JAK2 Mutation and Its Assessment in Relation to Profile of Young Polycythemia Patients in India

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Abstract

Purpose The main aim of this study was to detect the presence of JAK2 mutation and its assessment in relation to the clinical, hematological, and mutational profile of young patients with established polycythemia.

Methods Cross-sectional observational study was undertaken over a period of 1 year in a tertiary care center. Sixty patients were included in our study between the age 18 and 50 years with confirmed diagnosis of polycythemia vera. Reports of all the investigations including bone marrow biopsy and JAK2 mutation testing were assessed.

Results Presence of JAK2 mutation V617F was found in 38% patients, while bone marrow panmyelosis was present in 67% patients. Higher mean hemoglobin levels were observed in JAK2 mutation-positive patients, compared with those who were negative. Thrombosis-related complications were observed in five patients, all of whom were JAK2 mutation positive, while out of the 18 patients requiring phlebotomy, 15 patients were JAK2 mutation positive.

Conclusion JAK2 mutation V617F in young Indian population is seen in significantly less cases as compared with Western data, while bone marrow panmyelosis is frequently observed and thus a significant finding for diagnosing polycythemia in our setting. Median hemoglobin was greater for JAK2 mutation-positive cases and so were the phlebotomy requirements and thrombotic events occurrences. Forty-two percent patients had history of some exposure to high-altitude areas adhering to the fact that high altitude is an established risk factor for developing polycythemia and the same is reinforced by our study.
Introduction

Polycythemia vera (PV) belongs to a group of relatively indolent myeloproliferative disorders, usually characterized by trilineage hematopoietic cell hyperplasia. It is prone to thrombotic and hemorrhagic complications and many other symptoms and possesses a risk of progression to myelofibrosis (MF) or transformation to acute myeloid leukemia over time.1,2 It tends to affect the elderly, although it can occur in any of the age groups. PV is distinguished from secondary polycythemias by the presence of a low serum erythropoietin level.3

The etiology of PV is not fully understood, although nonrandom chromosome abnormalities are often associated. An important diagnostic criterion is the presence of JAK2 mutation.4 JAKs are cytoplasmic tyrosine kinases mediating signal transmission from cytokine receptors to the cell nucleus. More than 95% patients with PV express a mutation of the JAK2 (Janus kinase 2) gene, located on the short arm of chromosome 9.1 This mutated gene likely plays a role in the onset of PV. However, its precise role as the cause of the disease is still under study. Up to 10% of patients meeting the clinical criteria for PV do not express JAK2 V617F.5 Recently, a few of these patients were found to have mutations in JAK2 exon 12. Studies show a higher than 50% JAK2 V617F allele burden in PV has been associated with increased risk of fibrotic transformation. It is known that some patients with other myeloproliferative disorders such as idiopathic MF and essential thrombocytthemia (ET) might also express the JAK2 mutation.6 The diagnostic criteria referred for establishing the diagnosis were the World Health Organization (WHO) diagnostic criteria 20167,8 for PV (Table 1).

Diagnosis requires meeting either all three major criteria or the first two major criteria and the minor criteria. Major criteria number 2 (bone marrow [BM] biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels more than 18.5 g/dL in men (hematocrit 55.5%) or more than 16.5 g/dL in women (hematocrit 49.5%) if mutation criteria 3 and the minor criteria are present. However, initial MF (present in up to 20% of patients) can only be detected by performing a BM biopsy; hypercellularity may predict a more rapid progression to overt MF (post-PV MF).7–9

One of the risk factors identified for polycythemia is smoking. In smokers, occupation by carbon monoxide of binding sites on hemoglobin reduces the oxygen content of the circulating blood at any given PO2. Carbon monoxide also increases the oxygen affinity of the remaining hem sites making them more reluctant to release oxygen to the tissues. This reduced oxygen delivery to the renal oxygen sensor, responsible for erythropoietin release, may lead to polycythemia.10,11

Patients with polycythemia especially PV tend to develop thrombotic events like cardiovascular or cerebrovascular accidents and arterial and venous thromboembolism.1,2 With careful medical supervision and therapy, PV does not usually interfere significantly with everyday activities and employment. Among the treatment options, phlebotomy provides the best overall survival but can cause an increased risk of thrombosis during the first 3 years. Treatment with anticoagulants as low-dose aspirin (81 mg) decreases the risk of various thrombotic events, but is indicated only if thrombosis has occurred.2 Hydroxyurea can also be used for maintenance therapy in patients who are at a high risk of thrombosis or in those having difficulty in tolerating therapeutic phlebotomy. Other therapeutic options include treatment with interferon-α, psoralens with ultraviolet light in the A range or with anagrelide, which is used for ET.2,12,13 Another newer agent is ruxolitinib is a potent JAK1/JAK2 inhibitor approved by U.S. Food and Drug Administration in patients who are intolerant or not responding to hydroxyurea.13,14

Limited data are available about Indian patients of PV especially the under 50 years age group. Their profile and mutational analysis are important as their demographic profile differs significantly from Western population. Our study is an effort in this direction. More importantly, the JAK2 mutation is an extensive area of research in this disorder and has therapeutic implications; therefore, knowing its prevalence is of paramount importance.

Materials and Methods

A cross-sectional observational study was undertaken over a period of 1 year from July 2016 to June 2017. It was conducted in the Department of Haematology & Stem Cell Transplant Centre, in a tertiary care hospital after taking clearance from the institutional ethics committee. Sample size was estimated based on all the eligible patients presenting to the outpatient department, fulfilling the inclusion criteria for the study and consenting for the study during the study period. Total 60 patients were included in our study.

<table>
<thead>
<tr>
<th>Major criteria</th>
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<tr>
<td>1. Hemoglobin &gt; 16.5 g/dL in men, &gt; 16.0 g/dL in women, or hematocrit &gt; 49% in men, &gt; 48% in women, or increased red cell mass &gt; 25% above mean normal predicted value</td>
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<td>2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size).</td>
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<td>3. Presence of JAK2 V617F or JAK2 exon 12 mutation</td>
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</table>

<table>
<thead>
<tr>
<th>Minor criteria</th>
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<tr>
<td>1. Subnormal serum erythropoietin level</td>
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Data collection was done by scrutinizing patient’s case sheets after taking written informed consent from the patients. Individual parameters including demographic details, diagnosis, associated comorbid conditions, treatment given, and laboratory investigations were recorded. Results of BM biopsy and JAK 2 mutation testing were also recorded. Patients included in the study were all patients aged between 18 and 50 years with proven polycythemia as per the WHO definition.6–8 Those with insufficient data in medical records and not willing to share their information were excluded from the study. Total 60 patients were included in the study and results are expressed as percentage mean and SD.

ETHICAL CONSIDERATIONS: The study was conducted after obtaining ethical clearance from institutional ethical committee. Written informed consent from the patients was taken before data collection was done.

Results

Total 60 patients were included in our study; all the included patients were male. Various comorbid conditions including asthma, Budd–Chiari syndrome, dyslipidemia, hypertension, and diabetes mellitus were also found associated in 13 (22%) patients.

Looking for association with high altitude we observed that 10 patients (17%) patients were living in high-altitude areas, while 15 patients (25%) had history of some exposure to high-altitude areas and it was observed that in 9 patients (15%) history of smoking was also present. Our study found presence of JAK 2 mutation in 23 patients (38%), while BM panmyelosis was observed to be present in 40 patients (67%) (►Fig. 1).

As per our study, hemoglobin level of the patients was observed as per ►Table 2. A correlation was observed between presence of JAK2 mutation and hemoglobin levels, as higher mean hemoglobin levels were observed in JAK 2 mutation-positive patients (18.45 ± 4.204 g/dL) compared with JAK 2 mutation-negative patients (17.30 ± 3.903 g/dL) (►Fig. 2).

In our study out of the total 60 patients, thrombosis-related complications were observed in 5 patients all of whom were JAK 2 positive (►Fig. 3); out of the 18 patients requiring phlebotomy only 3 were JAK 2 negative, while rest 15 were JAK 2 positive (►Fig. 4).

The whole process has been summarized in the Consort Chart 1.

Discussion

This study was done in the patients admitted to the hematology department. Total 60 patients were included in the study. It showed male preponderance with all 60 male patients; some studies like ours also indicate that polycythemia affects slightly more men than women as observed in a study by Williams et al.6 Age distribution showed that 31 patients belonged to the age group between 31 and 40 years, 13 patients in the age group between 18 and 30 years, and remaining 16 in the age group between 40 years and above. Some of the patients were also reported to have various associated comorbid conditions such as asthma, Budd-Chiari syndrome, dyslipidemia, hypertension, and diabetes mellitus, but were found to have no clear association with the condition under study.

An important finding in our study was that out of 60 patients, 25 patients (42%) had at least some exposure to high-altitude areas or were living in such areas indicating a strong association of polycythemia to high-altitude area exposure. This finding was in accordance with the results in other studies and available literature, as in the study by Assi and Baz high altitude is implicated as one of the causes of polycythemia.12 Also, some patients were found to have history of smoking similar to findings in other studies.11

Another important finding was presence of JAK 2 mutation V617F in only 38% of our patients. A study by Kralovics

![Fig. 1](image1.png) Patients with JAK-2 mutation and bone marrow panmyelosis.

![Fig. 2](image2.png) Mean hemoglobin (Hb) levels in relation to JAK-2 mutation.

<table>
<thead>
<tr>
<th>S. no</th>
<th>Levels (g/dL)</th>
<th>No. of patients</th>
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<tbody>
<tr>
<td>1</td>
<td>15–17</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>17–19</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>19–21</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>21–23</td>
<td>5</td>
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<tr>
<td>5</td>
<td>23–25</td>
<td>1</td>
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![Table 2](image3.png) Hemoglobin levels
et al also stresses the importance of JAK2 mutation V617F in their study.\textsuperscript{15} Extensive studies of JAK2 V617F have indicated that its frequency within PV, idiopathic MF, and ET is variable, being most common in PV, and it is considered a major factor in the diagnosis of PV according to the WHO criteria,\textsuperscript{7–9} but in our study, 62\% of the patients tested negative for JAK2 mutation V617F.

In our study, BM panmyelosis was observed to be present in 67\% of the patients, pointing toward the fact that BM panmyelosis is an important diagnostic criterion in young patients in Indian setting, compared with JAK2 V617F for polycythemia. We advise BM studies along with JAK2 mutation studies in all Indian patients, or we might risk missing cases of PV.

A correlation was observed between presence of JAK2 V617F mutation and hemoglobin levels, as higher mean hemoglobin levels were observed in JAK2 V617F mutation-positive patients (18.45 ± 4.204 g/dL) compared with JAK2 V617F mutation-negative patients (17.30 ± 3.903 g/dL). In our study out of the total 60 patients, thrombosis-related complications were observed in 5 patients, all of whom were JAK2 V617F positive, while out of the 18 patients requiring phlebotomy only 3 were JAK2 V617F negative and rest 15 were JAK2 V617F positive, indicating that thrombotic complications as well as need of phlebotomy are greater for JAK2 V617F mutation-positive cases. Few other studies also comment on the thrombosis-related complications and use of phlebotomy in polycythemia patients.\textsuperscript{16–18} A study by Humphrey et al demonstrated that small volume phlebotomy (250 mL) can be safely done once every 2 months to lower the hematocrit in polycythemia patients\textsuperscript{16} another study by Assi and Baz states that phlebotomy should be used with caution in polycythemia due to fear of causing iron deficiency.\textsuperscript{12} Assessment of the treatment provided showed 30 (50\%)
patients required aspirin, while hydroxyurea was given to 20 (33%) of the patients, as per the requirement. Although several studies claim use of aspirin controversial, it is still recommended as the treatment option with phlebotomy in low-risk cases, and hydroxyurea in those with high risk who have no contraindications to this treatment.\textsuperscript{2,19,20} The drawback of our study was that no EXON12 study was done and also that as the study was done in Army hospital, all the patients included in the study were soldiers by occupation so that can be a cause of some bias in our results.

**Conclusions**

Our study uncovers that JAK2V617F mutation in young Indian population is seen in significantly less cases as compared with Western data, while BM panmyelosis is frequently observed and thus a significant finding for diagnosing polycythemia in our setting. This makes BM biopsy a significant investigation in the suspected cases lest we miss diagnosing any case. Median hemoglobin was greater for JAK2V617F mutation-positive cases and so were the phlebotomy requirements and occurrence of thrombotic events. Also, high altitude is an established risk factor for developing polycythemia and the same is reinforced by our study.

We recommend BM studies should be done in all patients to evaluate polycythemia in Indian settings. JAK2 mutation V617F should be done to predict phlebotomy requirements and complications. A high reading of hemoglobin should sensitize as an indirect indicator of JAK 2 mutation V617F positivity.

**Availability of Data and Material**

Available if required.

**Authors’ Contributions**

All authors provided significant contribution in the form of data collection, analysis and gave necessary inputs for the designing of the manuscript.
Ethics Approval
The study was conducted after obtaining ethical clearance from institutional ethical committee. Written informed consent from the patients was taken before data collection was done.

Source of Funding
None.

Conflict of Interest
None declared.

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13. JAKAFI® (ruxolitinib) Full Prescribing Information. Wilmington: Incyte Corporation; 2016