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Original Article

Pathology Section

Haemostatic Profile in Patients of Myeloproliferative Neoplasms-A Tertiary Care Centre Experience

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ABSTRACT

Introduction: Patients of MPN commonly present with abnormalities in laboratory coagulation tests that are consistent with hypercoagulable state. Some individuals with MPN exhibit a pattern of exclusive bleeding or thrombotic events; many others have both bleeding and thrombosis during the course of the disease.

Aim: This study was undertaken to assess the haemostatic defects and platelet functions in patients of MPN.

Materials and Methods: One year prospective study was conducted at a tertiary care centre in North India in Department of Pathology in collaboration with Department of Clinical Haematology. All recently diagnosed cases of MPN along with 30 age and sex matched controls were included. Patients on antiplatelet drugs, antimyeloproliferative treatment, vitamin K agonists or antagonists, OCPs, Platelet count <1,00,000/µl, high grade fever, liver disease, pregnancy were excluded from this study.

All the patients underwent screening investigations like CBC, peripheral smear evaluation, BT, PT, aPTT, Protein C and S measurement (clot based assay) and aggregation studies with ADP (5µM) (Optical Aggregometry with AGGRO/LINK 8 software and CHRONOLOG 700 aggregometer).

Results: In present study, 50 cases were included. There was an occult prothrombotic state, suggested by significantly ($p<0.001$) reduced levels of Protein C and Protein S, but no patient presented with frank thrombosis while 8 out of 50 patients had haemorrhagic manifestations ranging from subdural haematoma to pin point petechial haemorrhages. Patients of CML-CP, ET, PV, PMF, MPN-NOS showed significantly reduced maximal aggregation with ADP (5µM) when compared to control ($p<0.001$). MPV also showed a statistically significant increase in these patients.

Conclusion: Thrombohaemorrhagic complications significantly affect the morbidity and mortality of MPN patients. This can be assessed by the use of platelet aggregation studies, Protein C and S activities and other coagulation studies. Timely diagnosis of these prothrombotic/haemorrhagic states can decrease the morbidity in these patients.

Keywords: Haemorrhage, Platelet dysfunction, Thrombosis

INTRODUCTION

Life expectancy of patients with MPN is strongly compromised by the haemostatic complications, like vascular thrombosis and haemorrhages [1]. These patients present with arterial or venous thrombosis. Arterial thrombosis accounts for 60%-70% of events related to myeloproliferative neoplasms and it includes ischemic stroke, acute myocardial infarction, and peripheral arterial occlusion [2].

These patients can also present with deep venous thrombosis of the lower extremities, pulmonary embolism, intra-abdominal (hepatic, portal and mesenteric) and cerebral vein thrombosis. In PV patients, venous thrombosis is relatively common and constitutes approximately one-third of total events. Many a times splanchnic and cerebral vein thrombosis is the presenting feature in patients of MPN. Also, the prevalence of splanchnic and cerebral vein thrombosis is higher in these patients [2]. Thrombotic complications not only involve large blood vessels but also involve microvasculature causing digital ischemia, pregangrenous changes, erythromelalgia, dizziness, confusion, headache, seizures and visual disturbances [3].

Patients of MPN are in a hypercoagulable state even in the absence of frank thrombotic manifestations. This is proven by the increased levels of plasma biomarkers of haemostatic system activation [4-6].

This study was undertaken to assess the haemostatic defects and platelet functions in patients of MPN.

MATERIALS AND METHODS

This was a prospective study of one year duration from (August 2014 to July 2015) conducted in a tertiary care centre of North India. Fifty patients of MPN diagnosed after peripheral smear and bone marrow evaluation were included in this study. Patients taking vitamin K, antiplatelet/antithrombotic drugs, antimyeloproliferative drugs, patients with liver disease, high fever, patients on OCPs and pregnant patients were excluded from this study. Apart from this, 30 age and sex matched normal healthy individuals who were not receiving drugs interfering with platelet functions or coagulation were selected as controls.

All the patients underwent screening investigations like CBC, peripheral smear evaluation, BT, PT, aPTT, Protein C and S measurement (clot based assay using reagents from Tulip Diagnostics, India) and aggregation studies with ADP (5µM) (Optical Aggregometry with AGGRO/LINK 8 software and CHRONOLOG 700 aggregometer).

Continuous data were summarized as Mean±SD (standard deviation) while discrete (categorical), in number and percentage (%). Continuous groups were compared by Student's t-test. Categorical groups were compared by Chi-square (χ^2) test. A two-tailed $p<0.05$ was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0). Ethical clearance was obtained for this study.

RESULTS

A total of 50 cases were included in present study, out of which 42 were of CML, 2 each of CNL, ET, PMF and one each of PV and MPN-NOS. [Table/Fig-1] is showing the age distribution of cases. In the present study, out of 50 patients, 16 (32.0%) were females and 34 (68%) were males. Both patients of ET and single patient of PV were males. Of the total, 69% patients of CML were males, while sex distribution in CNL and PMF was 1:1. Thirty age and sex matched control were included.

Out of 50 patients, only 8 patients had haemorrhagic manifestations. None of patients presented with clinical frank thrombosis.

Haemorrhagic manifestations noted were epistaxis, melena, menorrhagia, gum bleeding, haematoma and petechial rashes. One patient of CML presented with subdural haematoma and one ET patient presented with petechial rashes mostly on lower limbs and forearms.

Difference in values for all the coagulation variables including platelet aggregation, in controls and cases was found to be statistically significant. Values of Maximal Aggregation with ADP 5μM, Protein C and Protein S in cases were found to be lower than that of controls while values of BT, PT, aPTT, MPV and PDW was found to be higher in cases as compared to controls [Table/Fig-2].

Maximal platelet aggregation, Protein C and S were found to be significantly lower in our patients of MPN as compared to controls ($p<0.001$). Mean platelet volume (MPV) was found to be significantly higher in our MPN cases as compare to controls ($p<0.001$).

Values of PDW showed that patients of myeloproliferative disorders have higher variation in size of their platelets. This variation was found to be more in ET patients; such variations represented as high values of standard deviation in our patients of MPN. Further differences in mean PDW of cases and controls were highly significant with p-value <0.01.

Age Group (years)	CML-(CP+BP)	CNL	ET	MPN-NOS	PMF	PV
Upto 10	2	-	-	-	-	-
11-20	1	-	-	-	-	-
21-30	6	-	-	-	-	-
31-40	20	-	1	1	-	-
41-50	10	1	1	-	1	-
>50	3	1	-	-	1	1
Total	42	2	2	1	2	1

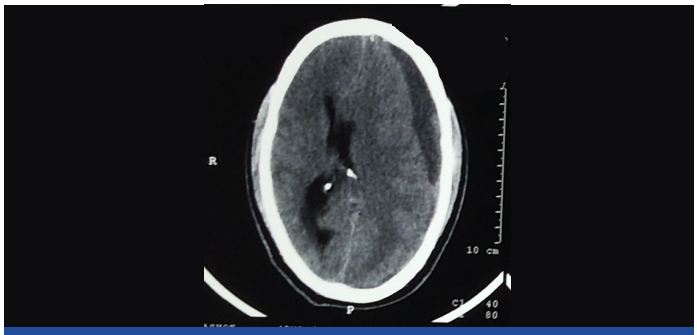
[Table/Fig-1]: Number of patents in each age group.

Variables	Control (n=30)	Cases (n=50)	Statistical Significance (Student t-test)	
	Mean±SD.	Mean±SD	't'	'p'
Bleeding time (BT) (in minutes)	2.73±0.64	4.74±2.90	-3.724	<0.001
Activated partial thromboplastin time (aPTT) (in seconds)	28.30±5.59	32.00±5.23	-2.984	0.004
Prothrombin time (PT) (in seconds)	12.50±1.36	13.41±1.67	-2.522	0.014
Maximal aggregation (%)	78.97±5.31	52.57±23.40	6.072	<0.001
Protein C (% activity)	96.03±10.20	60.67±22.88	7.987	<0.001
Protein S (% activity)	104.2±12.72	58.40±25.88	9.044	<0.001
Mean platelet volume MPV(fl)	8.73±1.10	10.45±1.67	-5.008	<0.001
Platelet distribution width PDW (%)	17.23±2.40	29.48±20.31	-3.282	0.002

[Table/Fig-2]: Comparison of various coagulation parameters between controls (n=30) and cases (n=50).

Variables	Non-Haemorrhagic (n=42)		Haemorrhagic (n=8)		Statistical Significance	
	Mean ± SD		Mean ± SD		t	'p'
Bleeding time (minute)	3.71	1.73	10.12	1.42	9.8420	<0.0001
aPTT (second)	31.57	5.06	34.25	5.90	1.3384	0.1871
PT (second)	13.39	1.69	13.50	1.69	0.1687	0.8667
Platelet count (cumm)	290381	142181	370000	253068	1.2653	0.2119
MPV (fl)	10.49	1.76	10.23	1.07	0.4019	0.6896
PDW (%)	27.93	18.25	37.60	29.09	1.2412	0.2206
Maximal aggregation (ADP 5μM) (%)	52.21	23.99	54.45	21.39	0.2458	0.8069
Protein C (% activity)	61.70	23.24	55.25	21.43	0.7274	0.4705
Protein S (% activity)	60.06	25.63	49.66	27.15	1.0426	0.3023

[Table/Fig-3]: Comparison between non-haemorrhagic and haemorrhagic cases.



[Table/Fig-4]: CT scan - chronic subdural hemorrhage in fronto-parietal region.

MPN-subtype	Number of cases	Maximal percentage aggregation (%)			
		Mean	Std. dev. (± SD)	Minimum	Maximum
CML-BP	1	79.00	-	79	79
CML-CP	41	55.02	22.81	12	105
CNL	2	67.00	2.83	65	69
ET	2	26.30	13.43	17	36
PV	1	44.00	-	44	44
PMF	2	20.00	4.24	17	23
MPN-NOS	1	23.00	-	23	23

[Table/Fig-5]: Subtypes of MPN and maximal platelet aggregation (ADP 5μM).

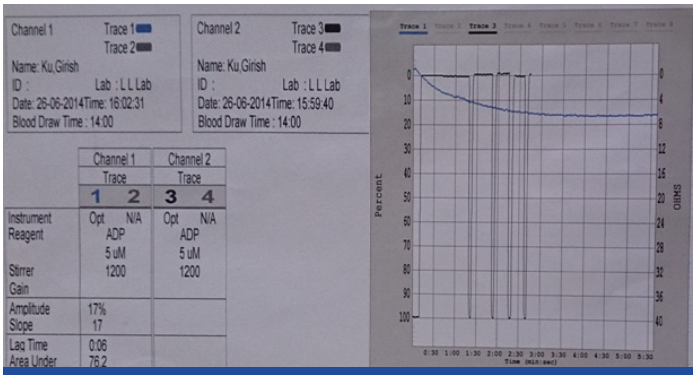
Out of 50 cases, only 8 patients had haemorrhagic manifestations. Difference between BT of haemorrhagic and non-haemorrhagic cases was statistically significant ($p<0.0001$) [Table/Fig-3]. Haemorrhagic manifestations seen in seven patients of CML included episodes of epistaxis, melena, menorrhagia, gum bleeding, haematoma and petechial rashes. One patient of CML presented with chronic subdural haemorrhage [Table/Fig-4].

Other variables showed only small differences between haemorrhagic and non-haemorrhagic MPN patients. Patients of CML-CP, ET, PV, PMF, MPN-NOS had reduced maximal aggregation with ADP (5μM) [Table/Fig-5,6].

DISCUSSION

Thrombohaemorrhagic complications in MPN are common morbid conditions. In present study there was an occult prothrombotic state, but no patient presented with frank thrombosis while eight out of fifty patients had haemorrhagic manifestations ranging from subdural haematoma to pin point petechial haemorrhages.

In present study, the only patient of PV, presented with symptoms suggestive of microcirculatory disturbances (headache, dizziness, pruritus) but no frank thrombosis was seen.



[Table/Fig-6]: Reduced platelet aggregation with ADP 5 µM.

One of ET patients presented with petechial rashes on the lower limbs; suggestive of qualitative platelet defect or defective vascular endothelial cells. Patients of PMF, CNL and MPN–NOS didn't show any thrombotic or haemorrhagic manifestations.

Another inference which can be made is that patients with normal or even elevated platelet count can present with raised BT and haemorrhagic manifestations; this finding gives us an insight of defective platelet functions in MPN as stated by previous workers [7-14]. Pathogenesis behind such findings includes increased expression of P-selectin and tissue factors on platelets in MPN patients which may lead to exhaustion of functional ability of platelets to aggregate. Another paradox is that very high number of platelets as seen in ET patients (8,73,000 and 5,60,000/mm^3) may consume all available large circulatory vWF multimers and results in increased bleeding time and haemorrhagic manifestations [15].

In present study, there were significantly reduced levels of maximal platelet aggregation with ADP (5µM) (52.57±23.40%) as compared to healthy controls (78.97±5.31%) with p<0.001 [Table/Fig-2]. Such results have been found in many studies as mentioned in [Table/Fig-7] [16-20]. All of these studies emphasize on the loss of secondary wave of aggregation and reduced maximal platelet aggregation.

These abnormalities can be justified by the defective arachidonic acid metabolism, reduced activation of platelets by agonists, acquired storage pool disease, decreased number of α2-adrenergic receptors, altered expression of specific platelet membrane Glycoproteins (GP) such as GPIIb/IIIa, GPIb/IX or increased number of GPIV molecules and increased expression of receptors for the Fc component of IgG, acquired von Willebrand's disease, a reduction of platelet procoagulant activity and an acquired form of Bernard-Soulier syndrome as reported in these disorders by other researchers [7-23].

In present study, activity of Protein C was found to be reduced very significantly [Tables/Fig-2,3] in MPN cases (60.67±22.88%) as compared to the mean activity in controls (96.03±10.20%) with p<0.001. Similar findings are seen in studies done by other workers and they found an acquired activated Protein C resistance in MPN cases [Table/Fig-8] [24-26].

Low levels of Protein C indicate the presence of "Prothrombotic State" in MPN patients even in the absence of frank thrombosis.

Protein S activity was also found to be reduced in MPN cases [Table/Fig-2] (58.40±25.88%) in the present study as compared to activity of Protein S in healthy controls (104.20±12.72%).

Such kind of differences have also been seen by other researchers. These results can be justified by the researchers which indicate that proteases from platelets and leukocytes are able to cleave Protein S in plasma [27]. Separate studies done by Marchetti M et al., and Falanga A et al., showed that increased neutrophil elastase levels in MPN patients degrade Protein S. Studies also showed that factor V also undergo similar degradation by elastases [28-30].

Falang A et al., suggested that increased blood coagulation in MPN patients is multifactorial and it involves abnormalities in platelets, erythrocytes, leucocytes and endothelial cells. Sekhar M et al., found splenic vein thrombosis to be one of the most common presentations of MPN. However, in our studies no such cases were found. Martinelli I et al., found that MPN patients are at increased risk of cerebral vein thrombosis [31-33].

MPV of MPN cases (10.45±1.67fl) was found to be statistically higher in comparison with that of controls (8.73±1.10) having p <0.001 [Table/Fig-2]. These higher values of MPV are suggestive of presence of large sized platelets in MPN patients.

Author	Platelet aggregation (abnormal cases / total cases)						Patient Population
	ADP		Collagen		Epinephrine		
Cesar JM (2005) [16]	6/55	11%	21/55	38%	32/55	58%	ET only
Avram S (2001) [17]	19/76	25%	-	-	-	-	All MPN
Waddell CC (1981) [18]	9/18	50%	7/18	38.9%	11/18	61.1%	All MPN
Pareti FI (1982) [19]	19/52	36.5%	9/52	17.3%	15/52	28.8%	All MPN
Russell NH (1981) [20]	9/17	52.9%	10/17	58.8%	-	-	All MPN
Present study	40/50	80%	-	-	-	-	All MPN

[Table/Fig-7]: Comparison of platelet aggregation (%) in different studies and in present study [16-20].

Author	Number of patients with deficiencies of PC and PS	Percentage
Bucalossi A (1996) [24]	22/81	27.16%
Amitrano L (2003) [25]		
Protein C	7/61	11.4%
Protein S	7/79	8.8%
Hoekstra J (2011) [26]	4/14	28.6%
Present Study		
Protein C	34/50	68%
Protein S	30/50	60%

[Table/Fig-8]: Comparison of Protein C and S activity in different studies [24-26].

PDW of MPN cases (29.48±20.31%) was compared with that of controls (17.23±2.40%) and was found to be significantly higher in MPN cases (p<0.01) [Table/Fig-2].

Higher value of PDW in MPN cases is an indicator of presence of variable sized platelets in MPN patients as seen in various studies [34,35].

In present study, MPV was correlated with maximal platelet aggregation (categorized as <70% and ≥70%); a statistically significant correlation was found (p<0.05) as patients with higher MPV had reduced maximal platelet aggregation.

LIMITATION

This study was of short duration and effect of MPN treatment on coagulation profile was not studied.

CONCLUSION

Thrombohaemorrhagic complications significantly affect the morbidity and mortality of MPN patients. These are multifactorial events that include the key role of platelets with their in vivo activation and role of vascular endothelial cells, leukocytes, and reduced activity of Protein C and S. Also, these patients are in a continuous hypercoagulable state even in the absence of frank thrombosis. Hence, patients of MPN, should be monitored and counselled appropriately keeping these complications in view.

ABBREVIATIONS

MPN: Myeloproliferative Neoplasm

BT: Bleeding Time

PT: Prothrombin Time

aPTT: Activated Partial Thromboplastin Time

ADP: Adenosine Diphosphate

MPV: Mean Platelet Value

CML-CP: Chronic Myeloid Leukamia in Chronic Phase

ET: Essential Thrombocythemia

PV: Polycythemia Vera

PMF: Primary Myelofibrosis

MPN-NOS: Myeloproliferative Neoplasm-Not Otherwise Specified

OCPs: Oral Contraceptive Pills

MPV: Mean Platelet Volume

CBS: Complete Blood Count

CNL: Chronic Neutrophilic Leukamia

PDW: Platelet Distribution Width

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