Original Article-I

Polycythemia Vera and Essential Thombocythemia – A Single Institution Experience

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ABSTRACT

Background: **In myeloproliferative disorders, occurrence of Janus kinase 2 [JAK2] mutation is a significant factor in pathogenesis in polycythemia rubra vera (PRV) and Essential thrombocythemia (ET). We studied the frequency of JAK2 mutation in patients with PRV and ET and to compare clinical and laboratory features of patients positive and negative for mutation.**

Patients and Methods: **Clinical and laboratory features of PRV and ET patients (WHO criteria) from 1997 to 2004 were included. After morphological diagnosis, presence or absence of JAK 2 mutation was done during follow up and follow up details were recorded.**

Results: **A cohort of 39 patients (ET-17, PRV -14, ET- PRV-8) were identified .The mean age of entire cohort was 55.5±14.5 years. Comparison of clinical and laboratory features showed JAK2 positive patients were older by a decade, had higher frequency of splenomegaly and higher values for hemoglobin (16.5±2.6 gm/dl) and neutrophil counts (14.5±4.8 thousand/µl).During the course of follow up (6 to 106 months; mean 25.5±28.6 months) frequency of thrombotic events in both groups (p value >0.05) was same, though time for occurrence for thrombotic event was shorter in JAK 2 positive patients, which is noteworthy.**

Conclusions: **The identification of JAK 2 mutation probably defines a sub entity in ET with aggressive behavior as evidenced by splenomegaly, higher total counts and transformation to PRV (n=6/8). The onset of JAK2 V617F mutation probably heralds progression to PRV.**

INTRODUCTION

Polycythaemia rubra vera (PRV) and essential thrombocythaemia (ET) are classified as Philadelphia -negative chronic myeloproliferative diseases (CMPD). In early 2005, a novel Janus kinase 2 (*JAK2*) mutation (*JAK2*V617F) was described in association with PRV, essential thrombocythemia (ET), and primary myelofibrosis (PMF)[1]. In reference to the Roman God Janus, who is depicted with 2 faces to signify beginning and ending, the coexistence of a catalytic and a negative auto regulatory domain in the same molecule led to the acronym JAK 2 (Janus Kinase 2) The JAK2 V617F point mutation makes the normal hematopoietic progenitor cells hypersensitive to thrombopoietin, erythropoietin, and myeloid progenitor cells, leading to trilinear hematopoietic myeloproliferation. Patients who have not been treated for the diseases are at great risk of morbidity and mortality as a result of thrombotic/ haemorrhagic events. However, if patients have been adequately treated, their prognosis is good and life-expectancy approaches normal. Most patients are diagnosed

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in the fifth and sixth decade of life, but the disorder is also seen in younger adults and, rarely, in children.

Over the last few decades the incidence of Ph-negative CMPDs has been increasing, probably more asymptomatic patients are diagnosed as a result of an increase in laboratory testing.[2] As there is paucity of literature in India, this article provides the frequency of JAK 2 mutation in patients with PRV and ET and also highlighting the comparison of clinical profile and laboratory features between patients who are positive against who are negative for mutations.

MATERIAL AND METHODS

The study included 39 patients with PRV / ET (ET- 17, PRV-14, and ET- PRV 8) enrolled from the Hematology clinic of the Department of Medicine, SJMCH Bangalore between 1995 and 2005. The diagnosis of PRV and ET was based on established criteria.[3,4] This included persistent thrombocytosis with a platelet count higher than 6,00,000/ml, hematocrit of > 40%, normal marrow iron store, and absent or minimal marrow fibrosis and exclusion of reactive causes such as MDS and chronic myeloid leukemia. A diagnosis of subsequent conversion to myelofibrosis was based on the presence of splenomegaly, peripheral smear showing tear drop cells and leukoerythroblastosis and marrow fibrosis. The diagnosis was confirmed by bone marrow aspirate and biopsies which were performed in all patients.

The values for Hb, WBC and platelet count were obtained by automated cell counter (sysmex corporation XT1800i). The marrow iron stores were assessed by Perl's stain and were graded from 1 to 5. JAK 2 was done by PCR method. The fibrosis was initially evaluated by Hemotoxylin and Eosin stain and ratified by reticulin stain. For diagnosis of thrombosis, ultrasound with Doppler and/ or MRI was done.

Selection of study group All patients who were on follow up for elevated Hb and platelets count, were studied for presence of JAK2 mutation. Retrospective analysis for JAK2 was done on all patients who were diagnosed prior to 2004 and were on follow up. The prospective analysis was done for patients who were diagnosed after 2004. This allowed a range of follow up from 6 months to 106 months.

DATA ANALYSIS

Data was entered in SPSS version 13. All relevant details including history, examination, investigations. were recorded. Comparison between categorical variables was performed by Chi square Test. Comparison between categorical variables and continuous variables were performed by Mann – Whitney or Kruskall wallis tests. Time to thrombosis was calculated by K-M taking interval from date of onset of symptoms to thrombotic episode and checked for significance by log rank test. P value < 0.05 was considered significant.

RESULTS

The study included 39 patients, the results of which are summarized in Table 1

The JAK 2 positive group was a decade older than the negative group. A slight female preponderance was seen in JAK 2 positive patients. 12 /21 patients presented with splenomegaly. Increased cellularity of the bone marrow was observed in all patients. Platelet counts were significantly higher in JAK 2 positive group.

Among the 14 patients who had thrombosis, only 5 had thrombosis at the time of presentation. Though the incidence of thrombosis occurring at the time of presentation did not differ between both JAK 2 positive and negative groups, it is note worthy that the time for occurrence for thrombotic event is shorter in JAK 2 positive patients

TABLE 1

	Entire cohort	JAK 2negative	JAK 2 positive	P value
Diagnosis	39	18	21	
ET	17	6	11	
PVR-ET	8	2	6	
PVR	14	10	4	
Demographics				
Age (years) (Mean)	55.5±14.5	45.4±15.2	58.4±12.5	0.014*
Sex				
Male	27	12	15	0.35
Female	12	5	7	
Clinical Features				
Palpable Spleen	16	4	12	0.009*
Asymptomatic	9	6	3	0.3
Thrombosis	14	8	6	0.5
Time for thrombosis during Follow up(Months)	51	60	39	0.04*
Lab Parameters				
Hemoglobin (gm/dl) ET -13.9±2.9,ET-PRV-17.8±1.9, PRV-18±1.8	14.5±2.4	15.3±4.5	16.5±2.6	0.08
Total WBC count (thousands) ET-12.7±6.1, ET-PRV-15.2±4.8, PRV-9.6±2.6)	12.1±5.2	9.3±4.2	14.5±4.8	0.05*
Platelet count (in lakhs) ET-7.2±2.8, ET-PRV-6.9±1.1, PRV-4.7±2.2	6.51	4.34	8.25	0.002*
Follow up in months (Mean) (Range = 6 -106)	25.5+28.6	12	40	0.016

ET- essential thrombocythemia PVR- polycythemia vera

A comparison of the laboratory features of JAK2 -positive and JAK2 negative ET patients showed that JAK2 positive group is

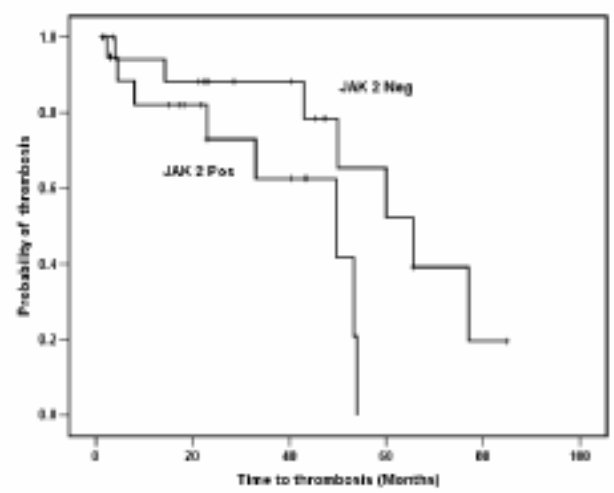

Figure 1-The figure compares the data for thrombosis between JAK2 positive and negative patients.

characterized by higher values for hemoglobin, and neutrophil counts Differences in JAK2-V617F expression, markedly lower in PVR than in ET probably reflecting different percentages of granulocytes carrying the mutation. Similar results were obtained for ET patients when the results were analyzed as two groups as ET and PVR. This probably indicates that JAK2 V617-positive ET patients, diagnosed according to the PVSG criteria, represent an early PRV mimicking ET suggesting that the JAK2 V617F mutation defines one disease entity with several sequential steps of ET – PV.

DISCUSSION

The identification of an acquired mutation of *JAK2* in patients with myeloproliferative disorders has raised questions about the relationship between mutation-positive and mutation-negative subtypes, timing of the *JAK2* mutation, and molecular mechanisms of disease progression. Review of literature on JAK2 V617F point mutation describes that the effect of mutation on the normal hematopoietic progenitor cells makes them hypersensitive to thrombopoietin, erythropoietin, and myeloid progenitor cells.[5] This will have three main clinical consequences during long-term follow-up. First, spontaneous growth of enlarged mature megakaryocytes in ET/PRV with overproduction of hypersensitive platelets results in a broad spectrum of platelet-mediated micro vascular circulatory disturbances, which are very sensitive to low-dose aspirin. Second, spontaneous growth of erythropoiesis with the overproduction of erythrocytes leads to classic PRV with increased hemoglobin, hematocrit, and red cell mass. This is associated with a high frequency of major arterial and venous thrombotic complications in addition to platelet-mediated micro vascular circulatory disturbances of thrombocythemia. Third, the slowly progressive myeloid (granulocytic) metaplasia in bone marrow and spleen is complicated by secondary myelofibrosis.

In the present study three issues are highlighted – (i) JAK 2 positive group being about a decade older than the negative group (ii). the laboratory profile being characterized by higher values for hemoglobin, TLC and platelet counts and (iii) eequal frequency of thrombotic events in both groups ($p > .05$). Several studies have reported on the occurrence of JAK2V617F in approximately 50% of patients with essential thrombocythemia and its presence has been associated with advanced age at diagnosis, higher hemoglobin and leukocyte levels, and increased rate of polycythemic transformation similar to the findings in the present study.[6,7,8] *JAK2* mutation positive ET patients differ from those who are negative as they have higher haemoglobin (Hb). This suggests that ET patients with *JAK2* mutation are closer to patients with PV. However, in the comparative studies between ET and PRV, the incidence of thrombosis has been reported more in PVR than ET.[6,7]. The identification of patients at risk for major thrombosis who need platelet-lowering therapy is important. Though the frequency of thrombosis is almost equal in both the groups, that the time for occurrence for thrombotic event is shorter in JAK 2 positive patients than negative patients.

The retrospective design of this study might be questioned concerning the time lapse occurring between the clinical diagnosis and the time when mutational analysis was performed, because of the possibility of a time-dependent change in mutational state. Our patients had the mutational analysis in a median of 497 days. However, a recent study[7] in ET patients found no evidence for significant modifications of JAK2 mutant clone burden over a median follow-up time of 47 months. None of 50 JAK2 negative ET patients, enrolled in the trial on re-evaluation after a median of 77 months, became mutation positive. Follow up of our patients is needed to determine if patients with JAK 2 negative essential thrombocythemia will progress to become JAK positive. There is no hard evidence for a time-dependent increase in clonal dominance, though there are some case reports.[8,9] Essential thrombocythemia may transform into other Philadelphia chromosome–negative myeloproliferative diseases, including PRV, myelofibrosis (MF), and acute myeloid leukemia (AML) or MDS.[10] Although conversion to PRV can occur, none of our patients developed PRV. However, because PRV may present with thrombocytosis and because hydroxyurea suppresses erythropoiesis, misdiagnosis of patients with PRV and prominent thrombocytosis as having ET may have occurred. At present there is no longitudinal study with a statistically consistent number of subjects and/or for a reasonable length of time to unequivocally support the thesis that JAK2 mutational status varies significantly with time.

In conclusion, the identification of JAK 2 mutation probably defines a sub entity in ET with aggressive biology as evidenced by splenomegaly, higher total counts and transformation to PRV (n=8). We are continuing this study to analyze more patients.

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