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A study on etiological factors in splanchnic venous thrombosis using JAK-2 mutation

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Abstract
Background: Splanchnic venous thrombosis is a rare disorder with multiple causes. The prevalence and cause of SVT vary in different parts of the world. Interaction between genetic and acquired risk factors is important in this disorder. Several conditions associated with hypercoagulability have been implicated in the causation of SVT.
Aim and Objective: To study etiological factors in splanchnic venous thrombosis using JAK-2 mutation
Methodology: This was a prospective study conducted in Department of Surgical Gastroenterology, Nizam's Institute of Medical sciences, Hyderabad from January 2013 to June 2014. Diagnosed cases of Budd-Chairi syndrome (BCS), portal vein thrombosis (PVT) and mesenteric vein thrombosis (MVT) admitted or attended outpatient department were included. Eighty five patients were included in study group. They were divided in two sub groups. Group I constitute initial 60 cases those who underwent Protein C, Protein S, ATIII, Homocystien and APL antibody evaluation. About 65 percent of patients were found to have unknown aetiology. So increase the yield of diagnostic tests we added FVL mutation and JAK-2 V617F studies in next 25 cases on trail basis. Group II constitute of those 25 cases with mutational analysis.
Results: The mean age group (in years) in study was 32.57 (13-75) years. Deficiencies of protein C activity were observed in 9 (15%) patients with splanchnic venous thrombosis. Protein C deficiency was most common etiological factor noticed in our study 16.25% (13/85). Further in subgroup analysis it was more common in MVT group 20.68% (6/29) compared to BCS and PVT group 14.28 and 10.71%. Protein S deficiency was seen in 11.76% (10/85) cases in our study group. PVT group had shown more incidence 14.28% (4/28) followed by MVT 13.79% (4/29) and BCS 7.14% (2/28). Antithrombin III deficiency was observed in 3 (10.71%) of 28 cases of Budd Chairi Syndrome, 2 (7.14%) of 28 cases of portal vein thrombosis and 2 (6.89%) of 29 cases of mesenteric vein thrombosis. After mutational analysis etiological factors were detected in 47.05% (40/85). Fifty three percent cases etiological factor was still not unknown.
Conclusion: Protein C is most common etiological factor in our study. Protein S was second most common etiological factor in study group. It has multifactorial aetiology can be the result of a combined effect of different pathogenesis mechanisms. Jack 2 mutations is not common in Indian Population.

Keywords: Protein C, protein S, antithrombin III, budd chairi syndrome, splanchnic venous thrombosis

Introduction
Abdominal vein thrombosis or splanchnic vein thrombosis is a rare, but life-threatening form of venous thrombosis and includes hepatic vein thrombosis Budd-Chiari syndrome, (BCS), portal vein thrombosis (PVT) and mesenteric vein thrombosis (MVT) [1]. BCS and PVT defined as primary if they are caused by a primarily venous disease, and defined as secondary if caused by compression or invasion by lesions originating outside the veins, such as benign or malignant tumors, abscesses, or cysts [2].
The annual incidence of BCS is 0.4 to 0.8 per million individuals in Western Countries [3, 4] and 0.1 per million in Japan [3]. BCS has a prevalence of 1.4 per million individuals in Western Countries [4] and 2.4 per million in Japan [3]. The annual incidence of superior mesenteric vein thrombosis is 2.7 per 100,000 individuals [5]. Isolated MVT without concomitant extra hepatic portal vein obstruction (EHPVO) and splenic vein thrombosis is rare [6].
MVT presentation can be acute, sub-acute, or chronic [7]. Acute thrombosis is associated with a bowel infarction in one-third of the patients and the mortality rate of MVT is 20% [4]. In most of the patients, the onset of MVT is characterized by acute abdominal pain. Other common symptoms include diarrhea, nausea, vomiting, and lower gastrointestinal bleeding [8]. MVT is associated with EHPVO in 65% of patients [9] and chronic presentation with no acute abdominal

pain and extensive venous collateral circulation is common [8].

In recent years several large-scale studies have been performed to study the underlying aetiological factors in these thrombotic disorders. Both inherited and acquired thrombophilia factors are frequently observed in these patients. Factor V Leiden mutation is frequently found in patients with BCS and prothrombin gene variant is seen more frequently in PVT. Myeloproliferative neoplasms (MPNs), including polycythemia vera and essential thrombocythemia, are underlying disorders in 30-40% of patients with abdominal vein thrombosis. Other aetiological factors are paroxysmal nocturnal haemoglobinuria (PNH), autoimmune disorders and hormonal factors. Primary splanchnic venous thrombosis is a multifactorial diseases as several prothrombotic disorders can be seen in same individual [10]. This justifies the need for a comprehensive thrombophilia screening in these subjects, even in the presence of known underlying predisposing factors or of obvious precipitating abdominal causes.

In the last decade the availability of advanced imaging procedures such as Doppler ultrasound, computer tomography (CT), and magnetic resonance imaging (MRI) allowed a better diagnosis of SVT in a large number of clinical settings ranging from fortuitous asymptomatic occlusions to acute abdomen. Moreover, the possibility of diagnosing SVT earlier results in a more prompt and effective therapy that translates in decreased morbidity and mortality [10].

SVT have been associated with various hypercoagulable states. Studies on etiopathologic factors for SVT are few, and most of them lack a complete workup for the prothrombotic state. To our knowledge, there is single study available on BCS and MVT from western India while other study was from northern India on BCS. There is no available data from southern India on etiological spectrum of SVT, so we conducted this study.

Objectives of the study

- To study etiological factors in splanchnic venous thrombosis using JAK-2 mutation

Material and Methods

This was a prospective study conducted in Department of Surgical Gastroenterology, Nizam's Institute of Medical sciences, Hyderabad from January 2013 to June 2014. Diagnosed cases of Budd-Chairi syndrome (BCS), portal vein thrombosis (PVT) and mesenteric vein thrombosis (MVT) admitted or attended outpatient department were included. Refereed cases from the departments of Medical Gastroenterology and General medicine were also included in the study.

Inclusion criteria

Patients who were diagnosed with primary BCS, PVT and MVT including acute and chronic venous thrombosis, at any age group who attended inpatient or outpatient care were included.

Exclusion criteria

All patients with secondary causes of venous thrombosis were excluded from study like, pregnancy, oral contraceptive use, nephrotic syndrome, hormone replacement syndrome, Local factors (omphalitis, pancreatitis, diverticulitis, cholecystitis) Portal vein axis injury (splenectomy, cholecystectomy, colectomy).

Diagnosis

Mesenteric venous thrombosis is diagnosed on the basis of US pulsed-Doppler and CECT Abdomen [10]. PVT was diagnosed in the presence of endoluminal material and absence of flow in the portal vein, or cavernous transformation of the vein as shown by duplex-Doppler ultrasound, or contrast enhanced CT scan or magnetic resonance imaging HVT was diagnosed according to previously published criteria i.e. small hepatic veins (HVs), large HVs, inferior vena cava (IVC), and combined obstruction of large HVs and IVC.

Blood sample collection

In patients presenting with acute thrombosis, samples were collected before starting conventional IV heparin. Patients who were already on oral anticoagulants are advised to withhold medication for two weeks and switch over to low molecular weight heparin 2500 international units subcutaneously once daily.

Blood sample had been collected about 10 ml. Complete blood picture, renal function test, liver function test, Prothrombin time checked along with work for thrombophilia- protein C, protein S, homocysteine, antithrombin III, anti-phospholipid antibody, prothrombin gene mutation, factor V Leiden mutation, JAK-2 mutation.

Patients were advised to undergo Upper GI endoscopy for to look for varices.

Eighty five patients were included in study group. They were divided in two sub groups. Group I constitute initial 60 cases those who underwent Protein C, Protein S, ATIII, Homocystien and APL antibody evaluation. About 65 percent of patients were found to have unknown aetiology. So increase the yield of diagnostic tests we added FVL mutation and JAK-2 V617F studies in next 25 cases on trail basis. Group II constitute of those 25 cases with mutational analysis.

Protein C

Protein C was tested by Photo-optical clot detection method. The patient sample is incubated with and without exogenous activated protein C (APC). Activated factor V (FVa) is broken down by APC, reducing conversion of prothrombin to thrombin and extending the clotting time. If factor V mutation is present, FVa breakdown is inhibited, leading to a shorter clotting time. Results are expressed as the ratio of clotting times obtained with and without exogenous APC.

Protein S

Clot detection method. Protein S in the patient sample enhances the anticoagulant action of activated protein C, resulting in a prolonged clotting time. The increase in clotting time is directly proportional to the percent of normal protein S activity.

Antithrombin III

ATIII activity was tested by chromogenic method. Antithrombin in the patients sample binds to thrombin; excess thrombin cleaves a synthetic thrombin substrate, the amount of which is inversely proportional to the amount of antithrombin activity in the plasma.

Homocysteine

Homocytein was detected by Competitive immunoassaymethod. The level of total homocysteine (i.e. protein-bound, oxidized, and free, reduced homocysteine) is measured in a competitive immune chemiluminometric assay.

Antiphospholipid antibody

APL ab was detected by ELISA test. The level of antibodies

directed against various plasma proteins that bind to phospholipid surfaces (eg, damaged endothelial membranes, monocytes, tumor cells, etc) is measured. The method is highly sensitive but not specific for β2-glycoprotein I antibodies. Individual tests for IgA, IgG, and IgM antibodies are available.

Factor V Ledin mutation analysis

FVLM was done by PCR assay. It detects oligonucleotide ligation, fluorescent detection. The 1691G>A factor V Leiden mutation is detected by amplification of the gene region with PCR, followed by oligonucleotide ligation and hybridization to colour-coded microspheres. Results are reported as no mutation detected, heterozygous positive, or homozygous positive.

JAK-2 V-617F

JAK-2 V617 F was detected by Reverse transcription PCR, sequencing. PCR and sequencing are performed as described above to detect the V617F mutation; if negative, an additional PCR and sequencing will be performed to detect JAK2 mutations in exons 12 and 13.

Ethics committee

The Study was approved by institutional ethics committee, reviews letter no EC/NIMS/1411/2013, 7th ECGS NO: 103/13. Informed consent was taken from all patients included in study.

Statistical analysis

Statistical analysis was performed using SPSS 17 software. Categorical variables were compared by Chi-square test when applicable. Continuous variables were analyzed by student t test or Mann Whitney U test when applicable. Continuous data was expressed as mean ± standard error of mean.

Results

Eighty five patients with splanchnic venous thrombosis (SVT) were evaluated for etiological workup during study period (January2013-June2014) in our Department of Surgical Gastroenterology and Department of Medical Gastroenterology Nizam's Institute Of Medical Sciences Hyderabad.

Division of patients according to diagnosis

These eighty five patients were divided as per site of thrombosis in three groups:

1. Budd Chairi Syndrome (BCS) n-28
2. Portal Vein Thrombosis (PVT) n-28
3. Mesenteric Venous Thrombosis n-29.

Table 1: Division of patients according to diagnosis

Diagnosis	Number of patients	%
Budd-Chari Syndrome	28	32.9
Portal vein thrombosis	29	34.5
Mesenteric venous thrombosis	28	32.9

Age at presentation

The mean age group (in years) in study was 32.57(13-75) years. The youngest patient was 13 years and the oldest was 75 years. About 50 percentiles of patients were blow 30 years of age group as shown in table no.2.

Table 2: Showing patient percentiles with age group

Percentiles	Age
25	22.5
50	30
75	40

Gender distribution

There were 45 male patients and 40 female patients (M: F: 1.12: 1). Of 28 patients with BCS, 9 (32%) were male, and 19 (67%) were female; 13 (46.42%) of 28 patients with PVT were male and 23 (79.3%) of 29 patients who suffered MVT were male. There was no statically deference found when compared gender distribution in inherited thrombophilic factors.

Table 3: Gender characteristics studied in BCS, PVT and MVT

Gender	Study n (%)	BCS n (%)	PVT n (%)	MVT n (%)
Male	45 (52)	9 (32.14)	13 (46.42)	23 (79.31)
Female	40 (48)	19 (67.85)	15 (53.58)	6 (20.79)

Table 4: Gender distribution of patients studied under various inherited thrombophilic factors

Test/Sex		Femalen (%)	Malen (%)	*p* value (†)
Protein C deficiency	Absent	34(85)	38(84.4)	0.569
	Present	6(15)	7(15.6	
Protein S deficiency	Absent	37(92.5)	38(84.4)	0.65
	Present	3(7.5)	7(15.6)	
Hyperhomocystinemia	Absent	36(90)	40(88.9)	0.337
	Present	4(10)	5(11.1)	
AT III Deficiency	Absent	37(92.5)	41(91.1)	0.843
	Present	3(7.5)	4(8.9)	
APL Ab	Absent	19(47.5)	28(62.2)	0.219
	Present	2(5)	1(2.2)	
FVLM	Absent	10(25)	15(33.3)	0.21
	Present	0(0)	0(0)	
JAK2V617 Mutation	Absent	9(22.5)	15(33.3)	0.207
	Present	1(2.5)	0(0)	

Numbers in parenthesis are row percentages; † indicate Pearson Chisquare test

The above table clearly shows that there are no significant difference between males and females suffering from various inherited thrombophilic factors. Protein C Deficiency was revealed in 6(46%) females and 7(54%) males.70%of males suffered from Protein S Deficiency. Two females of 3 individuals had circulating Anti Phospholipid Ab.

Eighty five patients were included in study group. They were divided in two sub groups. Group I constitute initial 60 cases those who underwent Protein C, Protein S, ATIII, Homocystien and APL antibody evaluation. About 65 percent of patients were found to have unknown aetiology. So increase the yield of diagnostic tests we added FVL mutation and JAK-2 V617F

studies in next 25 cases on trail basis. Group II constitute of those 25 cases with mutational analysis.

Deficiencies of protein C activity were observed in 9 (15%) patients with splanchnic venous thrombosis. Six (10%) of 60 patients of splanchnic vein thrombosis had Protein S deficiency. None of the 21 patients of Budd Chiari Syndrome had Protein S deficiency. Elevated homocysteine levels were associated with 5(8.33%) of 60 patients of splanchnic vein thrombosis, only 1 (4.76%) of 21 patients of Budd-Chairi Syndrome had hyperhomocysteinemia. Antithrombin III deficiency was observed in 1 (4.76%) of 21 cases of Budd Chairi Syndrome, 2 (9.09%) of 22 cases of portal vein thrombosis and 3(17.64%) of 17 cases of mesenteric vein thrombosis, thus comprising of 6(10%) subjects of 60 cases of splanchnic vein thrombosis with antithrombin III deficiency. Antiphospholipid antibody was observed in 1(4.76%) of 21 cases of Budd Chairi Syndrome and 1(5.88%) of 22 cases of mesenteric vein thrombosis, thus comprising of 2(3.33%) subjects of 60 cases of splanchnic vein thrombosis with Antiphospholipid antibody. Multifactorial aetiology was found in 7(11.66%) cases.

Definite etiological factor was found in 20(33.33%) cases.

Group I

Table 6(A and B): Differences in prevalence of protein C deficiency, protein S deficiency, antithrombin deficiency homocysteine and APL-abin 60 patients with Budd-Chiari syndrome (BCS), PVT and MVT

Table 6A

Test	Study (n-60)
Protein C deficiency	9 (15)
Protein Sdeficiency	6 (10)
ATIII deficiency	6 (10)
Hyper homocysteinemia	5 (8.33)
APL-ab	2 (3.33)
Multifactorial	7 (11.66)

Table 6B

Test	BCS (n-21)	PVT (n-22)	MVT (n-17)
Protein C deficiency	3 (14.21)	3 (13.63)	3 (17.64)
Protein S deficiency	0	2 (9.09)	4 (23.52)
ATIII deficiency	1 (4.76)	2 (9.09)	3 (17.64)
Hyperhomocysteinemia	1 (4.76)	1 (4.54)	3 (17.64)
APL-ab	1 (4.76)	0	1 (5.88)
Multifactorial	2 (9.52)	2 (9.09)	3 (17.64)

Group II

In this group two genetic mutational factors (JAK-2V617F, FVLM) were added in above battery of tests. This test was done on trail basis in latter 25 cases.

Table 7(A and B): Differences in prevalence of protein C deficiency, protein S deficiency, antithrombin deficiency, hyperhomocystenemia, APL-aband mutational analysis in 25 patients with Budd-Chiari syndrome (BCS), PVT and MVT

Table No.7A

Test	Study group (n-25)
Protein C deficiency	4(16)
Protein Sdeficiency	4(16)
ATIIIdeficiency	1(4)
Hyperhomocystenemia	4(16)
APL Ab	1(5.2)
FVLM	-
JAK-2V617	1(4)
Multifactorial incidence	3(12)

Table 7B

Test	BCS (n-7)	PVT (n-6)	MVT (n-12)
Protein C deficiency	1 (14.2)	-	3 (25)
Protein S deficiency	1 (14.2)	3 (50)	-
ATIII deficiency	1 (14.2)	-	-
Hyperhomocysteinemia	-	3 (50)	1 (8.3)
APL Ab	-	1 (25)	-
FVLM	-	-	-
JAK-2V617	-	1 (16.6)	-
Multifactorial incidence	1 (14.2)	2 (33.3)	-

Of 25 patients investigated later, there were 4(16%) patients each with protein C deficiency, protein S deficiency and homocysteinemia; 3(12%) of 25 cases had multifactorial incidence. Single patient in PVT group detected positive for JAK-2V617F. None of our patient in this group was detected for FVLM. In this group we could detect definite etiological factor in 52% (13/25) cases.

Table 8: Differences in prevalence of protein C deficiency, protein S deficiency, antithrombin deficiency, homocysteine and APL-ab, JAK-2 Mutation anf FVLM in study group

Table 8A

Test	Study (n-85)
Protein C Deficiency	13 (15.29)
Protein S Deficiency	10 (11.76)
ATIII Deficiency	7 (8.23)
Hyperhomocysteinemia	9 (10.58)
APL – Ab*	3 (6.0)

Table 8B

		Diagnosis			*p* Value
		BCS n (%)	PVT n (%)	MVT n (%)	
Protein C Deficiency	Absent	24(85.7)	25(89.3)	23(79.3)	0.569
	Present	4(14.3)	3(10.7)	6(20.7)	
Protein S Deficiency	Absent	26(92.9)	24(85.7)	25(86.2)	0.65
	Present	2(7.1)	4(14.3)	4(13.8)	
Hyperhomocysteinemia	Absent	27(96.4)	24(85.7)	25(86.2)	0.337
	Present	1(3.6)	4(14.3)	4(13.8)	
AT III Deficiency	Absent	25(89.3)	26(92.9)	27(93.1)	0.843
	Present	3(10.7)	2(7.1)	2(6.9)	
FVLM	Absent	7(25)	6(21.4)	12(41.4)	0.21
JAK2V617	Absent	7(25)	5(17.9)	12(41.4)	0.207
	Positive	0(0)	1(3.6)	0(0)	
APL-Ab	Absent	12(42.90)	14(50)	21(72.4)	
	Positive	1(3.6)	1(3.6)	1(3.4)	0.219

Numbers in parenthesis are row percentages

Deficiencies of protein C activity were observed in 13(15.29%) patients with splanchnic venous thrombosis. Ten (11.76%) of 85 patients of splanchnic vein thrombosis had Protein S deficiency. Only 2(7.14%) of 28 patients of Budd Chiari Syndrome had Protein S deficiency. Elevated homocysteine levels were associated with 9 (10.58%) of 85 patients of splanchnic vein thrombosis, only 1 (3.57%) of 28 patients of Budd-Chairi Syndrome had hyperhomocysteine -mia. Antithrombin III deficiency was observed in 2(7.14%) of 28 cases of Budd Chairi Syndrome, 2 (7.14%) of 28 cases of portal vein thrombosis and 3(10.34%) of 29 cases of mesenteric vein thrombosis, thus comprising of 7 (8.23%) subjects of 85 cases of splanchnic vein thrombosis with antithrombin III deficiency. Antiphospholipid antibody was observed in 1 (7.69%) of 13 cases of Budd Chairi Syndrome, 1 (6.66%) of 15 cases of portal vein thrombosis and 1 (4.45%) of 22 cases of mesenteric vein thrombosis, thus comprising of 3(6%) subjects of 50 cases of splanchnic vein thrombosis with Antiphospholipid antibody.

Multifactorial aetiology in SVT

Multifactorial aetiology was found in 11.76% (10/85) cases. Four (4.70%) cases had combined Protein C and S deficiency. Single patient in six various combinations were found in study group. Multifactorialaetiology was found in 11.76 % (10/85) cases. Five (5.88%) cases had combined Protein C and S deficiency. Two (2.35%) cases had Protein C and ATIII deficiency. One patient with hyperhomocystenemia had Protein C deficiency and other one had Protein S deficiency. Protein C, Protein S and ATIII deficiency was noticed in one patient.

Table 9: Number and percentage of patients having multifactorial etiology in SVT

Multifactorial etiology in SVT	n (%)
Protein C and Protein S deficiency	4 (4.70)
Protein C and ATIII deficiency	1 (1.17)
Protein S deficiency and Hyperhomocysteinemia	1 (1.17)
Anti-phospholipid antibody and ATIII deficiency	1 (1.17)
Protein C, Protein S deficiency and Hyperhomocysteinemia	1 (1.17)
Anti-phospholipid antibody and Hyper-homocysteinemia	1 (1.17)
JAK-2 V617F mutation and Hyperhomocysteinemia	1 (1.17)
Total	10 (11.76)

Discussion

Splanchnic venous thrombosis is a rare disorder with multiple causes. The prevalence and cause of SVT vary in different parts of the world. Interaction between genetic and acquired risk factors is important in this disorder. Several conditions associated with hypercoagulability have been implicated in the causation of SVT. Our study includes all three forms of splanchnic venous thrombosis i.e. BCS, PVT, MVT. This is unique feature of our study.

Almost 69% of cases were aged <40 years; hypercoagulable state was identified in 49.35%. The age of presentation ranges from 13 to 75 years, with mean age of patients 32.57 years and male to female ratio is 1.12: 1. Of 28 patients with BCS, 19 (67%) were female; 15 (53.57%) of 28 patients with PVT were female and 23 (79.3%) of 29 patients who suffered MVT were male.

Cond at *et al.* [13] had study population with equal gender distribution (M/F=77/64) M: F was 1.2 and mean age group 44 years. Janssen *et al.* had study population with mean age 40 yrs in BCS group and 51yrs in PVT group. Sex ratio in this study was M: F (1:2.2) M/F (16 /37) in BCS group and M: F (1:1.08) M/F (48 /52) in PVT group.

The prevalence of primary deficiencies in Protein C, Protein S, and Antithrombin III in BCS patients is difficult to determine, for several reasons. Firstly, the liver synthesizes these inhibitors of coagulation, and liver dysfunction related to BCS thus induces non-specific falls in the plasma levels of the inhibitors. Secondly, diagnosis of any primary deficiency is based on measurement of plasma protein level, because most mutations in the relevant genes are unique, rendering diagnosis using molecular biology techniques alone difficult. Finally, complete family screening is recommended to differentiate between inherited and false instances of deficiencies in Proteins C and S, but this is usually impractical.

In all of our patients with protein C and protein S deficiencies, an acquired deficiency was ruled out by normal liver function test results and a normal prothrombin time. None of our patients were receiving anticoagulant therapy at the time of protein C and protein S estimation.

Protein C deficiency

Protein C deficiency was most common etiological factor noticed in our study 16.25% (13/85).Further in subgroup analysis it was more common in MVT group20.68%(6/29) compared to BCS and PVT group 14.28 and 10.71%.

Protein C deficiency was found to have etiological factor in 1-9 % cases of PVT and BCS in previously published studies [11, 12].

Janssen *et al.* [10] showed Protein C deficiency was seen 7.6% and 9.3% in PVT and BCS respectively. Condat *et al.* [13] noticed 3% cases of Protein C deficiency. Egesel T *et al.* [14] shown Protein C deficiency in 26% cases of PVT. Denninger *et al.* [15] and Primignani *et al.* [16] could not found single case with protein C deficiency in PVT group.

Protein S deficiency

Protein S deficiency was seen in 11. 76% (10/85)cases in our study group. PVT group had shown more incidence 14.28% (4/28) followed by MVT 13.79% (4/29) and BCS 7.14% (2/28).

Protein C deficiency was found to have etiological factor in 1-7 % cases of PVT and BCS in previously published studies [11, 12].

Janssen *et al.* (2000) showed Protein S deficiency was seen 2.3% in PVT group. While Bhattacharya *et al.* [17] noticed Protein S deficiency 4% of PVT cases and 12% of BCS cases. B. Condat *et al.* [13] noticed 9% cases of Protein S deficiency. Egesel T *et al.* [14] had shown Protein S deficiency in 43% cases of PVT. Denninger *et al.* [15] and Primignani *et al.* [16] was noticed protein C deficiency in 30% and 2% cases PVT group respectively.

Antithrombin III deficiency

Antithrombin III deficiency was observed in 3 (10.71%) of 28 cases of Budd Chairi Syndrome, 2 (7.14%) of 28 cases of portal vein thrombosis and 2 (6.89%) of 29 cases of mesenteric vein thrombosis, thus comprising of 7 (8.23%) subjects of 85 cases of splanchnic vein thrombosis with antithrombin III deficiency in our study.

A-III deficiency was found to have etiological factor in 2-5 % cases of abdominal vein thrombosis in previously published studies [11, 12].

Janssen *et al.* (2000) [10] showed AT-III deficiency was seen 1% in PVT group. B. Condat *et al.* noticed 9% cases of AT-III deficiency. EgeselT *et al.* [14] had shown AT-III deficiency in 26% cases of PVT. Denninger *et al.* [15] and Primignani *et al.* [16] was noticed AT-III deficiency in 4% and 5% cases PVT group respectively. Studies conducted by Mohanty *et al.* [18] and Uskudar *et al.* [19], while working on patients with BCS showed

antithrombin III deficiency in 4.3%, 3.8% and 3%.

Hyperhomocystenemia

Elevated homocysteine levels were associated with 9 (10.85%) of 85 patients of splanchnic vein thrombosis, only 1 (3.57%) of 28 patients of Budd-Chairi Syndrome had hyperhomocysteinemia. Previous studies were shown 2-19% incidence of Hyperhomocystenemia in their cohort [11] Primignaniet *et al.* had shown Hyperhomocystenemiain 12% cases of PVT [20].

Antiphospholipid antibody

Antiphospholipid antibody test was done in only 50/85 cases. Three (3/50) patients were detected to have antiphospholipid antibody. In each group one patient was detected antiphospholipid antibody. In PVT group antiphospholipid antibody was detected in 6.66 % (1/15) cases. In BCS group antiphospholipid antibody was detected in 7.66 % (1/13) cases. In MVT group antiphospholipid antibody was detected in 4.45 % (1/22) cases.

Previous studies were shown antiphospholipid antibody was detected up to 20% cases of BCS and 10% cases of PVT. Mohanty *et al.* [18] had shown APL-ab was detected in 11.3% cases in BCS. Primignani *et al.* had shown APL-ab in 6 % cases of PVT [16].

Genetic mutational studies

After completing above mentioned tests, etiological factors were detected in 45.88% (39/85) cases in study group. Still 55% cases etiology was unknown, so last 26(BCS 8, PVT 6 and MVT12) cases were subjected for genetic mutational analysis i.e. Factor V Leiden mutation and JAK-2 V617 mutation.

JAK-V617F was detected in single 3.86% (1/26) patient in PVT group. This case was a middle aged lady, presented with pain abdomen detected to have PVT on USG abdomen followed by Doppler scan. She doesn't have any features of meyloprolifetative disorder on hematological or radiological investigations, so detected to have occult MPD.

MPNs are observed in 30-40% of patients with BCS or PVT, whereas this is rarely the cause of other types of VTE [21, 22].

While Mahmoud AE *et al.* had shown 23% cases with factor V Leiden positivity and 6.6% cases in PVT [18]. Mohanty *et al.* had shown FVLM was detected in 26.4% cases in BCS. Primignani *et al.* had shown FVLM in 23% cases of PVT [16]. After mutational analysis etiological factors were detected in 47.05% (40/85). Fifty three percent cases etiological factor was still not unknown.

Mutlifactorialetiology

Multifactorial etiology was noticed in 11.76 % (10/85) cases in our study. Various studies shown that multifactorial etiology seen in 10-50% cases. Although these studies considered primary as well as secondary etiological factors. Janssen *et al.* [10] had observed occurrence of either acquired or inherited thrombotic risk factors in 26% of the BCS patients and 37% of the PVT patients.. Mohanty *et al.* [18] had observed multifactorial etiology in 11.32% cases of BCS.

Conclusion

- Protein C is most common etiological factor in our study.
- Protein S was second most common etiological factor in study group.
- JAK-2 mutational study is not common in Indian population
- About 40% cases of Primary SVT are still idiopathic.
- Primary Splanchnic Venous thrombosis is disease of young age.
- It has equal gender distribution.
- It has multifactorial aetiology can be the result of a combined effect of different pathogenesis mechanisms.

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Conflict of interest

None

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