A rare cause of Pulmonary Hypertension - Protein S Deficiency.

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Abstract :
Protein S deficiency is a rare form of inherited thrombotic disorder. This is a case report of protein S deficiency presenting as chronic thromboembolic pulmonary hypertension. Middle aged male patient presented with features of pulmonary hypertension and right heart failure with past history of Mesenteric Venous Thrombosis (MVT) few years back. Investigations revealed mosaic pattern of attenuation in CT pulmonary angiogram and Protein S deficiency. Incidentally this patient belongs to Bombay blood group. Protein S is a vitamin K dependent plasma glycoprotein. It facilitates the anticoagulant activity of activated protein C. Protein S deficiency can cause both venous and arterial thrombosis. Among unselected outpatients presenting with venous thromboembolism (VTE) 2 to 3 may have low levels of protein S. Management of protein S deficiency includes lifelong anticoagulation.

Introduction :
Protein S Deficiency, an inherited thrombotic disorder (Autosomal Dominant), predispose the person to develop recurrent thrombotic events. More than 200 types of mutations are identified to cause either quantitative or qualitative defect in protein S. Homozygous state for defective PROS gene results in Neonatal Purpura Fulminans. Patients with heterozygous state for defective gene may present as either venous or arterial thrombosis. This article is about a patient with protein S deficiency, who presented with pulmonary hypertension due to chronic pulmonary thromboembolism.

Case presentation:
This 40 year old male, an office attendant, was admitted with complaints of progressive swelling of both lower limbs and scrotum for past 2 months; abdominal distension for 1 month and difficulty in breathing for 15 days. He also complained of extreme fatigability for past few months. His dyspnoea was exertional, fits to NYHA class III, but no history of orthopnoea or
Paroxysmal Nocturnal Dyspnoea (PND). No history of oliguria or dysuria. No history of pain with redness and warmth in lower limbs.

He was not a known diabetic or hypertensive. He denies history of Pulmonary Tuberculosis, asthma or COPD. In 2007, He was treated at a private hospital for mesenteric venous thrombosis. Initial symptoms were dyspepsia and abdominal pain. Ultrasonogram of abdomen showed splenomegaly and ascites. Upper GI endoscopy revealed congestive gastropathy with Grade 1 Oesophageal varices. That time CT abdomen with contrast revealed Superior Mesenteric Venous Thrombosis with small bowel Ischemia (Fig.1). Thrombus in the portal vein also was noted. Laparotomy was done, 60 cm of gangrenous proximal ileum was resected and ends brought out. Ileostomy closure was done after 1 year. Investigations revealed Protein S Deficiency (5.8 % Activity) but he was not started on any anticoagulant drugs that time.

Fig 1. Contrast CT Abdomen - Superior Mesenteric Venous Thrombosis (Red Arrow)

After that he developed recurrent episodes of severe anaemia and congestive cardiac failure and treated at various hospitals as short bowel syndrome and nutritional anaemia by packed red cell transfusion. His blood group is Bombay O Rh Positive. He was a smoker, amounting to 10 pack years of cigarette, quit smoking since 2007. He was not an alcoholic. He is born out of 2nd degree consanguineous marriage and his parents are alive and healthy. One of his two sisters has Bombay blood group, but no history of thrombotic events.

Married, has two healthy children of adolescent age.

On physical examination he appears middle aged with normal physique. He was anaemic, dyspnoeic and tachypnoeic with respiratory rate of 28 per minute. Bipedal pitting oedema, scrotal oedema and abdominal wall oedema were present (Fig 2). No clubbing or cyanosis was present. Jugular venous pressure was elevated 6cms above the sternal angle at 45 degree inclination with prominent 'a' wave. Abdomino jugular reflex was positive. His blood pressure was 110/70 mm of Hg; pulse rate was 97/min with regular rhythm. Examination of cardiovascular system revealed features of Pulmonary Hypertension with right heart failure, including right ventricular apex beat; left parasternal heave; palpable and loud P2; ejection-systolic murmur of grade 3/6 in pulmonary area and mid diastolic murmur of tricuspid origin. Respiratory system was clinically normal. Midline scar of previous laparotomy noted (Fig 2.) Liver was 4 cm below right costal margin and tender; no free fluid in the abdomen.

Central Nervous System: no focal neurological deficit.

Fig 2. Scrotal edema, Mid line Scar
ECG showed complete RBBB (Fig 4), chest film showed right atrial enlargement and main pulmonary artery dilatation (Fig.3). Lung fields were normal. Echocardiogram showed dilated main pulmonary artery, RA, RV dilatation and moderate tricuspid valve regurgitation. Doppler measurement showed pulmonary arterial hypertension. His pulmonary function tests were within normal limits.

**Fig 3.a** Chest X-Ray showing obliteration of pulmonary bay
**Fig 3.b** Chest X Ray taken 2 years back - No pulmonary artery dilatation

**Fig 4.** ECG - In Sinus rhythm with RBBB
CT Angiogram of pulmonary arteries showed dilatation of main pulmonary artery (31 mm) and its branches. Evidence of mosaic attenuation pattern was noted in bilateral upper lobe and middle lobe. (Fig 5)

**Fig 5.a.** CT Pulmonary Angiogram - Mosaic Attenuation with pulmonary artery dilatation
Ultrasound abdomen showed congestive hepatomegaly with minimal ascites. Complete haemogram, urine routine examination, renal functions and electrolytes were all in normal limits except for haemoglobin of 9.2 gm/dL. Liver function tests were within normal limits. Test for HIV, HBsAg and VDRL were negative. As there was no evidence of pulmonary disease, other causes of pulmonary hypertension were considered. The mosaic attenuation pattern in CT suggests areas of hyperaemia and oligemia due to chronic and recurrent pulmonary thromboembolism. As there is a history of mesenteric venous thrombosis, hypercoagulable state was considered. There was no evidence of acquired causes of hypercoagulability like HIV infection, nephrotic syndrome, malignancy, myeloproliferative disorders, inflammatory bowel disease etc. so he was investigated for inherited thrombophilic state. Diagnosis of protein S deficiency with recurrent venous thrombosis was made. He was started on subcutaneous Heparin, switched over after a week to oral anticoagulant therapy with warfarin. INR was planned to be maintained at 2 during steady state period. As he was in cardiac failure he was treated with back rest.
nasal oxygen, salt and fluid restriction, diuretics (Furosemide 20 mg bd and Spirotilactone 50 mg OD), Digoxin 0.125 mg 5 days a week, Aspirin 150 mg Od, and ACE inhibitors (Enalapril 2.5 mg bd). He recovered from cardiac failure and general condition improved.

**Discussion**

**Risk factors for venous thrombosis.** Virchow’s triad; three interrelated factors, namely stasis of blood flow; vascular endothelial injury and hypercoagulability explain pathogenesis of vascular thrombosis. Coagulation disorders that contribute to hypercoagulability can be divided into three risk-factor categories: situational, inherited, and acquired.

**Situational risk factors** are transient clinical circumstances that are associated with increased thrombosis risk, like surgery, prolonged immobilization, oral contraceptive pill (OCP) use, hormone replacement therapy (HRT), pregnancy, cancer chemotherapy, and heparin-induced thrombocytopenia (HIT). **Acquired risk factors** are no reversible disease processes that interfere with normal hemostasis or blood rheology. Examples include cancer, inflammatory bowel disease, nephrotic syndrome, vasculitis, antiphospholipid antibodies, myeloproliferative syndromes, paroxysmal nocturnal hemoglobinuria, and hyperviscosity syndromes. **Inherited risk factors** represent genetic mutations and polymorphisms that result in deficiency of a natural anticoagulant (e.g., protein C, protein S, or AT), procoagulant factor accumulation (e.g., prothrombin G20210A or the thermolabile variant of the enzyme methylene-tetrahydrofolate reductase), or coagulation factor resistance to inactivation by a natural anticoagulant (i.e., factor V G1691A, also known as factor V Leiden). Hyperhomocysteinemia and increased factor VIII functional activity can either be acquired in nature or have a genetic predisposition. Many patients who experience thrombosis are found to have a combination of more than one defect. Testing for an inherited hypercoagulable state is likely to uncover an abnormality in >60% of patients presenting with idiopathic (i.e., spontaneous or unprovoked) venous thrombosis.

**Protein S – An introduction.**

Protein S is a vitamin K–dependent plasma glycoprotein. It was named after the city of its discovery, Seattle, in 1979. In humans, protein S is encoded by the PROS1 gene situated in chromosome 3p11.1–11.2. It is principally synthesized in the liver, but other organs may be important sites for its synthesis, including the endothelium, kidney, testes, and brain. Protein S is synthesized as a precursor protein of 676 amino acids. Post translational modifications give rise to a mature secreted single-chain glycoprotein of 635 residues with three N-linked carbohydrate side chains. Protein S facilitates the anticoagulant activity of activated protein C (APC) and inhibits tissue factor activity by promoting...
the interaction between tissue factor pathway inhibitor and factor Xa.\(^8\)

**Clinical Manifestations.** Deep venous thrombosis and pulmonary embolism are the most common forms of VTE associated with protein S deficiency, although superficial vein thrombophlebitis and thrombosis in unusual sites also occur. About 50% of thrombotic events are unprovoked. Up to 25% of patients with protein S deficiency may experience arterial thrombosis, including stroke. In family studies, venous thrombosis occurred in 100% of protein S–deficient relatives of affected probands by 70 years of age. The estimated lifetime increased relative risk of thrombosis has been reported to be as high as 36-fold for protein S deficiency. Analysis of four prospective studies showed that the incidence of VTE in asymptomatic protein S-deficient relatives of symptomatic probands was 0.7 to 2.2 percent per year. About half of these events occurred during well-known risk periods, but incidence rates were decreased by prophylactic use of oral anticoagulants.\(^1^3\;\;8;\;14\).

J O’Sullivan, et al reported a case of Protein S Deficient 5 year old girl presented only with pulmonary hypertension.\(^1^5\)

In patients with homozygous or compound heterozygous protein S deficiency, neonatal purpura fulminans has been reported. warfarin-induced skin necrosis have
also been associated with protein S deficiency.

### Laboratory Features

<table>
<thead>
<tr>
<th>S.N</th>
<th>Investigation</th>
<th>Result</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prothrombin time (PT)</td>
<td>Test: 11 secs</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 11 secs</td>
<td></td>
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<td></td>
<td></td>
<td>INR: 1.0</td>
<td></td>
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<tr>
<td>2.</td>
<td>Activated partial thromboplastin time</td>
<td>Test: 32 secs</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>(aPTT)</td>
<td>Control: 32 secs</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Anti-cardiolipin antibody</td>
<td>IgM: 3.5 GPL U/mL</td>
<td>&lt;= 11: Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgG: 3.4 MPL U/mL</td>
<td>&lt;= 10: Negative</td>
</tr>
<tr>
<td>4.</td>
<td>Lupus Anticoagulants</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Antithrombin</td>
<td>93.5 %</td>
<td>Normal: 80 – 120%</td>
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<tr>
<td>6.</td>
<td>Anti nuclear antibody (ANA)</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Homocysteine (Plasma)</td>
<td>9.10 mol/L</td>
<td>Normal: 5.40 – 16.2 mol/L</td>
</tr>
<tr>
<td>8.</td>
<td>Protein S</td>
<td>54%</td>
<td>Normal: 93.5 – 126%</td>
</tr>
<tr>
<td>9.</td>
<td>Protein C</td>
<td>50%</td>
<td>Normal: 60 – 130%</td>
</tr>
<tr>
<td>10.</td>
<td>Activated Protein C Resistance (APC –</td>
<td>Negative</td>
<td>Rules out Factor V</td>
</tr>
</tbody>
</table>
|     | R)                                     |                 | Leiden mutation.
Free protein S antigen and APC cofactor anticoagulant activity are better parameters than total protein S antigen in screening for hereditary protein S deficiency. Protein S activity assays may be affected by coexisting APCR, although the second-generation assays in which factor V-deficient plasma is used as substrate have improved specificity. Assessment of total and free protein S plus protein S activity should allow classification of patients with protein S defects into type I, IIa, or IIb. In normal subjects, an excellent correlation is seen between free protein S antigen and anticoagulant activity. The lower limit of the normal range for free protein S is lower in females than in males (55% vs. 65%). Protein S is remarkably sensitive to hormonal status in females. The high frequency of acquired protein S deficiency makes identification of hereditary defects more difficult. Oral contraceptives and hormone replacement therapy decrease plasma protein S levels. Reduced levels of free protein S are regularly found during pregnancy, e.g., as low as 20–30% of normal, in patients who are taking oral anticoagulants, and in patients with disseminated intravascular coagulation, liver disease, nephrotic syndrome, inflammatory conditions, and acute thromboembolism. Protein S deficiency can occur in concert with the lupus anticoagulant and as a result of autoantibodies to protein S following varicella or other infections in children. Thus, these acquired conditions leading to low protein S levels should be excluded and tests repeated before making a diagnosis of hereditary thrombophilia.

Indian Scenario:
There are few case reports from India. Mendiratta V et al, New Delhi reported a case of protein S deficiency presenting as isolated distal cutaneous thrombosis. Mondal R et al, Kolkata reported a 7-year-old girl with deep vein thrombosis due to combined protein C and protein S deficiency. Hoorda A et al, Jaipur reported a case of protein S deficiency-related recurrent ischemic stroke in a 16-year-old girl. Shah I and, Bhatnagar S, Mumbai reported an 8-year-old Indian girl with portal cavernoma due to hereditary protein S deficiency. Goel PK and Batra A analyzed the prothrombotic tendency of individual patients by studying protein C and protein S levels. Eleven out of thirteen of their patients (84.6%) had protein S deficiency. Mohanty D, Mumbai studied in 53 Budd-Chiari syndrome (BCS) and 33 portal vein thrombosis (PVT) cases, and found 3 patients in BCS group (5.7%) and 1 patient in PVT group (3.03%) to have protein S deficiency.

Treatment:
The chief goal of therapy is to prevent recurrent VTE, because it is fatal in 5 percent of cases. Patients with a known thrombophilia who present with VTE should be treated with a standard regimen of heparin overlapped with warfarin until an international normalized ratio of 2.0 to 3.0 is obtained on 2 consecutive days. This regimen is sufficient for the prevention of skin necrosis, which may occur during the initiation of warfarin therapy in patients with a protein C and protein S deficiency. Warfarin therapy reduces the risk of recurrence by 90 to 95 percent, but the annual risk of fatal hemorrhage is 0.25 percent. Lifelong anticoagulation therapy is indicated in persons with protein S, protein C and Antithrombin deficiencies.

Bombay O Blood Group:
The antigens of the ABO system (A, B, and H) consist of
complex carbohydrate molecules. H antigen is an essential carbohydrate acceptor for either -1,3-Nacetylgalactosaminyltransferase (A transferase) or -1,3-galactosyltransferase (B transferase), which are both encoded by the ABO locus (9q34). In group A, B, or AB individuals, the A and B transferases convert precursor H antigen into either A or B determinants, respectively. In group O individuals, the O allele does not encode any functional transferase enzyme so that they continue to express terminal H structures only. In human tissues, H antigen can be synthesized by 2 distinct -1,2-fucosyltransferases. One is the H gene (FUT1)–encoded H enzyme that regulates expression of ABH antigens in red blood cells. The other is the Secretor gene (FUT2)–encoded enzyme that regulates expression of ABH antigens in the gastrointestinal tract and secretions. Individuals with the very rare Bombay phenotype are non-Secretors and also fail to express H transferase (FUT 1). Such people cannot synthesize A or B antigenic structures regardless of their ABO blood group genotype, and ABH antigens are absent from both their erythrocytes and secretions. ABO transferase enzymes are required to glycolate vonWillebrand factor. In Bombay blood group people, vWF levels are significantly lower than others due to enhanced degradation. Effect of abnormal vWF on coagulation is not known (21).

Conclusion:
In patients presenting with pulmonary hypertension, with evidence of chronic thromboembolism, both acquired and inherited factors play a role in persistent hypercoagulable state. As these are treatable cause of pulmonary hypertension they should be routinely investigated. Inherited Thrombophilia like protein S deficiency can result in recurrent life threatening thrombotic events. Clinical suspicion, early diagnosis and prompt anticoagulation might save life.

Bombay Blood Group is associated with abnormal vWF which is degraded faster than normal. But its effect on coagulation is not known.

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