Low frequency of factor V Leiden and prothrombin G20210A mutations in patients with hepatic venous outflow tract obstruction in northern India: a case-control study

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Background: Factor V Leiden (FVL) and prothrombin gene (G20210A) mutations are known to be associated with venous thromboembolism. Several studies have shown an association of these mutations with hepatic venous outflow tract obstruction (HVOTO). We studied the prevalence of these mutations among patients with HVOTO in northern India in comparison with healthy population. Methods: Genomic DNA from patients with HVOTO and healthy controls was analyzed for the presence of FVL and prothrombin gene G20210A mutations, using PCR and restriction-fragment length polymorphism. Results: Fifty-nine patients with HVOTO (age 5-69 years, median 27; 39 male) and 49 unrelated healthy controls from the same geographic region were studied. Of the 59 patients, 19 had a block in the hepatic vein, 7 in inferior vena cava, and 33 had mixed block. Presentation was with acute thrombosis in 9 patients and with long-standing obstruction in 50 patients. Among 49 controls, heterozygous and homozygous FVL mutations were observed in 2 and 0 subjects, respectively, with an allele frequency of 2% (2 of 98). In comparison, among 59 patients with HVOTO, four had heterozygous and none had homozygous FVL mutation, with an allele frequency of 3.4% (p=ns versus controls). The G20210A prothrombin gene mutation was not found in any of the patients or controls. Conclusion: FVL and prothrombin G20210A mutations appear to have no role in the pathogenesis of HVOTO in our patients with Budd-Chiari syndrome, consisting largely of those with long-standing obstruction of the inferior vena cava. [Indian J Gastroenterol 2005;24:211-215]

Hepatic venous outflow tract obstruction (HVOTO) is a heterogeneous group of disorders characterized by hepatic venous outflow obstruction at the level of the hepatic venules, the large hepatic veins, the inferior vena cava (IVC), or the right atrium. This condition was called Budd-Chiari syndrome (BCS). However, recently, there has been an attempt to classify hepatic venous outflow blocks into ‘primary hepatic vein thrombosis’ and ‘primary IVC obstruction’ since these may represent two different diseases with different onset, clinical manifestations and natural history.

Primary hepatic vein thrombosis occurs most commonly in the West, and is a severe disease with acute or sub-acute onset and a rapid downhill course, if untreated. There have been numerous studies dealing with etiological factors of primary hepatic vein thrombosis, which include myeloproliferative disorders (both overt and occult), factor V Leiden (FVL) mutation, protein C deficiency, protein S deficiency, antithrombin III deficiency, prothrombin gene mutation, paroxysmal nocturnal hemoglobinuria, anti-phospholipid antibody syndrome, pregnancy, oral contraceptives, and others. In contrast, primary IVC obstruction occurs more commonly in the Far East, is insidious in onset and appears to have a relatively slower clinical course. The etiology of this form of HVOTO is not clearly known. It appears that prothrombotic states are uncommon in patients with this form of HVOTO, though the data on this aspect are relatively limited.

In India, longstanding obstruction of the IVC with secondary involvement of the hepatic veins (HV) is the predominant cause of HVOTO. In the current study, we determined the frequency of the FVL and G20210A mutations in northern Indian patients with HVOTO, and compared it with the frequency of these mutations in healthy subjects.

Methods
One hundred patients with HVOTO, who had been treated in our hospital since 1988, were sent letters inviting them to participate in the study. The diagnosis of HVOTO was based on clinical and radiological criteria. Patients who visited the hospital in response to our letters were re-evaluated and their clinical presentation noted.

Two milliliters of blood specimen was drawn and stored in EDTA for genetic analysis. Blood was also obtained from 49 healthy adult subjects for use as controls; the subjects belonged to the same geographic region as the patients. All patients and con-
controls provided informed consent.

Detection of factor V Leiden mutation

Genomic DNA was extracted from peripheral blood leukocytes using the phenol-chloroform extraction technique, as described previously.\(^6\)

Extracted DNA was analyzed for the presence of FVL mutation using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), as described by Bertina et al.\(^7\) In brief, a 267-basepair (bp) segment of the factor V gene was amplified using oligonucleotide primers [5'-TGC CCA GTG CTT AAC AAG ACC A-3' and 5'-TGT TAT CAC ACT GGT GCT AA-3']. The PCR product (10 \(\mu\)L) was incubated with 4 U of DNA restriction enzyme \(M_{\text{nl}} I\) (New England Biolabs, Beverly, MA, USA), at 37\(\degree\)C for 16 h, and run on a 2% low-melting-point agarose (Sigma, MO, USA) gel electrophoresis. The restriction pattern was read under ultraviolet light after staining the gel with ethidium bromide. The wild type DNA gives 3 bands (163, 67 and 37 bp), whereas heterozygous FVL mutation gives four bands of 200, 163 and 67 and 37 bp, and homozygous FVL mutation gives two bands (200 and 67 bp).

Detection of prothrombin gene mutation

The method used to detect prothrombin gene mutation of interest has been described previously.\(^8\) In brief, a 345-bp fragment containing G20210A was amplified by PCR using 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 \(\mu\)L) was incubated with 20 U of \(H_{\text{ind}} III\) (New England Biolabs), at 37\(\degree\)C for 16 h. The resulting DNA fragments were separated using 3% low-melting-point agarose (Sigma, MO, USA) gel electrophoresis and read using ultraviolet light. Wild-type DNA was expected to yield a solitary 345-bp band, heterozygous G20210A mutation was expected to yield two bands of 345 and 322 bp, and homozygous mutation to yield only a band of 322 bp.

Statistical methods

Inter-group comparisons were done using the chi-squared test and Epi-Info version 6 software (Centers for Disease Control; Atlanta, GA, USA).

Results

Of the 100 patients with HVOTO who were invited, 59 (59%) agreed to participate. The baseline characteristics of these 59 patients are shown in Table 1. Of these 59 patients, 52 (88%) were from northern Indian states; 5 patients were from other parts of the country (one each from West Bengal, Maharashtra, Madhya Pradesh, Andhra Pradesh and Tamil Nadu) and 2 were from Nepal. None of the patients was taking oral contraceptive pills or had acute post-partum hepatic vein thrombosis. Forty-nine age- and sex-matched controls were included in the study after obtaining informed consent.

Of the 59 patients studied, 4 (6.8%) had FVL mutation. All these four patients were heterozygous for the FVL mutation and none was homozygous, yielding an allele frequency for FVL mutation of 4/118 (3.4%). Of the 49 controls, two (4%) were heterozygous for FVL mutation, with an allele frequency of 2/98 (2%). There was no significant difference in the allele frequency of FVL mutation between the patients with HVOTO and controls. The characteristics of patients who were found to have FVL mutation are shown in Table 2. Of the 9 patients with acute hepatic vein thrombosis, one had evidence of FVL mutation, and among 50 patients with long-standing HVOTO, three had this mutation. The mutation was detected in one of 19 patients with block in the HV, none of 7 with block in the IVC, and 3 of 33 with block in both HV and IVC. A representative gel picture is shown in Fig. 1.

No patient (0/53) or control (0/46) tested positive for the G20210A prothrombin gene mutation (Fig. 2).

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Table 1: Clinical and radiological characteristics of patients with hepatic venous outflow tract obstruction

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range) years</td>
<td>27 (5-69)</td>
</tr>
<tr>
<td>Male: Female</td>
<td>39:20</td>
</tr>
<tr>
<td>Clinical presentation (onset of block)</td>
<td></td>
</tr>
<tr>
<td>Acute (&lt;6 months)</td>
<td>9 (15.3%)</td>
</tr>
<tr>
<td>Chronic (≥6 months)</td>
<td>50 (84.7%)</td>
</tr>
<tr>
<td>Site of block</td>
<td></td>
</tr>
<tr>
<td>HV alone</td>
<td>19 (32.2%)</td>
</tr>
<tr>
<td>IVC alone</td>
<td>7 (11.9%)</td>
</tr>
<tr>
<td>Combined HV and IVC</td>
<td>33 (55.9%)</td>
</tr>
<tr>
<td>Blocks in other venous territories</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Nature of block</td>
<td></td>
</tr>
<tr>
<td>Segmental</td>
<td>22 (37.3%)</td>
</tr>
<tr>
<td>Membranous</td>
<td>37 (62.7%)</td>
</tr>
<tr>
<td>Treatment for HVOTO</td>
<td></td>
</tr>
<tr>
<td>Dilation / stenting / TIPS</td>
<td>55</td>
</tr>
<tr>
<td>No treatment offered</td>
<td>2 (spontaneous recanalization 1; advanced hepatocellular carcinoma 1)</td>
</tr>
<tr>
<td>Awaiting treatment</td>
<td>2</td>
</tr>
</tbody>
</table>

HV = hepatic vein(s); IVC = inferior vena cava; TIPS = transjugular intrahepatic porta-systemic shunt
Table 2: Characteristics of patients with hepatic venous outflow tract obstruction and factor V Leiden mutation

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Onset</th>
<th>Site of obstruction</th>
<th>Type of obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>#3</td>
<td>30</td>
<td>F</td>
<td>Chronic</td>
<td>Combined HV and IVC</td>
<td>Membranous</td>
</tr>
<tr>
<td>#8</td>
<td>27</td>
<td>M</td>
<td>Chronic</td>
<td>Combined HV and IVC</td>
<td>Segmental</td>
</tr>
<tr>
<td>#29</td>
<td>25</td>
<td>F</td>
<td>Chronic</td>
<td>HV alone</td>
<td>Segmental</td>
</tr>
<tr>
<td>#58</td>
<td>19</td>
<td>M</td>
<td>Acute</td>
<td>Combined HV and IVC</td>
<td>Segmental</td>
</tr>
</tbody>
</table>

HV = hepatic vein(s); IVC = inferior vena cava

Discussion

Our data show that FVL mutation was no more frequent among patients with HVOTO than in healthy subjects, and that G20210A prothrombin gene mutation was not found in any of the patients or controls. These data suggest that these inherited hypercoagulable states do not contribute to the development of HVOTO in the northern Indian population.

Deficiencies and functional abnormalities of antithrombin III, protein C and protein S are well-recognized causes of thromboembolic disease. In 1993, Dahlbäck and colleagues reported a patient with recurrent venous thromboembolism, whose plasma failed to show normal prolongation of activated partial thromboplastin time (APTT) on addition of activated protein C (APC), which is known to inactivate clotting factors Va and VIIIa, thus preventing efficient generation of thrombin. This trait had an autosomal dominant inheritance, and was caused by the presence of an abnormal factor V. The abnormal APC-resistant, dysfunctional factor V, now known as factor V Leiden, arises from a G→A mutation at nucleotide 1691 (CGA→CAA) in the factor V gene, resulting in replacement of arginine at position 506 by glutamine (Arg506Gln).

In Europe, the frequency of FVL mutation among patients with HVOTO is quite high (23%-31%). In the Netherlands, FVL mutation was found in 25.6% of patients with BCS and 2.9% of controls; thus individuals with this mutation had a relative risk of 11.3 for developing BCS. Similarly, in a study from the UK, the frequency of FVL mutation was higher in patients with BCS than in controls (23% vs 6%). Also, in an uncontrolled study from France, 31% of BCS patients had FVL mutation.

In HVOTO patients from the Far East, thrombotic states are less common. In a study from China, the frequency of FVL mutation was not increased in sporadic BCS patients.

There have been previous studies on the frequency of FVL in Indian patients. Two studies, one from western India and the other from northern India, found an increased frequency of FVL mutation in patients with HVOTO (14 of 53 [26%] and 5 of 29 [17%] patients, respectively). In contrast, a study from northern India found the frequency of FVL mutation to be no more frequent among patients with HVOTO than in healthy subjects.
FVL mutation to be similar among patients with BCS (7/121; 5.8%) and controls (3.2%). We too failed to find significant increase in the frequency of FVL mutation among patients with HVOTO.

Our failure to find FVL mutation among patients with HVOTO could be related either to the relative infrequency of FVL mutation in our population or to a real difference in the role of this mutation in creation of HVOTO in the Indian and European populations. We believe that the latter is more likely since the FVL mutation has been found in healthy subjects in most Indian studies at a frequency that is not widely different from that in Europe.

There are several morphologic and pathophysiologic differences between the HVOTO seen in the West and in the East. In the West, HVOTO occurs primarily as acute or sub-acute thrombosis of the hepatic veins, which in some patients may progress to involve the IVC. In contrast, in the Far East, HVOTO occurs primarily as long-standing IVC obstruction, usually by a membranous structure. Though the exact pathogenesis of this form of obstruction is not known, it is believed to arise from organization and replacement by fibrotic tissue of repeated thromboses in the IVC. Hepatic veins may get involved due either to ostial block by the IVC membrane or to secondary thrombosis due to venous stagnation. The process is usually insidious and chronic, and most patients present after cirrhosis has set in.

In our group of 59 patients, 50 had chronic HVOTO akin to that seen in the Far East, and only 9 had acute HVOTO. The failure to find an increased frequency of FVL in our study and in another study from northern India indicates that FVL may not predispose to chronic HVOTO. The different results in a previous Indian study that found an increased frequency of FVL mutation in HVOTO may have been related to inclusion of a large number of patients with acute hepatic vein thrombosis (23 of 53) in the other report showing such association, the number of patients with acute thrombosis was not provided.

The prothrombin gene mutation is another known cause of thromboembolism. In the Netherlands, 4.7% of 43 patients with HVOTO and 2.3% of 474 controls had prothrombin gene mutation, providing an odds ratio of 2.1 (95% confidence interval 0.4-9.6). None of our patients or controls had the G20210A prothrombin gene mutation. In fact, this mutation has not been found in any Indian study. It thus appears that this mutation may not be present in the Indian population.

We have previously looked for anti-cardiolipin antibodies in patients with BCS and found little evidence of a pathogenetic role for these in the causation of BCS. We could not study factors like protein C, protein S and antithrombin III deficiency; assays for these required discontinuation of anticoagulants, which the patients were receiving following angioplasty, and this was considered unethical.

Our data lend support to classification of patients with HVOTO into two distinct subpopulations, namely, 'primary hepatic vein thrombosis' and 'obliterative hepatocavopathy'. Our data, as also previous studies, suggest that HVOTO due to IVC obstruction may not be related to disorders of coagulation, and that there is need for further studies on the causation of this disease.

In conclusion, we did not find an increased frequency of FVL mutation or prothrombin gene mutation in a group of patients with HVOTO with predominant long-standing membranous obstruction of the IVC, as compared to controls. Thus inherited abnormalities of coagulation pathways like FVL and G20210A prothrombin gene mutations are unlikely to be responsible for this disease.

References


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**FVL and prothrombin mutations in HVOT obstruction**

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