmonary thromboembolism. In a study done by Jose J *et al* on the Indian population with angiographically documented CAD, fibrinogen levels (estimated using the Clauss method) were higher ($P<0.0001$) than in controls and further levels were higher in patients with triple-vessel disease when compared to those with single or two vessel disease. Similarly, a study done by Deepa R *et al* on the South Indians on the relation between prothrombogenic risk factors and CAD, showed significant relationship between serum fibrinogen levels and CAD both in diabetic and non-diabetic patients thus, substantiating the fact that serum fibrinogen may be an independent risk factor for ischaemic heart disease. However, further studies are needed to establish its role as a thrombophilic factor.

Hyperhomocysteinemia is an independent risk factor for thrombosis. In 1969, McCully demonstrated that premature atherosclerosis and arterial thrombosis is associated with severe hyperhomocysteinemia[2]. The genetic cause includes an alanine-to-valine substitution at amino acid 677(C677T) in the gene encoding for the enzyme methylene tetrahydrofolate reductase (MTHFR) which results in production of a thermolabile variant with reduced enzymatic activity (T mutation). This enzyme is involved in the metabolism of homocysteine. In 1997 D'Angelo *et al* suggested that the genetic defect may be present in 1.4–15% of Caucasians[26]. A meta-analysis of 40 observational studies involving 11,162 patients showed homozygous individuals had a 16 % higher odds of coronary heart disease compared with controls (odds ratio 1.16, 95% CI 1.05–1.28)[2]. In case-control study involving 289 patients, Den Heijer and colleagues demonstrated that mild hyperhomocysteinemia is an independent risk factor for venous thromboembolism. They showed that a plasma homocysteine concentration of more than 22 µmol per liter increased the matched odds ratio for deep venous thrombosis to 4.0[2,26].

The most common acquired thrombophilic disorder is antiphospholipid syndrome (APS)[27]. APS is a multisystem autoimmune disorder which may be either primary or secondary to other autoimmune diseases like SLE. It occurs more commonly in females and is characterized by arterial and venous thrombosis and pregnancy–related complications like recurrent abortions, especially in the late first trimester or early second trimester, pre-eclampsia, eclampsia, fetal growth retardation, and premature delivery mediated by lupus anticoagulant (LA), anticardiolipin (aCL) antibody (IgG, IgM, and IgA), and anti-beta-2 glycoprotein I antibody[28]. Arterial thrombosis could affect any of the organ systems while venous thrombosis commonly presents as DVT. Saxena R *et al* while screening for aetiology of young DVT found 5.3% and 2.8% of patients with DVT to have aCL and LA respectively[29]. A similar study from Western India, by Ghosh K *et al* reported

the incidence of aCL and LA to be 9.9% and 8.3% respectively in patients with DVT in the young[12]. In our study, of the 18 patients, 2 female patients had APLA syndrome (11.1%) and both presented with digital gangrene and had history of abortion.

Polycythemia rubra vera was detected in 2 patients (11.1%). It is a myeloproliferative disorder which can present with vascular thrombosis in 30% of the patients. 95% of the patients are positive for JAK2 (V617F) mutation. One of the patients (>50 years) presented for the first time with splenic vein thrombosis, while the other patient had DVT, MI and digital gangrene of finger tips at different times.

Thus, the spectrum of thrombophilic disorders is extremely varied. A high index of suspicion is needed for diagnosis. Thrombophilia screening should be done in patients with recurrent venous thrombosis, young patients (< 45yrs) without obvious acquired risk factors like prolonged immobilization, recent surgery, cancer etc, thrombus in unusual sites (portal and hepatic veins, superior mesenteric vein, cortical veins etc) and both arterial and venous thrombosis as is evident from the above cases.

All patients should receive a minimum of at least three months therapy with a standard regimen of heparin or low molecular weight heparin (LMWH) overlapped with oral anticoagulant until an international normalized ratio (INR) of 2.0 to 3.0 is obtained on two consecutive days[30]. The optimum duration of anticoagulation therapy following the first episode of thrombosis is not known. The decision depends on the patient's risk of recurrent thromboembolism, the risk of severe bleeding due to anticoagulation, the cost and inconvenience of anticoagulation. The annual incidence of major bleeding complications associated with anticoagulant therapy is 7.2 events per 100 person-years and the risk of fatal bleeding is 1.31 per 100 person-years, with a case-fatality rate of 13.4%[31], which decreases over time during the course of treatment but increases with age. Among elderly patients, it has been reported that the rate of serious or fatal bleeding is even higher, at 7% to 9% per year[34]. The risk of recurrent thromboembolism depends on the presence of acquired or congenital risk factors and declines over time. About 5% of the recurrences are fatal[32] as in our cases. Using these numbers, one could calculate that long-term oral anticoagulation would benefit the subgroups of patients in which the annual incidence of recurrence is above 10%, such as those who have already had a recurrence of venous thromboembolism, life-threatening episode, a strong family history, presence of more than one thrombophilic factor or who have the lupus anticoagulant. More prospective trials are needed to investigate the value of prolonged anticoagulant therapy in such patients, but until the data is available, extended prophylaxis must be considered in such patients.

CONCLUSION

Thrombophilia, an abnormality of blood coagulation that increases the risk of thrombosis is caused by hereditary and acquired factors. It generally presents with thrombosis in young, thrombosis in unusual sites, recurrent thrombotic episodes and affects several family members. Testing for heritable thrombophilia involves a range of complex coagulation based tests along with genetic testing. Deficiencies of protein C and protein S are seen as strong risk factors for thrombosis. The risk is higher in patients with combined thrombophilic defects. They also carry a higher risk of recurrence. Morbidity and mortality in these patients results not only from the first episode of thrombosis but also from the recurrence of thromboembolism. Recurrent venous thrombosis can be prevented by prophylaxis with oral anticoagulants, but these drugs can cause fatal bleeding. Choosing the optimal duration of prophylaxis entails balancing the risk of recurrent thrombosis after the discontinuation of anticoagulant therapy against the risk of haemorrhagic complications. Screening of the family members of the index case should be offered wherever possible, though may not be cost-effective and may not accurately predict recurrent events. This study is presented to make the physicians familiarize with the myriad manifestations of the disease. High index of suspicion is required in appropriate clinical settings to diagnose this entity at the earliest and ensure prompt treatment, which can result in almost complete recovery.

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