Background: Extra-hepatic portal vein obstruction (EHPVO) caused by portal vein thrombosis (PVT) mainly occurs among the elderly in Western countries.\(^1,2\) In regions, including India, \(^3,4\) where this condition is common, it predominantly affects children and adolescents and is the commonest cause of portal hypertension in these age groups, presenting most often with well-tolerated variceal bleeding.\(^1,5\)

The etiopathogenesis of PVT is unclear. In recent studies, inherited or acquired prothrombotic factors, or local factors that provide a thrombogenic stimulus, have been identified in several patients.\(^2\) Thus, in some reports, idiopathic PVT formed only about 10% of the cases studied.\(^6,7\)

Inherited prothrombotic disorders include protein C deficiency, protein S deficiency, antithrombin III deficiency, and mutations in the genes for coagulation factor V (the factor V Leiden [FVL] mutation), prothrombin (PTHFR G20210A) and methylene tetrahydrofolate reductase enzyme (MTHFR C677T). The FVL mutation (G → A in codon 506) is associated with activated protein C resistance and thrombosis at unusual sites.\(^8\) The PTHR G20210A mutation leads to production of increased amount of prothrombin,\(^9\) and is associated with increased risk of venous thrombosis.

Data on the frequency of prothrombotic conditions in patients with PVT in India are limited. We have previously reported our experience with deficiencies of protein C and protein S, and the presence of anti-cardiolipin antibodies in these patients.\(^10\) In the current study, we looked at the possible role of FVL and prothrombin G20210A mutations in patients with PVT.

Methods

Sixty-one patients presenting with portal hypertension due to EHPVO were included in the study. The diagnosis of EHPVO was based on demonstration of (a) evidence of portal hypertension on endoscopy and (b) either a portal vein block or a portal cavernoma at abdominal ultrasonography. Patients with suspicion of chronic liver disease on history, clinical examination, biochemical tests or imaging studies were excluded. Patients with history suggestive of pancreatic disease or of ingestion of oral contraceptive drugs, or ultrasonographic evidence of liver tumor were excluded. Forty-nine healthy persons were studied as control subjects. All patients and controls provided informed consent; in case of children, consent was obtained from one of the parents.

For each study subject, genomic DNA was extracted from 2 mL of blood using phenol-chloroform extraction.\(^11\)

Detection of factor V Leiden mutation

Extracted genomic DNA was tested for the presence of FVL mutation using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).\(^8\) In brief, a 267-basepair (bp) segment of the factor V gene was amplified using specific primers (5'-TGC CCA GTG CTT AAC AAG ACC A-3' and 5'-TGT TAT CAC ACT GGT GCT AA-3'), as previously described.\(^5\) The PCR product (10 μL) was
digested with 4 U of DNA restriction enzyme MnlI (New England Biolabs, Beverly, MA, USA), at 37°C for 16 h, subjected to 2% low melting point agarose (Sigma, MO, USA) gel electrophoresis, and viewed under ultraviolet light after staining the gel with ethidium bromide. The digested amplicon from wild-type DNA gives 3 bands of 163, 67 and 37 bp, respectively; in contrast, heterozygous and homozygous FVL mutations show four (200, 163, 67 and 37 bp) and two (200 and 67 bp) bands, respectively.

**Detection of prothrombin gene mutation**

For detection of G20210A prothrombin gene mutation, we used a PCR-RFLP method that has been described previously. In brief, a 345-bp genomic DNA fragment encompassing a part of the prothrombin gene that contains the mutation was amplified by PCR using specific primers (5'-TCT AGA AAC AGT TGC CTG GC-3' and 5'-ATA GCA CTG GGA GCA TTG AAG C-3'). The PCR product (10 μL) was digested with 20 U of Hind III (New England Biolabs, Beverly, MA, USA), at 37°C for 16 h, subjected to 3% low melting point agarose gel electrophoresis and read using ultraviolet light. Using this method, the wild-type DNA yields a solitary 345-bp band, heterozygous G20210A mutation yields two bands of 345 and 322 bp, respectively, and homozygous mutation only one band of 322 bp.

**Statistical method**

Inter-group comparisons were done using the chi-squared test.

**Results**

Sixty-one patients with PVT (age range 2-42 years [median 11]; 47 male) were studied. Of these, 49 (80%) were children below the age of 18 years. Most belonged to the states of Uttar Pradesh (n=48) and Bihar (n=7). The Table shows the clinical, laboratory and radiological characteristics of these patients. Four patients had history of umbilical sepsis. No patient had history of thrombosis at any other site or of family history of venous thrombosis.

Of the 61 patients studied, 1 (1.6%) had FVL mutation (Fig 1). This patient, a 7-year-old boy with onset of disease at 4 years of age, was heterozygous for the FVL mutation, yielding an allele frequency for FVL mutation of 1/122 (0.8%). Of the 49 control subjects, two (4.1%) were heterozygous for FVL mutation, with allele frequency of 2/98 (2.0%; p=ns versus controls).

None of the patients (0/61) or control subjects (0/46; not tested in 3 controls) had the G20210A prothrombin gene mutation (Fig 2).

**Discussion**

EHPVO is a common cause of portal hypertension in India. The disease is particularly common among children. In most patients with this disease, obstruction of the portal vein arises from PVT; in an occasional patient, another cause, viz., agenesis, atresia or stenosis of the portal vein, or tumor or cyst encroaching on portal vein may be responsible. In many patients with PVT, the cause of thrombosis...
remains unknown. Our data show that FVL and G20210A prothrombin gene mutations were uncommon among patients with PVT in India.

Several causes have been proposed for PVT among children. It was initially proposed that umbilical sepsis or catheterization of the umbilical veins in the neonatal period were responsible for PVT. However, such history is available in only a minority of patients with PVT. Further, PVT is uncommon on follow-up in patients with umbilical infection or umbilical vein catheterization. Thus, the underlying cause remains unknown in most patients with PVT, hampering efforts at prevention of this disease.

In recent years, presence of congenital or acquired prothrombotic conditions that predispose a person to venous thrombosis has been an interesting hypothesis for the causation of PVT. Of the congenital prothrombotic states, FVL mutation and prothrombin gene mutation have received a lot of attention in PVT. FVL mutation, which is present in 2%-7% of normal Western population, is associated with a 5-10 fold and 80-fold higher risk of thrombosis among heterozygotes and homozygotes. However, the frequency of this mutation is relatively lower (<1%) among Blacks and Asians. Similarly, a point mutation in 3’-untranslated region of the prothrombin gene (nt 20210; G → A), which results in a 30% increase in plasma prothrombin levels, is associated with an increased risk of thrombosis. Heterozygotes account for 18% of familial and 6% of sporadic cases with venous thrombosis in Western countries.

Studies on these mutations in patients with PVT have reported conflicting data. Thus, two studies from Switzerland and Egypt reported that FVL was more frequent among patients with PVT, whereas other studies did not find such a difference. Similarly, three studies including two from Italy and one from France found a higher frequency of prothrombin gene mutation in patients with PVT. Unfortunately, most of the negative studies have had small sample size. Failure to find an increased frequency of FVL and prothrombin gene mutations in our study, which had a large sample size, contributes significantly to the available information on the subject.

Congenital prothrombotic states may be expected to be more important in children with PVT than in adult patients with this disease. Unfortunately, data from children with PVT have been limited, with only three studies providing data either exclusively or predominantly from children. Our study included 49 children with PVT, and thus is the largest study in the pediatric age group. Of the three previous studies, two studies from Brazil showed results similar to ours. However, a study of 40 children from Egypt found a higher frequency of both FVL and prothrombin gene mutations in PVT.

Our failure to show FVL and prothrombin gene mutations in a predominantly pediatric group with PVT in India suggests that congenital prothrombotic states are unlikely to be the cause for the high rate of PVT in this population.

Recently, two other Indian groups have looked at FVL mutation in EHPVO, and they found this mutation in 3 of 26 and none of 112 patients, respectively. One of these studies also looked for the prothrombin gene mutation and failed to find it in patients studied.

The variable frequency of these mutations in patients with PVT in various studies may be related to ethnic differences. For instance, FVL mutation is known to be less common among healthy Blacks and Asians as compared to the populations of European descent. Similarly, prothrombin gene mutation has not yet been reported from India, whether among patients with thrombosis at various sites or among healthy controls included in such studies.

Our study is limited by the fact that we did not study the presence of protein C or S deficiency or anti-cardiolipin antibodies. However, we have looked at these abnormalities previously in another group of patients with PVT and shown these to be common.

In conclusion, our data show that FVL and...
prothrombin gene mutations play little role in the causation of PVT in India, where this condition is the commonest cause of portal hypertension among children.

References


