Frequency of JAK2 and MPL Mutation in BCR/ABL Negative Myelofibrosis in KPK

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Author’s Contribution
1 Conception of study
2,3 Experimentation/Study conduction
2,3 Analysis/Interpretation/Discussion
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1 Critical Review
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Abstract

Introduction: BCR-ABL1-negative myeloproliferative disorders are a sub-group of myeloproliferative neoplasms (MPNs) that consist of polycythemia Vera (PV), Essential thrombocythemia (ET), and Primary Myelofibrosis (PMF).

Objective: To determine the frequency of JAK2 (p.V617F) and MPL (p.W515L) mutation in primary myelofibrosis in the KPK province of Pakistan.

Materials & Methods: Fifty patients with PMF were enrolled in the study. JAK2 mutation status was determined using allele-specific primers and MPL mutation was detected by the direct Sanger sequencing technique. The data was analyzed using BioEdit by aligning the sequence data with reference genome hg19 assembly.

Results: Among 50 patients, 41 patients were diagnosed with PMF, while 9 patients had secondary myelofibrosis i.e. Post PV-MF and Post ET-MF. Out of the 41 PMF patients, 2 patients had MPL gene variation, while one of the Post ET –MF had an MPL gene variation. Forty-eight (96%) were positive for JAK2 mutation. Five patients who had MPL mutation also showed JAK 2 mutation. Two of the MPL-positive patients were also positive for JAK2 mutation.

Conclusion: We reported the rate of recurrence of JAK2 mutation in 96% of the cases and MPL exon 10 mutations in 6% of the cases.

Keywords: JAK2, MPL, Myeloproliferative neoplasms, BCR-ABL1, Genetic diagnosis.
 Myers proliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders that results in an increase in the number of erythrocytes, megakaryocytes, or granulocytes in blood circulation.\(^1\) According to World Health Organization (WHO) 2008 criteria and the 2016 revision, classical MPNs include chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).\(^2,3\) Among these classes, CML is BCR-ABL1 positive while PV, PMF, and ET are negative.\(^4\) The clinical profile of the patients includes cytopenia of one or more lineages, myelofibrosis, thrombosis, varying degrees of extramedullary hematopoiesis, and risk of transformation to acute leukemia.\(^5\)

Besides morphological evaluation, genetic diagnosis is a major criterion for MPNs. The major discovery in molecular diagnostics of BCR-ABL1 negative MPN occurred with the identification of p.V617F mutation in the JAK2 gene whose frequency is 95% in PV patients and 50-60% in ET and PMF patients (6-8). There are a few reports in this context from developing countries including Pakistan so the present study aimed to improve the knowledge on the clinical and molecular profile of BCR-ABL1 negative in the population of Khyber Pakhtunkhwa (KPK) province of Pakistan.

### Table 1: Allele-specific and Sanger sequencing primers

| Mutation | Primer | Primer Sequences |
|----------|--------|------------------|
| JAK2: p.V617F | JAK2-Ex13 F | AAAGGGACCAAAGCACATTGT |
| JAK2 | JAK2-Ex13-R(G) | GTTTTACTTACTCTCGTCTCCACACAC |
| MPL: p.W515L | JAK2-Ex13-R(T) | GTTTTACTTACTCTCGTCTCCACACA |
| MPL_Ex10 F | MLL | CCGAAGCTCGACCTTTTTG |
| MPL | MLL_Ex10 R | ACAGGAGCAGACACAGATGC |

### Results

A total of 50 individuals were recruited for this study. Out of these 50 patients, 41 (82%) were diagnosed with Primary myelofibrosis (PMF), while 9 (18%) patients had SMF. These SMF patients included 6 (12%) post-PV cases and 3 (6%) post-ET. The patient with PMF (range 16-80) had a mean age of 49.17 while patients with SMF (post-PV and post-ET) had a mean value of 58.11 (range 41-70). Among the 50 individuals, 16 (32%) were female while 34 (68%) were male. The patients presented with wide-range symptoms that included fever (72%), pallor (62%), easy bruising (12%), splenomegaly (98%), bleeding gums, and hepatomegaly (6%). The hematological parameters of the study population are presented in Table 2.
Table 2: The hematological parameters of the study population

| Diagnosis  | Hb (g/dl) | Hct (%) | MCV (Fl) | MCH (pg) | MCHC (g/dl) | WBC (X 10^9/L) | Platelet count (X 10^9/L) |
|------------|-----------|---------|----------|----------|-------------|-----------------|--------------------------|
| PMF (n=41) | 8.92±2.6  | 32.46±7.73 | 70.55±8.04 | 22.08±3.2 | 30.2±2.66 | 21.2±7.59 | 273.27±204.6 |
| Post ET (n=3) | 11.3±2.4 | 42.33±2.51 | 72.8±4.8 | 72.8±4.8 | 31.1±1.96 | 22.5±22.3 | 265.00±97.00 |
| Post PV (n=6) | 11.88±4.8 | 37.33±10.94 | 66.95±6.9 | 22.6±3.6 | 30.5±3.04 | 21.4±17.5 | 272.17±230.04 |

The allele-specific PCR performed for JAK2 mutation detection showed the presence of a mutation in 96% (n=48) of the sampled individuals. Sequencing data analysis of exon 10 of the MPL gene revealed the presence of a missense variation, p.W515K, in heterozygous state in two affected individuals (M7, M33) (Figure 1). Another heterozygous nonsense variant, c.1545G>A (p.W515*), was also identified in another affected individual (M14) (Figure 1). Both variants were predicted to be a disease caused by mutation taster and are known to be associated with the pathogenicity of myelofibrosis. Out of the 41 patients who had PMF, two patients tested negative for JAK2 mutation. All the patients with SMF tested positive for JAK2 mutation. Table 3 represents the hematological profile of patients positive for MPL gene mutation.

Table 3: Hematological profile of patients positive for MPL gene mutation

| ID  | Age/Gender | Diagnosis | JAK2 Mutation | Cytogenetic abnormality | Hb (g/dl) | WBC Count (X 10^9/L) | Platelet count (X 10^9/L) |
|-----|------------|-----------|---------------|-------------------------|----------|----------------------|---------------------------|
| M7  | 27/M       | PMF       | Negative      | Nil                     | 16.3     | 16.4                 | 161                       |
| M14 | 48/F       | PMF       | Positive      | Nil                     | 6.4      | 77.6                 | 134                       |
| M33 | 58/M       | POST-ET   | Positive      | Nil                     | 13.8     | 13.7                 | 153                       |

Figure 1: Sequencing data analysis of exon 10 of the MPL gene. Individual M7 and M33 are heterozygous for a missense variation, p.W515K and individual M14 are heterozygous for a nonsense variant p.W515*.

Discussion

Genetic data is an important parameter for the classification of classical BCR-ABL1 negative MPNs. The missense variant in JAK2, p.V617F, is a best-characterized mutation in BCR-ABL1 negative MPNs with a prevalence of >95% in PV, and 50% in ET and PMF. It is a gain-of-function mutation that results in constitutive activation of the JAK-STAT pathway which leads to cytokine independent growth. MPL is the second candidate for MPNs and is involved in the downstream signaling pathway that is required for mutant JAK2-mediated transformation of myeloprogenitor cells. In this study, we recruited 50 individuals of MPNs out of which 82% (n=41) were diagnosed with PMF and 18% (n=9) were diagnosed with SMF (post PV and post-ET). Forty-eight patients tested positive for JAK2 mutation while only three patients (M7, M14, M33) were found to be heterozygous for MPL mutation (p.W515*, p.W515K). All SMF patients were positive for JAK2 while 95% (n=39) of PMF were positive for JAK2 mutation. One of the PMF patients, M7, was negative for JAK2 and positive for the MPL p.W515K variant. The other two patients, M14 and M33, were positive for both JAK2 and heterozygous MPL mutation. The MPL W515 mutations were not found in Post-PV, which was suggestive that in this disease, JAK2 is obvious and is associated with the phenotype of red cell development.
Preferably, a pot when considering stem cell transplantation. Deciding treatment recommendations, predominantly higher these mutations may promote the diagnosis to a prognostic evidence, mainly in MF. The occurrence of these mutations may promote the diagnosis to a higher-than-expected risk group, which may help in deciding treatment recommendations, predominantly when considering stem cell transplantation. Preferably, a potential assessment of mutational profiles would recognize patterns of new mutations with disease progression and clonal evolution. This measures the effect of disease duration and therapy on the development/evolution of these mutations, and helps clinicians in determining whether or not monitoring allele variance frequency is necessary for all myeloid neoplasm patients.19

Table 4: Prevalence of JAK2 and MPL mutations in different populations

| S. No. | Population | Prevalence of JAK2 Mutation | Prevalence of MPL Mutation | Reference |
|--------|------------|----------------------------|---------------------------|-----------|
| 1      | China      | 85% in PV, 58.4% in ET and 65.8% in PMF | 1.2% in ET, 2.7% in PMF | 12        |
| 2      | France     | -                          | 1% in ET                  | 13        |
| 3      | Italy      | 64.7%                      | 3% of ET and 1% PMF       | 14        |
| 4      | Turkey     | 43.9% in ET, 54.5% in PMF  | 2.6% in PMF               | 16        |
| 5      | Pakistan   | 91% in PV, 63% in ET and 60% in PMT | -                       | 17        |

Mutation screening studies conducted in different populations showed less occurrence of MPL mutation in MPN patients. Studies conducted on Iranian and Egyptian MPN patients didn’t reveal any MPL mutation.10,11 In china, 929 patients were screened for JAK2 and MPL mutations. They reported prevalence of 1.1% (1.2% in ET, 2.7% in PMF) patients for MPL mutation and 67.4% (85% in PV, 58.4% in ET and 65.8% in PMF) of patients for JAK2 mutation.12 In another study from china, MPL pathological variant was identified in 1% of ET patients.13 Study conducted in France showed the prevalence of MPL variant in 3% of ET and 1% of PMF patients14 while the prevalence in Italy and Spain is reported to be 64.7% and 4% for JAK2 and MPL variant respectively in PMF patients.15 Mutation screening on Turkish MPN patients reported MPL mutation frequency of 2.6% across PMF patients while they didn’t find any in ET patients while the JAK2 mutation frequency in ET patients was 43.9%, and in PMF patients it was 54.5%.16 A study conducted in KPK provide of Pakistan reported the prevalence of JAK2 mutation in 75.75% (n=50) patients among which 91% (n=29) were of PV, and 63% (n=12) were of ET and 60% (n=9) were of PMT.17 Another study from Pakistan reported positivity for JAK2 p.V617F mutation in 15% (n=16) of the screened patients while JAK2 exon 12 mutation and MPL were found to be negative in all screened patients (n=90).18 (Table 4) These studies suggest a lower prevalence of MPL gene mutation in PML and our data supports the previous findings.

The driver mutational profile complements the diagnostic approach to MPNs. The effect of the driver mutational profile on diagnosis and the identification of certain somatic mutations provide significant prognostic evidence, mainly in MF. The occurrence of these mutations may promote the diagnosis to a higher-than-expected risk group, which may help in deciding treatment recommendations, predominantly when considering stem cell transplantation. Preferably, a potential assessment of mutational

Conclusion

With help of allele-specific PCR, we were able to identify JAK2 mutation (p.V617F) in 96% (n=48) of the recruited patients out of which 39 were diagnosed with SMF. Heterozygous MPL mutations in exon 10 were identified using sanger sequencing in only 3 patients. The frequency of MPL variant was less in our study population which supports the previous data.

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