Original Article

Standardization of real-time quantitative polymerase chain reaction for detection of the JAK2V617F mutation in BCR-ABL1 negative myeloproliferative neoplasms: A Tertiary Care Centre experience

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ABSTRACT

Background: Identification of JAK2V617F mutations has led to a significant development in our understanding of the pathogenesis and therapy of BCR-ABL1 negative myeloproliferative neoplasms (MPNs). However, not all cases of BCR-ABL1 negative MPNs carry JAK2V617F mutations. The present study was undertaken with an aim to standardize the real-time quantitative polymerase chain reaction (PCR) for the detection of JAK2V617F mutations and to find the prevalence of Janus kinase 2 (JAK2) mutations in MPNs in the Indian scenario. **Materials and Methods:** Real-time quantitative PCR was used to detect the JAK2V617F mutation. Standardization of the detection procedure was carried out using recommended guidelines to ensure the accuracy and reproducibility of results. Forty-nine patients of BCR-ABL1 negative MPNs were included in the study. **Results:** The JAK2V617F mutation was detected in 63.3% patients of BCR-ABL1 negative MPNs. On classification of these BCR-ABL1 negative MPNs, JAK2V617F mutation was detected in 78.3% patients with polycythemia vera (PV), 62.5% patients with essential thrombocythemia (ET), and 44.4% patients with Primary myelofibrosis (PMF). **Conclusion:** Role of detection of JAK2 mutations in BCR-ABL1 negative MPN has begun to be described and can be used in the diagnosis of PV, ET, and PMF along with other criterias. In future, it may be suitable for treatment monitoring and prognostication.

Key words: BCR-ABL1 mutation, essential thrombocythemia, JAK2V617F mutation, myeloproliferative neoplasm, polycythemia vera, primary myelofibrosis

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells leading to an excessive production of blood cells because of hypersensitivity or independence from normal cytokine regulation.[1,2] This myeloproliferation results from the absence of feedback regulation by mature cells thereby decreasing cytokine levels. They include chronic myeloid leukemia (CML) which is characterized by BCR-ABL1 fusion gene, and BCR-ABL1 negative MPNs-which have recently been shown to carry somatic mutations of Janus kinase 2 (JAK2), located on chromosome 9p24.[3] The BCR-ABL1 negative MPNs involve the 3 main myeloid lineages but predominate in one of them: The erythroid lineage for polycythemia vera (PV), the megakaryocyte (MK)/platelet

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lineage for essential thrombocythemia (ET), and the MK/granulocytic lineages for primary myelofibrosis (PMF). They are associated with increased numbers of mature blood cells, except in PMF, in which abnormalities in MK differentiation might be responsible for the marrow fibrosis. A continuum exists between the BCR-ABL-negative MPN and myelodysplastic syndromes (MDS), as exemplified by chronic myelomonocytic leukemia, classified as MPN/MDS by the World Health Organization (WHO). For many years, the diagnosis of BCR/ABL-negative chronic myeloproliferative disorder (CMPD) was mainly based on clinical symptoms, cytomorphology and histomorphological findings. The identification of JAK2V617F mutations in the year 2005 has led to a significant development in our understanding of the pathogenesis and therapy of BCR-ABL1 negative MPNs. In most of the MPNs featuring JAK2 mutations, the amino acid-phenylalanine substitutes for valine at position 617 (JAK2V617F). This leads to a constitutive activation of the downstream signaling pathways causing abnormal proliferation of the myeloid lineage. However, not all cases of BCR-ABL1 negative MPNs carry JAK2V617F mutations. In the western population, this mutation is found in almost all PV cases and nearly half of PMF and ET cases. In other cases of BCR-ABL1 negative MPNs, mutations have been described in exon 12 of JAK2, MPL, IDH, ASXL1, CBL, TET2, and IKZF1.[3] A number of JAK2 inhibitors are now undergoing clinical trials for the therapy of BCR-ABL1 negative MPNs. The present study was undertaken with an aim to standardize the real-time quantitative polymerase chain reaction (RQ-PCR) for the detection of JAK2V617F mutations and to find the prevalence of JAK2 mutations in MPNs in the Indian scenario.

MATERIALS AND METHODS

The study was conducted in a Tertiary Care Hospital from the year 2010 to 2012. All patients diagnosed as MPNs according to WHO classification-2008 based on history, clinical examination, and hematological studies and who were tested to be BCR-ABL1 negative by quantitative real-time polymerase chain reaction (PCR) were included in this study. Patients diagnosed as MPN but treated previously were excluded from the study. A total of 49 patients were included in the study, of which 23 cases were diagnosed as PV, 8 cases as ET, and 18 cases as PMF.

Standardization of JAK2V617F mutation detection procedure was carried out using guidelines put forth by Bench *et al*.[4] This is important to ensure the accuracy and reproducibility of results. 3 ml of peripheral blood sample was collected in ethylene diamino tetraacetate anticoagulation tube. DNA extraction was done on the same day of collection using QIAamp DNA Mini Kit (Catalogue no: 51304, QIAGEN, Hilden, Germany) using the spin protocol according to the manufacturer's instructions. Detection of JAK2V617F mutation was done using Ipsogen JAK2 Mutascreen kit (Catalogue No: 673023, QIAGEN, Hilden, Germany) and Rotor-gene 3000. This kit uses the principle of multiplex real-time PCR where two allele-specific probes labeled with fluorescent dyes at the 5′ end, namely, 6-carboxyfluorescein (6-FAM) and VIC and quencher dyes at the 3′ end bind to wild-type and mutant-type allele, respectively. The probe if attached to the target sequence, is cleaved by the 5′→3′ exonuclease activity of Taq DNA polymerase, separating the reporter dye from the quencher and thus releasing detectable fluorescence. The VIC or FAM fluorescence signal indicates the presence of the targeted sequence in the sample (wild-type allele, mutated allele, or both).

All test samples were run in duplicate along with positive, negative controls, cut-off sample, and water to test for the accuracy of each run. Ongoing internal quality runs are regularly performed for this test. The temperature profile used for the PCR run was 50°C for 2 min, 95°C for 10 min followed by 50 times cycling at 92°C for 15 s and 60°C for 1-min. Acquisition of FAM and VIC fluorescence in channel Cycling A green and yellow, respectively was done. A scatter plot of fluorescence data with VIC fluorescence on X-axis and FAM fluorescence on Y-axis was plotted. The normalized ratio of each sample was calculated using FAM/VIC ratios using which the status of JAK2 mutation was interpreted for each sample according to the manufacturer's instructions.

RESULTS

A total of 49 patients were included in the study. The age range of the patients was 32–67 years (mean age 58 years). Of the 49 patients included in the study, 23 patients (47%) were diagnosed as PV, 18 patients (37%) were diagnosed as PMF, and 8 patients (16%) were diagnosed as ET using WHO 2008 criteria for MPNs. The JAK2V617F mutation was detected by multiplex PCR in 63% patients (31 of 49 patients) of BCR-ABL1 negative MPNs. On classification of these BCR-ABL1 negative MPNs, JAK2V617F mutation was detected in 78.3% (18 of 23), 62.5% (5 of 8) patients with ET, and 44.4% (8 of 18) patients with PMF [Table 1].

Table 1: Positivity of JAK2V617F mutation in BCR-ABL1 negative patients as detected by multiple PCR

Category of BCR-ABL1 negative patients	Number of patients	Number of patients (%) positive for JAK2 mutation on multiplex PCR
PV	23	18/23 (78.3)
ET	8	5/8 (62.5)
PMF	18	8/18 (44.4)
Total	49	31/49 (63)

PCR: Polymerase chain reaction, ET: Essential thrombocythemia, PMF: Primary myelofibrosis, JAK2: Janus kinase, PV: Polycythemia vera

Table 2: Summary of various studies on prevalence of JAK2V617F mutation in chronic MPNs

Study	Polycythemia vera (%)	Essential thrombocytosis (%)	PMF (%)	Method of detection
James *et al.*, 2005[4]	89	43	43	Sequencing
Levine *et al.*, 2005[5]	74	32	35	High throughput DNA sequencing
Kralovics *et al.*, 2005[6]	65	23	57	Microsatellite mapping and DNA sequencing
Baxter *et al.*, 2005[7]	97	57	50	Allele-specific PCR; molecular cytogenetic study; microsatellite PCR; affymetrix single nucleotide polymorphism
Sazawal *et al.*, 2010[8]	82	70	52	Allele-specific PCR
Suksomyos *et al.*, 2012[10]	81	60	70	Allele-specific PCR; PCR-RFLP
Chatterjee *et al.*, 2015	78	63	44	Real-time PCR

PMF: Primary myelofibrosis, JAK2: Janus kinase 2, MPNs: Myeloproliferative neoplasms, PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism

DISCUSSION

The zone of MPNs has seen a number of developments in the last two decades. The introduction of WHO 2001 classification brought a complete change in the classification criteria of PV. The subsequent description of acquired mutation of tyrosine kinase JAK2 in MPN in 2005 has further asserted the fact that the entities included under MPNs are closely related to each other.[5] Detection of JAK2V617F mutation is now widely used for the diagnosis of MPN. However, because of different types of detection methods used and lack of standardization of the detection methods, results may vary. Recognition of JAK2V617F mutations has also led to the development of interest in the field of recognizing JAK 2 inhibitors that can be used in patients with MPN. According to studies conducted in the West, JAK2V617F mutations are reported in 65–97% of PV, 23–57% of ET, and 30–56% of PMF.[6-8] Studies to determine the prevalence of this mutation in various MPN in the Indian population are needed.

In this study, the JAK2V617F mutation was detected in 63% of BCR-ABL1 negative MPNs. This is comparable to studies done by Sazawal *et al*. in the Indian population and by Suksomyos *et al*. on Thai patients that reported 68% and 68.8% prevalence of JAK2V617F mutation in BCR-ABL1 negative MPNs.[9,10] Both these studies had used allele-specific PCR and PCR-restriction fragment length polymorphism. The Indian study had also included previously diagnosed patients on follow-up.

The prevalence of JAKV617F mutations in PV, ET, and PMF, was 78.3%, 62.5% and 44.4%, respectively in this study. Previous studies have reported a wide variation in the prevalence of JAK2V617F mutations amidst various categories of BCR-ABL1 negative MPNs [Table 2]. Studies by Kralovics *et al*. and Levine *et al*. had reported the prevalence of JAK2V617F mutation in PV to be 65% and 74%, in ET to be 23 and 32%, and in PMF to be 57 and 35%, respectively.[6,7] The values reported in these two studies are lower than those reported in this study and in studies by Baxter *et al*., Sazawal *et al*. and Suksomyos *et al*.[8-10] This variation can be explained by the difference in the methods of detection used. The last mentioned studies had used allele-specific PCR, which has an approximate sensitivity of 0.1–5%.[4]

The presence of JAK2 mutations in BCR-ABL1 negative MPN have been compared to the presence of a BCR-ABL1 mutation in CML. Fifty years after BCR-ABL1 discovery in 1960's in CML, its detection is used not only for diagnosis but also treatment monitoring and imatinib mesylate, which inhibits the tyrosine kinase activity of BCR-ABL1 is used to treat CML. Role of detection of JAK2 mutations in BCR-ABL1 negative MPN has just begun to be described and may be expected to have similar implications. For the above reasons, detection methods of JAK2 mutations need to be standardized and routinely used in BCR-ABL1 negative MPNs for diagnostic purposes. In future, they may be suited for treatment monitoring and prognostication.[11,12]

CONCLUSION

The JAK2V617F mutation was detected in 63.3% of patients with MPN in this study. The significant prevalence of this mutation in MPNs support the recommendation that peripheral blood mutation screening for JAK2V617F be incorporated into the initial evaluation of patients with suspected MPN. However, since the mutation may be absent in a few cases of PV, ET, and IMF, it cannot be used as a single test for making the diagnosis. It should be done in addition to other tests such as red cell mass, serum erythropoietin, and bone marrow biopsy before excluding the diagnosis of suspected CMPD. It is advisable that as in the West, detection of this mutation may have a significant role in diagnosing BCR-ABL negative CMPD and in identification of subsets who would respond to JAK2 inhibitor therapy.

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Conflicts of interest

There are no conflicts of interest.

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