Objective: To study the prevalence of thrombophilic conditions in patients with acute and chronic portal vein thrombosis (PVT) and to compare it with those in patients suffering from deep vein thrombosis (DVT) after lower limb arthroplasty and in healthy subjects.

Methods: Twenty-six patients with spontaneous PVT (20 chronic, 6 acute) with normal liver function and not receiving anticoagulants were evaluated for thrombophilic conditions. Levels of protein C, protein S and antithrombin were compared with those in 50 healthy controls. Factor V gene ‘Leiden’ mutation (FVL) and high homocysteine levels were looked for in patients with PVT and in 18 patients developing post-arthroplasty lower limb DVT despite anticoagulation.

Results: Of 26 patients with PVT, 19 had at least one thrombotic condition (acute PVT 5/6, chronic PVT 14/20) and 12 had more than one such condition; in comparison, of 18 patients with DVT, eight had one thrombophilic condition and one had two such conditions (p=0.03). Patients with PVT had significantly lower levels of protein C, protein S and antithrombin than healthy subjects and those with DVT. Six patients had Factor VIII levels above 150%; four had elevated homocysteine levels and three had detectable anti-cardiolipin antibodies. Three patients with PVT (acute 2, chronic 1) were heterozygous for FVL mutation.

Conclusions: Underlying thrombophilic conditions are common in Indian patients with spontaneous PVT. In many patients, multiple thrombophilic conditions are present and these may play a role in the pathogenesis of PVT.

The term thrombophilic condition includes several heterogenous disorders that either increase the clotting tendency or inhibit thrombolysis. These have increasingly been implicated in the etiology of thrombosis of the portal or mesenteric venous system.\textsuperscript{1,2} In India, portal vein thrombosis (PVT) in the absence of cirrhosis is a common cause of portal hypertension and variceal bleeding.\textsuperscript{3} Various factors such as umbilical sepsis, childhood diarrhea and dehydration have been implicated in the pathogenesis of chronic PVT.\textsuperscript{4} However, Sherlock et al\textsuperscript{5} failed to find progression to PVT in any of 300 infants with umbilical vein catheterization and sepsis whom they followed up. Acute portal or mesenteric vein thrombosis is an uncommon cause of the acute abdomen and may be life-threatening. In studies from Western countries, over 60% of patients with PVT had underlying thrombophilic conditions,\textsuperscript{6} though it is unclear whether the frequency and nature of these conditions differs from those responsible for postoperative deep vein thrombosis (DVT).

We studied the frequency of various thrombophilic conditions in patients with acute or chronic PVT who did not have liver cirrhosis, and compared these with those in patients undergoing lower limb arthroplasty who developed DVT despite prophylactic low-molecular-weight heparin therapy.

Methods

This prospective study was carried out between March 2001 and September 2003 with the approval of the ethics committee of P D Hinduja Hospital. Patients with PVT referred to this hospital, documented on Doppler ultrasonography or CT portovenography, were included. Exclusion criteria were: abnormal serum levels of liver enzymes or prothrombin time, evidence of chronic liver disease, other causes of portal hypertension, malignancy, chronic pancreatitis, splenectomy, use of anticoagulant drugs (in such cases, tests could be done after cessation of these drugs for 1 week), oral contraceptive pill intake, active variceal bleeding, ascites, and myeloproliferative disorders. Among patients with chronic PVT alone, those presenting within a year of the index event and those receiving sclerotherapy were excluded.

PVT was considered as acute if it was accompanied by abdominal pain, and CT scan showed a clot in the portal vein with surrounding contrast enhancement. Portal thrombosis in the absence of an acute attack of pain and associated with a demonstrable cavernoma on imaging studies was designated as
chronic PVT.

Twenty-six patients, including 20 with chronic PVT (mean age 20.2 years [SD 9.3], range 9-47) and 6 with acute PVT (40.3 years [SD 11.3], 25-53), were studied. One patient with chronic PVT gave history of umbilical sepsis. No patient had family history of venous thrombosis. Patients with chronic PVT did not report thrombosis at any other site.

In two patients with acute PVT, tests were done immediately after diagnosis and prior to administration of anticoagulant drugs; these were repeated after 6 months. In one patient, these were done 3 months after the acute attack (the patient was not receiving anticoagulants). In the remaining three patients, tests were done after stopping anticoagulant drugs for at least one week.

Blood specimens were collected after overnight fast and were tested for thrombophilic conditions.

Coagulation tests
Protein C activity was measured by a clotting-based assay. Free protein S level was determined by an enzyme-linked immunosorbent assay. Antithrombin activity was determined by a chromogenic assay. All these test kits were obtained from Diagnostica Stago (Asneries, France).

Factor VIII activity was measured by one-stage clotting assay using factor VIII-deficient plasma (Diagnostica Stago, Asneries, France). The presence of lupus anticoagulants (LA) was determined using three methods: dilute activated partial thromboplastin time (APTT reagent – Instrumentation Lab, USA), kaolin clotting time (platein LS – Biomerieux, Durham, North Carolina; kaolin – Sigma Aldrich, USA) and dilute Russel viper venom time (Diagnostica Stago, Asneries, France). Positive results with the dilute Russel viper venom time and kaolin clotting time were considered significant even if the dilute APTT was negative.

Biochemical tests
Serum homocysteine level was determined using fluorescence polarization immunoassay. Paroxysmal nocturnal hemoglobinuria screening was done using three methods: acidified serum test (Ham’s test), sucrose lysis test (SLT) and urinary hemosiderin. Anticardiolipin antibody was determined by indirect competitive immunoassay for semiquantitative determination of IgM and IgG antibodies in human serum (Pharmacia Diagnostics, Freiburg, Germany).

Molecular assays
DNA was extracted from peripheral blood leukocytes by a ‘salting out’ procedure; it was dissolved in Tris-EDTA buffer (TE; pH 8.0) and stored at -70°C.

Factor V ‘Leiden’ (FVL) and prothrombin G20210A mutations were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of the amplified products. The primers used for detection of the FVL (G1691A) mutation (forward: 5’-AAA CAC AGA AAA TGA TGC CCA G-3’; reverse: 5'-TGC CCC ATT ATT TAG CCA GGA G-3’) and of the prothrombin gene G20210A mutation (forward: 5'-TCT AGA AAC AGT TGC CTG GC-3’; reverse: 5’-ATA GCA CTG GGA GCA TTG AAG C-3’) were obtained from Genset Singapore Biotech, Singapore. The PCR products for determination of FVL and G20210 mutations were digested with MnlI and Hind III restriction enzymes, respectively (MBI Fermentas, Vilnius, Lithuania). The digested PCR products were separated by electrophoresis in 15% and 10% polyacrylamide gel, respectively, followed by staining with ethidium bromide and visualization under ultraviolet light.

All tests with abnormal results were repeated for confirmation; for quantitative results, an average of the two values so obtained was used. In 4 patients, all positive tests were repeated 4-8 months later with similar results, confirming the reproducibility of test results.

Controls
The normal ranges for plasma levels of protein C, protein S and antithrombin were calculated from blood specimens of 50 healthy persons presenting to the health checkup facility of the hospital. Values beyond 2 SD of the mean were considered abnormal. Results of 18 patients developing DVT after joint replacement surgery, with no evidence of thrombosis prior, who underwent tests for thrombophilic conditions were used as prothrombotic controls for proteins C and S antithrombin, lupus anticoagulant, factor V ‘Leiden’ gene mutation and homocysteine levels.

Statistical analysis
Comparisons were made using the Fisher’s exact test, chi-squared test and Mann-Whitney U test, as appropriate. For correlation between various measurements, linear regression analysis was used. Analysis was done using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). p<0.05 was considered significant.
Results

Proteins C and S and antithrombin levels (Figs 1-3)

Of the 26 patients with PVT, 12 had deficiency of protein C, nine had deficiency of protein S and eight had antithrombin deficiency. Of these, 2 patients each had a factor deficiency in the group with acute PVT, and 10, 7 and 6 patients, respectively, in the group with chronic PVT. Cut-off values for these assays based on levels in 50 healthy controls were 67%, 65% and 71%, respectively, marginally lower than those indicated by the manufacturers.

The PVT group had significantly lower levels of proteins C and S and antithrombin than the healthy controls, and significantly lower protein C and antithrombin levels had no correlation with simultaneously measured levels of either serum albumin or fibrinogen (in 15 patients in whom such data were available), which were used as surrogate markers of protein synthesis in the liver. Factor VIII (Fig 4) and homocysteine (Fig 5) levels

Of the 26 patients, 6 showed elevated factor VIII levels (>150%). Four patients had elevated homocysteine levels.

Anticardiolipin antibodies (Fig 6) and lupus anticoagulant

Three patients, all with chronic PVT, had elevated levels of IgG anti-cardiolipin antibody. Four patients (acute PVT 1, chronic PVT 3) tested positive for these levels were lower in the healthy controls.
**Genetic factors** play a large role in the pathogenesis of thrombophilic conditions in Western countries.

**Presence of multiple thrombophilic factors**

In the chronic PVT group, 14 (70%) patients had a thrombophilic state. Of these, 7 had one factor, 4 had 2 factors, 2 had 3 factors and 1 had 4 factors. In the acute PVT group, 5 of the 6 patients showed thrombophilic conditions. All had more than one factor (Table).

Eight patients with DVT showed the presence of one factor and only one patient showed two factors (protein C and hyperhomocysteinemia). In contrast, 19 patients with PVT showed the presence of any one factor, of whom 12 had more than one factor (p=0.03).

**Discussion**

Our data showed that a large proportion of patients with PVT had at least one thrombophilic condition.

Our study was designed to avoid false-positive results. In patients with venous thrombosis, the thrombotic process may deplete proteins C and S and antithrombin. To obviate this, we tested the patients at least one year after the index event in case of chronic PVT and away from the time of variceal sclerotherapy. In 4 of the 6 patients with acute PVT too, the specimens were obtained well after the event. Of the remaining two patients who were tested at the time of diagnosis, repeat tests confirmed the findings in one; the other patient had factor V ‘Leiden’ gene mutation, which is not influenced by thrombosis. Secondly, reduced hepatic protein synthesis due to associated liver dysfunction may influence the levels of protein C, protein S and antithrombin. We therefore excluded patients with overt liver dysfunction.

![Anticardiolipin antibody levels in patients with portal vein thrombosis. Dotted line represents upper limit of normal](image)

**Table: Clinical characteristics and outcome in patients with PVT**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age / gender</th>
<th>Previous/other venous thrombosis</th>
<th>Time of specimen collection</th>
<th>Thrombophilic conditions</th>
<th>Treatment</th>
<th>Current state of portal vein</th>
<th>Duration of follow up</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53 / M</td>
<td>Recurrent PVT on anticoagulation</td>
<td>6 mo later</td>
<td>Pr S deficiency, FVL mutation, high factor VIII (200%)</td>
<td>Bowel resection</td>
<td>Recanalized</td>
<td>2 y</td>
<td>Lost to follow up</td>
</tr>
<tr>
<td>2</td>
<td>31 / M</td>
<td>DVT</td>
<td>Immediate</td>
<td>Pr C and S deficiency, homocysteinemia</td>
<td>Conservative</td>
<td>Cavernoma</td>
<td>2 y</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25 / M</td>
<td>Pulmonary embolus</td>
<td>Immediate; repeat 6 mo later</td>
<td>AT deficiency, high factor VIII (327%), lupus anticoagulant</td>
<td>Bowel resection</td>
<td>Cavernoma</td>
<td>10 mo</td>
<td></td>
</tr>
</tbody>
</table>

**Time of specimen collection is shown with respect to time of portal vein thrombosis**
tries.20-24 The factor V ‘Leiden’ gene mutation accounts for the majority of these cases. However in India,25,26 the frequency of this factor in the population in general and in thrombophilic conditions in particular is low. In our study, 3 patients showed this mutation. The numbers are, however, too small to state the role of this mutation in spontaneous PVT. The factor II G20210A mutation was absent in all our patients. Though our sample size was small, it is interesting that in another Indian study none of the 86 patients and 242 healthy controls from India had this mutation.27

As the prevalence of thrombophilic conditions may show regional variations, it is best to compare these results with other published Indian literature. The frequency of protein C deficiency in our series is comparable to that in a previous report from India.28 In that study, protein S levels were measured only if protein C was normal by a clotting assay; hence no comparable data are available. That study also found a higher prevalence of anticardiolipin antibodies than that in our study. Another study reported presence of lupus anticoagulant in two of 30 children with PVT29 and absence of antithrombin deficiency.30 Reports from the West support a high frequency of deficiencies of protein C, protein S and antithrombin in “idiopathic” PVT.2,31,32

Considerable debate has been generated on whether the deficiencies of protein C, protein S and antithrombin are the cause or effect of PVT.31,32,33 A few studies have found deficiencies in the immediate family members of patients with these deficiencies, indicating that the abnormalities are primary.32 Other reports too suggest that hepatic dysfunction is not the cause of these deficiencies,1,32 as was observed in our study as well. The levels of these proteins do not increase after shunt surgery, suggesting that splenic sequestration is not the cause.32 One recent study suggested that improvement of portal perfusion by a mesenterico-Rex shunt may improve levels of these factors;31 however, it included patients with a high initial prothrombin time, an atypical finding in extrahepatic PVT, and not present in any patient in our series.

The striking feature of our study is the high proportion of patients with multiple thrombophilic factors. Besides protein C, protein S and antithrombin deficiencies, 14 of the 26 patients had other thrombophilic factors. It has been well described that venous thrombosis is a multifactorial state.1 This seems especially true for mesenteric vein thrombosis as the majority of patients having thrombophilic conditions showed the presence of more than one such factor. Our study supports this argument.

All factors were not available for comparison between the PVT and DVT groups, and factor VIII levels were not compared since the arthroplasty group included patients with diseases like rheumatoid arthritis and fracture, which may be associated with elevated factor VIII levels. However, it is clear that unlike post-arthroplasty DVT, where mechanical factors play a significant role, spontaneous PVT appears to be more dependent on the simultaneous occurrence of multiple thrombophilic factors.

Our findings have therapeutic implications. The exact period of anticoagulation in patients with PVT has not been defined. It is reasonable to suggest lifelong anticoagulation if multiple thrombophilic conditions were to coexist.34 Similarly, anticoagulation is necessary in those undergoing portal systemic shunt surgery, commonly used to treat non-cirrhotic portal hypertension, where thrombophilic conditions are present, to prevent shunt blockage.

In conclusion, thrombophilic conditions are widely prevalent in Indian patients with spontaneous thrombosis of the portal venous axis. A wide spectrum of these states is present. The presence of multiple thrombophilic factors may play an important role in its pathogenesis.

References

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