JAK2V617F Mutation in Patient with Splanchnic Vein Thrombosis

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Abstract Splanchnic vein thrombosis is an uncommon life-threatening form of venous thrombosis. It is one the common complication among MPN's. In the western studies the prevalence of JAK2V617F mutation among SVT patient is high and ranges from 7 to 59%. The frequency of this mutation among Indian SVT patients is heterogenous. This was a prospective case control study. A total 52 cases of SVT and 40 controls were screened for JAK2V617F mutation along with other routine thrombophilic risk factors. Out of total 52 cases, 10 had BCS, 2 had MVT and rest 40 were of PVT/EHPVO. The JAK2V617F mutation was seen in two cases and not in controls. Among the thrombophilic markers, heterozygous FVL mutation, PC, PS and presence of APA were seen in 2, 3, 1 and 3 cases respectively. In addition, eight cases also showed deranged risk factors (5 inherited and 3 acquired), however the repeat testing was not performed due to loss of follow up. Among controls, one person showed presence of APA and one person showed multiple thrombophilic risk factor deficiency. JAK2V617F mutation was observed in 3.8% among north Indian SVT patients. The frequency of mutation is on the lower side as compared to the available Indian data. The other thrombophilia markers (both inherited and acquired) are more frequent (18%) and

patients should be routinely screened for these thrombophilia markers.

Keywords JAK2 V617F · Myeloproliferative neoplasms · Splanchnic vein thrombosis · Thrombophilia

Introduction

JAK2V617F an acquired mutation in the Janus kinase 2 (JAK2) gene located on the short arm of chromosome 9. It leads to substitution of valine to phenylalanine at position 617 in the pseudokinase domain of JAK2 protein [1]. This mutation is common in BCR-ABL1-negative myeloproliferative neoplasms (MPN's), and detected in is detected up to 95% of cases with polycythemia Vera and 50–60% in essential thrombocythemia and primary myelofibrosis [2]. In Western Literature, the incidence of thrombosis among BCR-ABL1 negative MPN's ranges from 12 to 39% in PV, 11 to 25% in ET and up to 7% in patients with MF. The arterial involvement is more common as compared to the venous one [3, 4].

Splanchnic vein thrombosis (SVT) is one of the complications of MPN's. In the absence of common causes of SVT such as cirrhosis and solid cancers; MPNs are a contributing factor in at least 30–40% of patients with SVT [5]. The prevalence of SVT among JAK2V617F positive MPN ranges from 7 to 59%. Approximately 25–65% of these cases of MPN may not be apparent at initial presentation and these patients display normal blood counts [6, 7]. The Indian data on JAK2V617F mutation in SVT patients is heterogenous and ranging from 1 to 27%. The studies with higher prevalence had included the cases of BCR-ABL1 negative MPN's having thrombosis [8–14]. The present study was planned to find out the frequency of

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JAK2V617F mutation in patients with the SVT as the initial presenting symptom.

Materials and Methods

This was a prospective case control study from coagulation laboratory of Hematology department. The study was approved by the Institute ethical committee with reference no. INT/IEC/2015/217 dated 15.7.15. A total 52 cases of SVT and 40 controls were screened for JAK2 V617F mutation along with other routine thrombophilic risk factors. The patients were diagnosed with splanchnic vein thrombosis (SVT), based on abdominal imaging (Doppler ultrasound/contrast-enhanced CT/magnetic resonance venography). The exclusion criteria included were the patients on oral anticoagulation and the onset of the symptoms within 4–6 weeks.

Approximately, 10 ml of peripheral venous blood sample was collected in vacutainers containing 0.109 M (3.2%) trisodium citrate anti-coagulant (9:1) and 3 ml of blood in dipotassium EDTA vacutainer. The citrated blood sample was centrifuged at 3600 rpm for 10 min, to obtain platelet poor plasma (PPP) that was stored at -20°C for thrombophilia work-up. The Protein C, Protein S, Antithrombin-III assays and Lupus anticoagulant assays were performed on fully automated coagulation analyzer (ACL TOP 500 CTS®, Instrumentation Laboratory, Bedford, MA, USA). The anti-Cardiolipin antibody and anti-B2-Glycoprotein1 antibody levels were tested on fully automated chemiluminescent analyzer (ACUSTAR, Instrumentation Laboratory, Bedford, MA, USA). The Lupus anticoagulant, anti-Cardiolipin antibody and anti-B2-Glycoprotein 1 antibody tests were kept together in a group of antiphospholipid antibodies (APA) for interpretation. The EDTA anticoagulated blood sample was used to measure complete blood count including Hemoglobin (Hb), Total Leucocyte count (TLC) and Platelet count (PC) on hematology blood cell counter (Coulter LH750; Beckman Coulter, Miami, FL, USA) within 30 min of sample collection. The same blood sample was used for genomic DNA extraction for Factor V Leiden (FVL) gene mutation and JAK2V617F mutation. The FVL mutation was detected by restriction fragment length polymorphism (RFLP) PCR using 5'-TGCCCAGTGCTTAACAAGACCA-3' primer and the 5'-CTTGAAGGAAATGCCCCATTA-3' primer and MnlI as a restriction enzyme. The JAK2V617F mutation was detected by and amplification-refractory mutation system (ARMS) PCR using 4 primers: forward outer, 5'-TCC TCA GAA CGT TGA TGG CAG-3'; reverse outer, 5'-ATT GCT TTC CTT TTT CAC AAG AT-3'; forward wild-type specific, 5'-CAT TTG GTT TTA AAT TAT GGA GTA TAT G-3'; and reverse mutant

specific, 5'-GTT TTA CTT ACT CTC GTC TCC ACA AAA-3'.

Results

The study comprised of 52 SVT patients and 40 healthy controls. There were 27 males and 25 females in the patient group (male to female ratio of 1.08:1) with a mean age of 33.64 years (4–78 years). The control group comprised of 29 males and 11 females (male to female ratio of 2.6:1) and a mean age of 33.5 years (25–53 years). Out of total 52 cases, 10 had Budd–Chiari syndrome (BCS), 2 had mesenteric vein thrombosis (MVT) and rest 40 were of portal vein thrombosis/extrahepatic portal venous obstruction (PVT/EHPVO). Among the thrombophilic markers, heterozygous FVL mutation, PC, PS and APA were seen in 2, 3, 1 and 3 cases respectively. All these cases were considered abnormal after repeat testing over a period of 1 month (PC and PS) and 3 months (APA). Three cases showed multiple inherited risk factors deficiency most likely due to secondary liver associated causes since the prothrombin time was also prolonged in these cases. In addition, eight cases also showed deranged risk factors (5 inherited and 3 acquired), however the repeat testing was not performed due to loss of follow up. Among controls, one person showed presence of anticardiolipin antibody and one person showed mild derangement of PC and AT with deranged PT. This person later found to have deranged liver function tests. The frequency of thrombophilic risk factors among patients and controls are mentioned in Table 1. The complete blood count comprising of Hb, TLC and PC was measured among cases and controls. The mean Hb, TLC and PC was higher in controls as compared to the cases (Table 2).

The frequency of JAK2V617F mutation was 3.8% (two cases) among cases and 0% among controls (Table 2). Both these cases harbor heterozygous mutation. One of these cases was a 37-years male with EHPVO and multiple collateral along with splenomegaly. The liver function tests (LFT) were deranged. The CBC values show anemia (Hb:

Table 1 The thrombophilic risk factors in study population

Risk factors	Patients	Controls
Factor V Leiden (Heterozygous)	2	Nil
Protein C deficiency	3	Nil
Protein S deficiency	1	Nil
Antithrombin deficiency	0	Nil
Antiphospholipid antibodies	3	1
Multiple risk factors	3	1

Table 2 The complete blood counts and JAK2V617F mutation status among cases and controls

	Cases (Mean)	Controls (Mean)
Hb (gm/dl)	11.7	14.2
TLC ($\times 10^9/l$)	7.6	7.7
PC ($\times 10^9/l$)	188.5	245
JAK2 V617F mutation	2/52	0/40

9 gm/dl; TLC: $7.8 \times 10^9/l$ and PC: $350 \times 10^9/l$), however later this case was diagnosed as Iron deficient Polycythemia Vera (PV) after bone marrow examination. The other case was 35-years female with BCS. Her LFT were also deranged. Her CBC values showed a High Hemoglobin (18 gm/dl) with normal PC count ($394 \times 10^9/l$) and raised TLC ($25.5 \times 10^9/l$) and hence diagnosed as PV.

Discussion

SVT is frequently associated to prothrombotic conditions such as inherited thrombophilia (factor V Leiden, PC, PS and AT deficiencies) or acquired risk factors. The acquired factors include MPNs, APA and oral contraceptive use [15]. The JAK2V617F mutation is a diagnostic marker of clonality in the majority of patients with BCR-ABL-1 negative MPN. However, there remains uncertainty about the role of JAK2V617F mutation in patients without overt signs of an MPN. The present study was planned to find out the frequency of JAK2V617F mutation among patients presenting with SVT.

In the western literature the prevalence of SVT among JAK2V617F positive MPN ranges from 7 to 59%. Among these the prevalence of JAK2 V617F ranged from 17 to 34% in portal vein thrombosis and from 40 to 58% in the Budd–Chiari syndrome [15–19]. This wide range was due to varied patients sample size, heterogeneity in the inclusion criteria and different assay with different sensitivities for the detection of JAK2V617F. In contrast, the data from India had less cases of JAK2V617F mutation among SVT patients and ranged from 1 to 27.5% [8–14]. The frequency among PVT/EHPVO cases ranged from 2 to 22.8% and among BCS ranged from 0 to 25% (Table 3). In the present study, the JAK2V617F mutation was seen in 3.8% (2/52) of SVT cases. The frequency among PVT/EHPVO and BCS were 2.5% (1/40) and 10% (1/10) respectively. The results are comparable with the Indian data and within the range. Among these 2 cases one case was a clear-cut case of MPN while other one was an overt case of MPN. As compared to the western studies, the JAK2V617F mutation among Indian patients with SVT is on the lower side. The CBC

values should be closely observed if the Hb and PC are on the higher side than the JAK2V617F mutation study must be performed. The SVT cases, where the CBC values are within normal range or on lower side, the testing should be optional and only after the clinical assessment.

There are many studies showing association of high JAK2V617F allele burden in MPN's with the thrombosis. [20–23]. However, a few studies denied this claim and found no association. [24, 25]. The data on allele burden for JAK2 V617F mutation in venous thrombosis is sparse. The study by Pardanani A et al. in 2007 comprised of 210 patients of non-splanchic venous thrombosis in the absence of overt myeloproliferative disorder. In this study, 4 patients (2%) carried the JAK2V617F mutation. The median JAK2V617F allele burden ($JAK2_{V617F}/JAK2_{V617F} + JAK2_{WT}$) for the four patients was 5.1% (range = 2.2–5.3%). The authors suggest that JAK2V617F mutation contributes to a hypercoagulable state, although the strength of the contribution and its role with other thrombophilia factors remains to be enlighten [26]. Goodyer M et al. in 2016 had studied 171 JAK2 V617F-positive patients and found 17 cases with SVT. Among these, seven (44%) had a normal peripheral blood count. The data did not have any statistically significant difference in the JAK2 V617F allele burden between those patients with a latent MPN (median 9.6%, range 6.8–55.5%) and those with an overt MPN (median 19.8%, range 3.1–62.1%) ($P = 0.475$) [27]. In the present study, semiquantitative PCR was performed and our patient was heterozygous for the JAK2V617F mutation. The allele burden was not calculated since qPCR was not performed.

The prevalence of reported deranged thrombophilic factors in Indian SVT patients ranged from 20 to 60% (Table 4) [11, 12, 14, 28–30]. In the present study, we had a frequency of 17% (9 cases) and among these heterozygous FVL mutation, PC, PS and APA were seen in 3.8%, 5.8%, 1.9% and 5.8% cases respectively. The frequency of FVL is less but following the similar trend as reported earlier from north Indian studies [12, 21, 22]. The protein S deficiency in this study are much lower as compared to the earlier studies. One of the reasons for this is the use of newer test; Free protein S Antigen instead of functional activity assay. This test is more specific for the detection of protein S deficiency. The PC deficiency is within the reported range while the AT deficiency was not seen in our study. The similar also happened in few earlier studies also [11, 21]. The small sample size may be another reason. The APA were seen in 5.8% of cases. These patients were considered positive only after a repeat testing over a period of 3 months. The inherited thrombophilia factors deficiencies in SVT patient's needs to be interpreted with a caution as many of these patients have deranged liver function. In such cases the multiple factors deficiencies

Table 3 Indian Studies on JAK2V617F mutation in SVT patients

References	Total no of cases (JAK2+; %)	Controls (JAK2+)	PVT/EHPVO (JAK2+; %)	BCS (JAK2+ ; %)	Others (JAK2+; %)
Shetty et al. [8]	215 (19; 8.8)	111 (0)	78(7; 8.9)	137 (12; 8.7)	
Amarapurkar et al. [9]	58 (16. 27.5)	23 (0)	35 (8; 22.8)	20 (5; 25)	3 (3; 100)
Sazawal et al. [10]	52 (10; 19)	20 (0)	32 (6; 18.7)	20 (4; 20)	
Sawant et al. [11]	30 (4; 13)	–	23 (3;13)	7 (1;14)	
Mutreja et al. [12]	52 (7;13.5)	90 (0)	24 (4; 16)	27 (3; 11)	
Rai et al. [13]	90 (1; 1.1)	–	50(1; 2)	40 (0)	
Shukla et al. [14]	202 (32; 15.8)	–		202 (32; 15.8)	
Present study	52 (2; 3.8)	40 (0)	40 (1;2.5)	10 (1; 10)	2 (0)

Table 4 Indian studies with thrombophilia workup in SVT patients

References	Total patients (Abnormality; %)	FVL (%)	PS (%)	PC (%)	AT (%)	APA (%)
Mohanty et al. [20]	88 (26;29.5)	14 (15.9)	3 (3.4)	7(7.9)	2 (2.2)	–
Bhattacharyya et al. [21]	105 (23;21.9)	6 (5.7)	6 (5.7)	11 (10.5)	0	–
Ahluwalia et al. [22]	142 (47; 33)	2 (1.4)	19 (13.4)	7 (4.9)	3 (2.1)	16 (11.3)
Sawant et al. [11]	30 (6;20)	0	5 (16.6)	0	0	1 (3.4)
Mutreja et al. [12]	69 (42; 60)	2/46 (4.3)	5 (7.2)	19 (27.5)	7 (10.1)	9/47 (19.1)
Shukla et al. [14]	202 (93; 46)	27 (13.4)	10 (4.9)	9 (4.5)	10 (4.9)	37 (18.3)
Present study	52 (9; 17)	2 (3.8)	1 (1.9)	3 (5.8)	0	3 (5.8)

should be considered as acquired. In our study we have encountered three cases with multiple factors deficiencies. In addition, A repeat testing should be performed for confirmation of abnormal results before categorizing that case as abnormal. In the present study, we had 8 (5 inherited and 3 acquired) such cases. In these cases, the repeat testing was not performed and hence were excluded from final data interpretation.

The limitation of the study is the sample size. We were not able to perform JAK2 V617F mutation in further cases due to financial limitation and lack of manpower in the study.

In conclusion, JAK2 V617F mutation was seen in 3.8% SVT cases and not in controls. The routine thrombophilia markers (both inherited and acquired) are much more frequent (17%) and patients should be routinely screened for these markers. The screening for JAK2 V617F mutation should be performed in cases with elevated CBC values.

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Compliance with Ethical Standards

Conflict of interest There is no conflict of interest amongst any of the authors.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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