Clinical Spectrum of Primary Polycythaemia and Its Complications - Experience from a Single Center in Karnataka

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ABSTRACT

BACKGROUND
Awareness and diagnosis of Philadelphia Negative Chronic Myeloproliferative Disorders has now improved and there is a need for more epidemiological data from India.

METHODS
This is a retrospective study of patients of polycythaemia conducted at clinical haematology services, BMCRI, Bengaluru from 2010 to 2017.

RESULTS
88 patients of polycythaemia were retrospectively studied. 84.1% were male and 15.9% were female. Their ages ranged from 19 to 79 years. 75 (85.23%) had Polycythaemia Rubra Vera (PRV). JAK-2 (V617F) mutation was positive in 33.33%. The commonest presentation was with unexplained erythrocytosis in 50 (66.66%), thrombosis in 20 (26.66%) and with bleeding in 2 (2.66%). 22 thrombotic events occurred in 20 PRV patients. Cortical sinus thrombosis was seen in 27.3%, cerebrovascular accidents in 22.8%, portal vein thrombosis in 13.6%, pulmonary embolism in 9.1%, central retinal artery occlusion in 13.6%, myocardial infarction in 4.5% and digital infarction in 9.1% patients. 3 cases of PRV presented with diplopia. No other definitive cause for ocular palsy could be found. The JAK 2 positive group was slightly older than the negative group and had higher frequency of splenomegaly ($p<0.05$) and higher values for haemoglobin ($p<0.001$) and neutrophil counts ($p<0.001$) and platelet counts ($p<0.05$).

CONCLUSIONS
Patients with thrombosis, erythrocytosis, thrombocytosis and haemorrhage should be suspected to have myeloproliferative disorders like PRV and investigated. Ophthalmoplegia is a rare presentation and should raise the suspicion for polycythaemia. There is a higher probability of splenomegaly and higher values for haemoglobin and neutrophil counts and platelet counts in JAK 2 positive group.

KEY WORDS
Polycythaemia Rubra Vera (PRV), Philadelphia Negative Chronic Myeloproliferative Disorders, Ph-Negative CMPD / MPN, JAK-2 Mutation, Ophthalmoplegia, Thrombosis

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BACKGROUND

Abnormal proliferation of all hematopoietic bone marrow elements and an absolute increase in red cell mass is characteristic of Polycythaemia Vera[1-4]. It should be suspected when men present with a haemoglobin (Hb) greater than 18.5 g/dL and in women with a Hb greater than 16.5 g/dL.

JAK2 mutation is now a major diagnostic and clonal marker for Ph-negative CMPD in the revised WHO classification and important in designing personalized treatments in coming times,[1,2] The abnormal proliferation in PV is due to constitutive activation of the JAK-STAT pathway and majority have the V617F mutation[5,6,7] Discovery of non-receptor tyrosine kinase JAK2 mutations (exon 14, JAK2 V617F and exon 12) in 2005,[5-15] has furthered our understanding of the molecular mechanisms of Ph-ve CMPD such as PV, essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients. Incidence of JAK2 V617F mutation reported has varied from 65-97% for PV, 23- 57% for ET and 35-57% for idiopathic myelofibrosis (IMF).[5-15]

JAK2 V617F mutation is a G-C to T-A transversion, leading to valine to phenylalanine substitution at codon 617. This is a gain of function mutation causing the release of auto-inhibitory action of JH2 and recruits STAT (Signal Transducer and Activator of Transcription) in the complete absence or in presence of only trace quantities of hematopoietic growth factors. This in turn leads to activation of a number of downstream pathways like JAK/STAT, PI3K/AKT and MAPK/ ERK.[6-15]

Secondary causes of polycythaemia must be ruled out. EPO overproduction occurs in hypoxia, tumours (e.g., kidney, brain, hepatoma, uterine fibroid, and pheochromocytoma), renal artery stenosis, and renal cysts. Other causes include androgen therapy, congenital erythrocytosis, EPO receptor hypersensitivity, auto-transfusion (blood doping), and self-injection of EPO. Familial PV has been associated with mutations of the EPO receptor, high oxygen affinity haemoglobins and 2, 3- BPG deficiency.

Ph-negative CMPDs are being more often diagnosed, probably because more asymptomatic patients are diagnosed as a result of an increase in knowledge and more vigilant screening. There are only a few studies on the epidemiology of polycythaemia from India; this study provides insight on the presentations of polycythaemia patients in this part of the country and the incidence of JAK 2 (V617F) mutation in our PRV patients.

METHODS

This was a retrospective study of patients of polycythaemia referred to Clinical Haematology OPD from February 2010 to September 2017 at Victoria Hospital, BMCRI, Bangalore. The study was approved by Institutional Ethics Committee and informed consent was obtained.

Complete blood count with peripheral smear, liver function test, renal function test, arterial blood gas analysis, chest X-ray, abdominal ultrasound, and serum erythropoietin (EPO) levels were done in all patients for diagnosis. Bone marrow study and JAK 2 (V617F) mutation analysis, by PCR and gel electrophoresis in the peripheral blood leucocytes, was done at diagnosis in all PRV patients. Relevant tests such as Renal Doppler, abdominal computed tomography (CT) scan, intravenous pyelogram, pulmonary function tests, endocrine evaluation to look for secondary causes of polycythaemia were done as required.

For diagnosis of thrombosis, ultrasound with Doppler and/or MRI was done. CT scans and MRI (Magnetic Resonance Imaging) venography of the brain were done in all 3 patients of PRV with ophthalmoplegia. Extensive investigations were done in all patients with thrombotic events and ophthalmoplegia to exclude other identifiable explanations like trauma, infection, screening for hypertension, inflammatory causes, autoimmune risks with autoantibody profile and antiphospholipid antibodies, serum homocysteine levels, lipid profile and fasting and post prandial blood sugar levels. Those with concomitant risk factors were excluded from this data.

Polycythaemia vera was diagnosed as per revised WHO Criteria 2016[16]

Major Criteria

1. Hb > 16.5 gm/dL or HCT > 49% (in males) & Hb > 16 gm/dL or HCT > 48 % (females).
2. Hyper cellular Bone marrow biopsy (for age) with trilinear proliferation.
3. Positive report for JAK2V617F or JAK 2 exon 12 mutation.

Minor Criterion

1. Subnormal Erythropoietin (EPO) levels.

Diagnosis is confirmed if all the 3 major criteria are met or 2 major and minor criteria is positive. Bone marrow biopsy is not required in patients with sustained absolute erythrocytosis Hb> 18.5 gm/dL or HCT> 55.5% (males), Hb> 16.5 gm/dL or HCT> 49.5 % (females), if maturation criteria is present) Secondary polycythaemia was diagnosed as Hb > 16.5 gm/dL or HCT > 49% (m) and Hb > 16 gm/dL or HCT > 48 % (f) with presence of secondary underlying causes and not fitting into the diagnostic criteria for Vera.

Statistical Analysis

Statistical analysis was done using SPSS software. Quantitative data was expressed as mean ± standard deviation (SD) and compared using student t test. P value < 0.05 was considered significant. Categorical data was expressed as percentages and compared using chi square test. MS word and excel were used to generate tables and graphs.

RESULTS

88 patients of polycythaemia were retrospectively studied, 74 (84.1%) of them were male and 14 (15.9%) were female. Male to female ratio was 4:1. Age of patients ranged from 19 to 79 years. Mean age was 45.16 ± 14.22 years. 75 (85.23%) of the patients were found to have PRV. Among them 60 (80%) were male and 15 (20%) female. Serum EPO levels were low in 35 (46.66%) patients of PRV. JAK-2 (V617F) mutation was positive in 25 (33.33%). The mean age of patients who were

positive for JAK-2 mutation was 47.76 ± 12.64 years. Secondary polycythaemia was diagnosed in 13 (14.77%), among whom 12 were male and 1 female.

Our Study	JAK-2 Positive	JAK-2 Negative	P Value
Hb (gm/dL)	20.79 ± 1	19.64 ± 1	<0.001
WBC (Thousand) per mm³)	11.79 ± 3.27	7.28 ± 1.8	<0.001
Platelet count (lakhs/mm³)	4.96 ± 2	3.80 ± 0.94	<0.05
Splenomegaly	15/25 (60%)	15/50 (30%)	<0.05

Table 1. Clinico-Haematological Parameters of JAK-2 Positive and Negative Patient Groups

	Our Study	Cecil Ross et al	Sudha Sazawal et al
No. of cases	75	Entire MPD-39 PRV-14+8	34
Age	45.16 ± 14.22	55.5 ± 14.5	52 ± 14.3
Sex M/F	60/15	27/12	26/8
Thrombosis	22 events	14	1
Haemorrhage	2	-	3
Hb (gm/dL)	19.9 ± 1.06	18 ± 1.8	17.3 ± 2.92
WBC (Thousand per mm³)	8608.10 ± 3136.38	9.6 ± 2.6	29.9
Platelet count (lakhs/mm³)	4.14 ± 1.45	4.7 ± 2.2	3.68

Table 2. Comparison with Literature

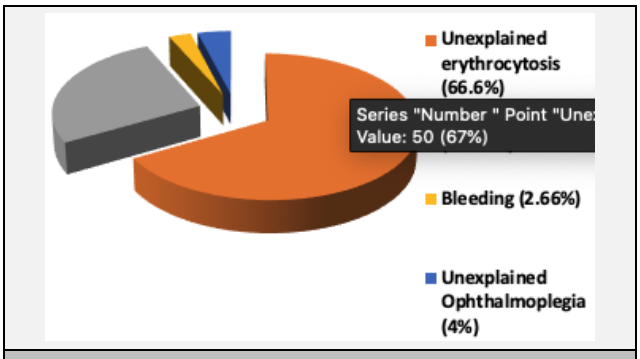

Figure 1. Modes of Presentation in Polycythaemia Vera

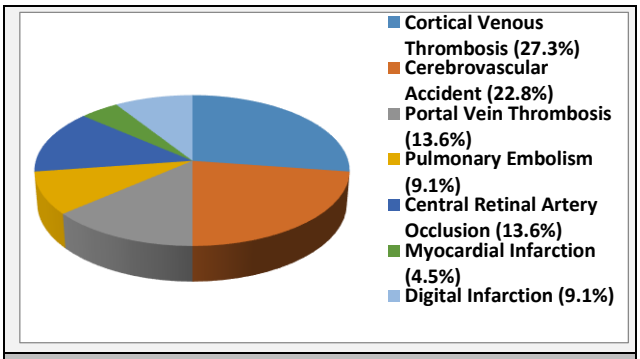

Figure 2. Types of Thrombotic Events

72(96%) of the PRV patients had conjunctival plethora and ruddy cyanosis. Splenomegaly was seen in 30(40%) of whom 60% of them were JAK-2 mutation positive. 15/25 (60%) patients were JAK-2 mutation positive and 15/50 (30%) patients were JAK-2 mutation negative.

The commonest presentation among patients of PRV was with unexplained erythrocytosis in 50 (66.66%) and thrombosis was seen in 20 (26.66%) and bleeding in 2 (2.66%). Both the patients with bleeding had thrombocytosis (platelet counts were 10 L/mm³ and 8.6 L/mm³ respectively), were females and JAK-2 mutation positive. (Figure 1)

Twenty-two (22) thrombotic events occurred in the 20 PRV patients. Cortical sinus thrombosis was seen in 6 (27.3%), cerebrovascular accidents in 5 (22.8%), portal vein thrombosis in 3 (13.6%), pulmonary embolism in 2 (9.1%), central retinal artery occlusion in 3 (13.6%), myocardial infarction in 1 (4.5%) and digital infarction in 2 (9.1%) patients. (Figure 2)

Three (4%) cases of PRV presented with diplopia. On examination two patients had restriction of lateral gaze, while one had restriction of down gaze on adduction, pointing towards 6th nerve and 4th nerve palsies respectively. Hb levels were > 18 in all three cases and PCV> 45. CT and MRI venography of the brain revealed no abnormalities. LP-CSF was normal. ANA and ANCA were negative. Serum erythropoietin was reduced in one, normal in the other two. JAK2 mutation was positive in two patients. Platelet count was elevated in all 3. Definitive cause of ocular palsies could not be found out in all our 3 cases. Patients were started on, aspirin, hydroxyurea, short term steroids and underwent phlebotomies to which there was complete response.

The mean Hb was 19.9 ± 1.06 gm/dL and the Mean TLC was 8608.10 ± 3136.38/mm³ and the mean platelet count was 4.14 ± 1.45 lakh/mm³. The mean Hb of the JAK-2 mutation positive PRV patients was 20.79 ± 1 gm/dL. Their Mean TLC of was 11,794.11 ± 3271.89/mm³ and the mean platelet count was 4.96 ± 2 lakh/mm³. The mean Hb of the JAK-2 mutation negative PRV group was 19.64 ± 1 gm/dL. Their Mean WBC count was 7287 ± 1876.9/mm³ and the mean platelet count was 3.8 ± 0.9 lakh/mm³. Follow up during 4.8 yrs. showed that 2 patients had transformed to myelofibrosis.

DISCUSSION

PRV is a clonal disorder of the pluripotent stem cell which can differentiate into red blood cells, granulocytes, and platelets. There is erythropoietin (Epo) -independent in vitro erythroid colony formation. Homozygous JAK2 mutation causing pronounced kinase activity leads to trilinear megakaryocyte, erythroid, and granulocytic myeloproliferation, myeloid metaplasia, and is associated with 'Classical PV' complicated by thrombotic events and platelet-mediated microvascular thrombotic syndrome of thrombocythemia.[17-24] The hypersensitive platelets produced by spontaneously proliferating enlarged megakaryocytes in the bone marrow of ET and PV patients spontaneously activate and secrete their products, forming aggregates that transiently plug the microcirculation, or result in occlusive platelet thrombi in arterioles or small arteries leading to transient ischemic attacks and thrombotic complications respectively.[19-24] Acquired Von Willebrand disease associated with extreme thrombocytosis is the major cause for bleeding.

Serum EPO levels should be low to normal in patients with PRV but high in patients with secondary polycythaemia, although there may be some overlap. Presence of JAK2 V617F mutation along with a low EPO level favours the diagnosis of PRV. When JAK2V617F mutation test is negative but the EPO level is low, testing for other mutations of JAK2 identifies a minority of patients with PRV. All the other patients having a normal or elevated EPO level should be evaluated for secondary polycythaemia. Arterial oxygen saturations of less than 92% points towards secondary polycythaemia.

Males were more affected than females. The mean age of our patients was lower than other studies[25,26] (Table 2). 26.66% of the patients presented with thrombosis and investigations revealed polycythaemia. Hence basic investigation for an underlying aetiology should be done in all patients with thrombotic events, irrespective of their age and other risk factors like hypertension, which can also occur due to erythrocytosis. There was a higher probability of splenomegaly ($p<0.05$) and higher values for haemoglobin ($p<0.001$) and neutrophil counts ($p<0.001$) and platelet counts ($p<0.05$) in JAK 2 positive group similar to other studies[25,26]. (Table 1)

Treatment for PV should be risk-adapted[3,4]. Patients are stratified into low-risk and high-risk categories based on age (if more than 60 years) and previous history of thrombosis. Patients who are younger than 60 years with no history of thrombosis with platelet count below 1,500 x 10^9/L, and the absence of cardiovascular risk factors like smoking, hypertension, congestive heart failure are considered low risk. They are treated with phlebotomy alone. High-risk patients require phlebotomy plus cytoreductive therapy or interferon. Hydroxyurea is used as first-line therapy. Aspirin as well as cytoreductive therapy is needed to control thrombocytosis, leucocytosis and extra medullary haematopoiesis. Interferon is safe and recommended in women of childbearing age and in patients who cannot tolerate hydroxyurea.

The goal of therapy is a haematocrit of 45% for men and 42% for women and is associated with a four-fold decrease of major cardiovascular events and prevention of thrombotic complications but does not prevent the microvascular circulation disturbances and occlusive thrombotic complications because thrombocythaemia persists. Aspirin induces irreversible inhibition of platelet cyclo-oxygenase (COX-1) activity and aggregation, thereby relieving the peripheral, cerebral and ocular ischemic disturbances [19,20,21,22].

JAK2 inhibitors are being evaluated in clinical trials for PV; however, the initial enthusiasm hoping these drugs could selectively target mutant cells and cause a molecular remission similar to Imatinib in CML (Chronic Myeloid Leukemia) has proved unreal.

CONCLUSIONS

Patients with thrombosis, erythrocytosis, thrombocytosis and haemorrhage should be suspected to have myeloproliferative disorders like PRV and investigated. Ophthalmoplegia is a rare presentation and should raise the suspicion for polycythaemia. There is a higher probability of splenomegaly and higher values for haemoglobin and neutrophil counts and platelet counts in JAK 2 positive group.

JAK2 mutation analysis should be a part of initial evaluation of patients suspected with BCR-ABL negative CMPD. Other known JAK2 mutations like exon 12-15, MPN and CALR also should be evaluated, which was not available to us at that point of time. JAK2 mutation analysis can be useful in situations like unusual and/or extensive thrombotic complications like cortical sinus thrombosis, abdominal thrombotic events, as a surrogate marker to diagnose CMPDs.

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