AMLS Archives of Medical Laboratory Sciences

Original Article

Prevalence of JAK2 V617F Mutation in Iranian Patients with Myeloproliferative Neoplasms

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Received: September 9, 2020, Accepted: November 21, 2020

Abstract

Background and Aim: Multiple lines of evidence have been suggested that JAK2 is likely the main candidate gene responsible for the pathogenesis of myeloproliferative neoplasms. The V617F mutation in the pseudokinase domain of JAK2 protein has been detected in a majority of patients. We aimed to evaluate the frequency of this somatic missense substitution among Iranian patients with myeloproliferative neoplasms.

Methods Peripheral blood samples were collected from patients with myeloproliferative neoplasms across different regions of Iran. The JAK2 V617F mutation was identified by allele-specific PCR. To confirm the PCR results, randomly selected positive and negative samples were sequenced.

Results: Among 72 identified patients, 45 (62.5%) were found to harbor JAK2 V617F. The frequencies of the mutation ranged 100% for primary myelofibrosis, 75% for chronic myelogenous leukemia, 67% for polycythemia vera, 62.5% for myelodysplastic/myeloproliferative neoplasms, and 52% for essential thrombocythemia. Our findings revealed that the mutation was more common among men in comparison with women and the correlation between the mutation and gender was statistically significant (p-value<0.01). Additionally, the presence of JAK2 V617F was associated with older ages (p-value =0.009).

Conclusion: The JAK2 V617F mutation was detected in 62.5% of patients with myeloproliferative neoplasms. We have shown that this single acquired point mutation was presented in at least half of the patients. Hence, it seems that the identification of JAK2 V617F mutation in myeloproliferative neoplasms can be very effective in disease diagnosing and management.

Keywords: Myeloproliferative Disorders; Myeloproliferative Neoplasms; JAK2 V617F; Mutation; Iran.

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Please cite this article as: Hamid M, Shahbaz Z. Prevalence of JAK2 V617F Mutation in Iranian Patients with Myeloproliferative Neoplasms. Arch Med Lab Sci. 2020;6:1-7 (e5). https://doi.org/10.22037/amls.v6.32758

Introduction

The myeloproliferative neoplasms (MPNs) are hematological malignancies due to clonal proliferation of the myeloid cell lineages which affect pluripotent hematopoietic stem cells in the bone marrow to produce more platelets, red blood cells, and white blood cells (1, 2). According to the 2016 Revised WHO classification, MPNs includes eight subcategories: 1) chronic neutrophilic leukemia (CNL), 2) chronic myelogenous leukemia (CML), 3) polycythemia vera (PV), 4) BCR-ABL1–positive, 5) primary myelofibrosis (PMF), 6) essential thrombocythemia (ET), 7) eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES), 8) mastocytosis (MCD) and unclassifiable MPNs (3, 4).

A single somatic G > T base conversion in exon 12 of Janus-associated Kinase-2 (JAK2) gene results in the amino acid substitution of valine (V) to phenylalanine (F) at position 617. This change in the pseudokinase domain is thought to alter JAK2 protein conformation that leads to constitutive Janus-associated Kinase- signal transducers and activators in the JAK-STAT signaling pathway (5, 6). JAK2 V617F is occasionally detected in leukemia or other bone marrow disorders and related disease (7). We don't know yet how one particular mutation can be associated with several different conditions (8). Recent studies have shown that a significant proportion of non-CML patients

have acquired JAK2 V617F. This mutation has a variable frequency in different disease conditions with the highest percentage in PV samples ranging from 65% to 97% (9). The mutation is also found in a slightly lower but still substantial percentage of ET (30%-57%) and chronic idiopathic myelofibrosis (CIMF) (35%-95%) (10, 11). On the other hands, JAK2 V617F is relatively uncommon in other blood disorders such as typical CML, myelodysplasia, acute leukemias without previous chronic myeloproliferative diseases (CMPDs), chronic myelomonocytic leukemia (CMML), chronic lymphocytic leukemia (CLL), and acute lymphoblastic leukemia (ALL) (9, 10, 12). In vitro and mouse model studies have been shown that JAK2 V617F enhances the survival and proliferation of BaF3 cells and also increases the constitutive phosphorylation of tyrosine, leading to cytokine hypersensitivity and erythrocytosis stimulation (13-15). Based on these findings, it is likely that the JAK2 V617F mutation has an important role in the pathogenesis and manifestations of these disorders (15). In previous studies from Western Europe, North America, and Middle-Eastern countries, the frequency of JAK2 V617F was reported more than 95% in patients with PV, and approximately 50% in patients with ET and PMF (16, 17). It has been shown previously that V617F mutation could be readily detected by AS-PCR. When the mutant cell population is low, detection of the JAK2 V617F mutation using the AS-PCR method is easily performable, rapid, sensitive, and also cost-effective (18). Hence, here, we aimed to estimate the frequency of JAK2 V617F mutation in patients with MPNs using AS-PCR.

Methods

Participants

This study was approved by the Ethics Review Committee of Pasteur Institute of Iran and conducted on patients referred to the Oncology Center of Imam Khomeini Hospital from May 2010 to September 2012. Peripheral blood samples were obtained from 112 patients following obtaining written informed consent. Forty patients had hematological and/or clinical features suggestive but not diagnostic of MPN and were excluded from the study. Then a total of 72 unrelated patients who had an elevated count of platelets, red blood cells, and white blood cells were included in the study. The patients had a negative test result for BCR-ABL translocation by either cytogenetic or PCR methods. The diagnosis was confirmed according to the 2016 World Health Organization (WHO) classification (3).

Molecular Study

The molecular study was done at the Pasteur Institute of Iran. Genomic DNA was extracted by the salting-out method. Amplification was done using Allele-specific polymerase chain reaction (AS-PCR) by two forward and 1 reverse designed primers according to the target DNA sequence of the JAK2 gene (Table1).

Table 1. Forward (F) and reverse (R) Primer sequences for AS-PCR and product size

Allele Types	Forward Primer (5'→3')	Reverse Primer (5'→3')	Product size
Wild Allele	AGCATTTGGTTTTAAATTATGGAGTATATT	CTGAATAGTCCTACAGTGTTTTCAGTTTCA	364 bp
Mutant Type Allele	ATCTATAGTCATGCTGAAAGTAGGAGAAAG		203 bp

PCR reaction was performed in a total volume of 25 µL containing approximately 25 ng DNA, 12.5 µL of PCR Master Mix 2X (Roche, Germany), 0.5 µl of each primer.

The PCR time and temperature program on the thermal cycler (Eppendorf) was an initial denaturation step at 94°C for 6 min, 40 cycles of 40 sec. at 94°C, 45 sec. at 56°C, 45 sec. at 72°C, and a final extension step of 72°C for 10 min. The samples were identified by electrophoresis of the AS-PCR products on a 2% agarose gel. To confirm the results in the early stages, several positive and

negative samples were sequenced by the Sanger sequencing method.

Statistical Analysis

Statistical analysis was done to determine any correlation between mutation and age by t-test. The correlation between mutation and sex was analyzed by $\chi 2$ test. p-value of less than 0.05 was considered statistically significant.

Results

Using AS-PCR, 45 (62.5%) of 72 patients with MPNs, were found to harbor JAK2 V617F mutation. The agarose gel electrophoresis and the chromatogram of Sanger sequencing results for wild and mutant alleles are presented in Figure 1.

Demographic feature and distribution of JAK2 V617F mutation

Demographic features and cancer types in the samples are presented in Table 2. The JAK2 V617F positive ranged 100 % (2 of 2) for PMF, 75 % (3 of 4) for CML, 67% (22 of 33) for PV, 62.5% (5 of 8) for myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and 52% (13 of 25) for ET.

A summary of these results according to the MPN categories is given in Table 3.

Table 2. Demographic feature and disease subtypes in patients

Characteristics	Number (%)
Gender (Men/Women)	43(60%)/29(40%)
Essential thrombocythemia (ET)	25(35%)
Polycythemia vera (PV)	33(46%)
Primary myelofibrosis (PMF)	2(3%)
Chronic myelogenous leukemia (CML)	4(5%)
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)	8(11%)

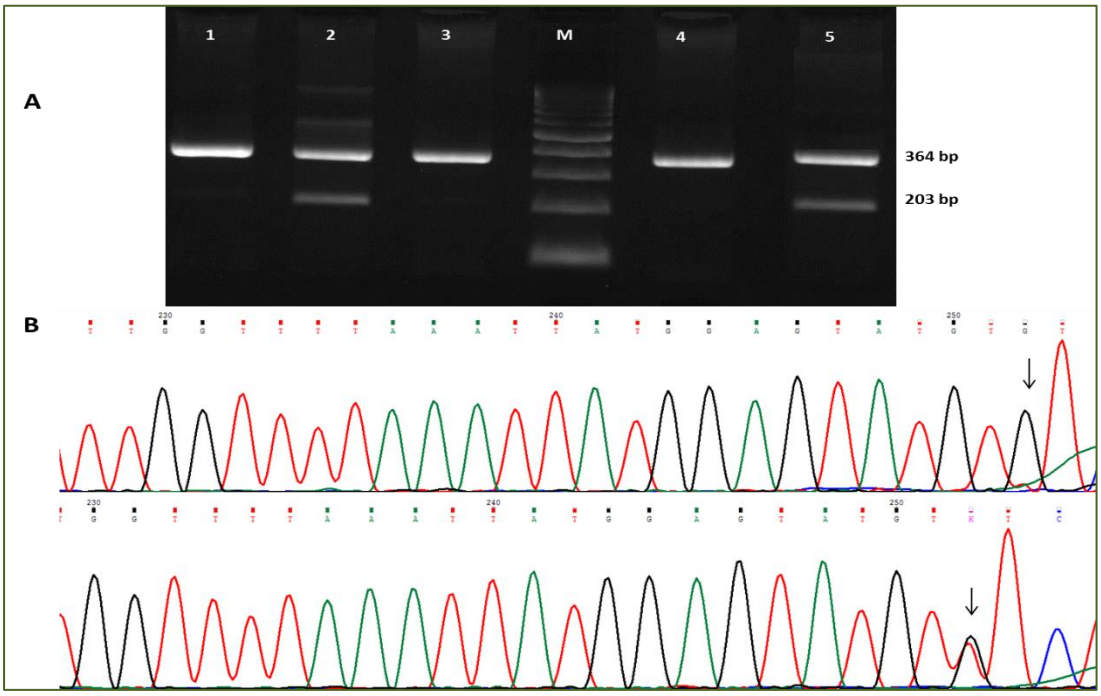

Figure 1. Identification of the JAK2 V617F mutation by AS-PCR and DNA sequencing methods. A) Gel electrophoresis result; 1: Wild type, 2: Mutant type, 3: Wild type, M: 100 bp DNA marker, 4: Negative control, 5: Positive control. B) Sanger sequencing results; Up one is related to wild type sequence, down one is related to the mutant type sequence.

Table 3. Incidence of JAK2 V617F mutation in patients with MPN, and gender-related distribution of JAK2V617F mutation in different disease subtypes

Subtype of disease	Number of patients	JAK 2 positive (%)	Gender in JAK2 positive patients (Men/Women)
Chronic myelogenous leukemia (CML)	4	3 (75%)	3/0
Polycythemia vera (PV)	33	22 (67%)	14/8
Essential thrombocythemia (ET)	25	13 (52%)	7/6
Primary myelofibrosis (PMF)	2	2 (100%)	1/1
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)	8	5 (67%)	4/1
Total	72	45 (62.5%)	29/16 (64%/36%)

Gender-related distribution of JAK2V617F mutation

The JAK2 V617F mutation was more common among men in comparison with women (29 out of 45 mutation-positive patients were men). The correlation between JAK2 V617F and gender was statistically significant (p-value <0.01; Table 3).

The median age of positive patients with MPNs was 53.2±18.7 years and that of the JAK2 V617F negative was 41.2±16.7 years. The presence of the mutation was associated with higher age (p-value =0.009). We found this mutation not only in the PV, ET, and PMF but also in patients with MDS/MPN.

Discussion

Multiple lines of evidence have been suggested that JAK2 is likely the main candidate gene responsible for the pathogenesis of MPN (19). Patients with JAK2 V617F mutation in their hematopoietic cells respond well to signal transduction therapy. Therefore, it is very important to diagnose this mutation in patients with MPNs (20). JAK2 V617F mutation does not happen in all MPNs which could be count as an advantage in distinguishing patients with a true primary malignancy from those patients with hematological profiles and other clinical features, such as erythrocytosis or thrombocytosis (21).

In the current study, the prevalence of the JAK2 V617F mutation in patients with MPNs was high, which maybe the result of the small sample size. However, our results were inconsistent with the findings of several previous studies (22, 23).

Since 2005, JAK2 V617F has been detected and reported in different CMPDs. There are some case report publications claimed the presence of JAK2 V617F mutation in CML (24). However, Murugesan et al. (2006) did not detect JAK2 V617F mutation in patients with CML and Non–Myeloproliferative disorders (12). On the other hand, in the study performed by Pahore et al. (2010), 26.7% of CML patients had shown the presence of JAK2 V617F mutation (24). Karimzadeh et al. (2011) reported that 19% of CML patients carrying JAK2 V617F (25). In general, it seems that the high frequency of JAK2 V617F mutation in CML patients in the current study (about 75%), was in contrast with previous research that showed this mutation rarely occurred in CML. The high frequency of JAK2 V617F harboring patients with CML may be due to the sample size and analytics method related to bias.

Previous studies have been reported the frequency of JAK2 V617F mutation in patients with ET in a range between 25 to 50 %. In the reports by Baxter et al. (26) and Kralovics et al. (27) JAK2 V617F frequency in patients with ET was 57% and 23%, respectively. These findings were confirmed by subsequent studies (28, 29). In the present study, the frequency of JAK2 V617F in patients with ET was 52 % which is in coincidence with previous studies (30).

Although the number of PV patients examined in the current study was relatively high, the JAK2 V617F mutation in our PV patients has a lower frequency compared to previous studies (n=22, 67%) (31, 32). This discordance may be related to

ethnic variation. This statistical deviation maybe corrected by selecting a larger sample size.

Also in the current study, the frequency of V617F mutation in patients with PMF was estimated using allele-specific PCR method to be 100 %. This high-frequency yield maybe the sample size and analytics method related to bias.

Previous surveys have shown that V617F mutation could be found in one-third of MDS/MPN patients. For example, Langabeer et al (2007) reported that 33% of patients with MDS/MPN had V617F mutation (33). We found here 67% of our MDS/MPN patients carrying V617F variation. This discordance may be due to a higher number of studied patients and higher sensitivity of clinical criteria used for diagnosis.

The JAK2 V617F was much more frequent in men than in women patients in the present study. This finding is not incompatible with other reports, in which the proportion of women with JAK2 V617F mutation was higher than men (34). In another study with the same sample size, the frequency of JAK2 V617F mutation in men and women was equal (35). It has been suggested that when considering all patients with myeloproliferative disorders, allelic burdens are significantly lower in women than in men (36). In addition, the prevalence of JAK2 V617F mutation in MPNs was associated with a higher age range ($P<0.01$), especially with PV patients. JAK2 V617F mutation allelic frequency has a great impact on phenotype severity and therapy results in patients with MPNs. Several studies have shown that ET and PMF patients with positive V617F test results manifested with older diagnosis age, higher hemoglobin level, and leukocyte count, but also lower platelet number (37, 38). In a suggested model, the JAK2 V617F allele burden progressively increases alongside changes in phenotype, with lower allele burden inducing isolated thrombocytosis and higher levels being accompanied by increases in hemoglobin level, leukocytosis, and splenomegaly, and ultimately with the fibrotic transformation(39, 40). JAK2 mutation can challenge the treatment process of patients with MPNs. A study has proven that V617F-positive and negative patients with ET responded to treatments in different ways. For example, V617F-positive patients are extra responsive to Hydroxyurea therapy while are not to Anagrelide treatment (40-41). The Hydroxyurea treatment outcome is the decreased incidence of arterial thrombosis in V617F-positive patients, while Anagrelide treatment (plus aspirin in each arm) does not have this effect. It is noteworthy that such differences in drug effect are not documented in V617F-negative patients. These findings suggested that Hydroxyurea is a more effective treatment compared with Anagrelide in JAK2 V617F positive ET patients, whereas no significant difference, in consequence, is observed between these two treatments in V617F-negative ET patients (42, 43). This finding cannot be generalized to PV and patients with PMF.

Conclusion

In summary, our findings suggest that it is essential for patients with MPN to have JAK2 V617F mutation examined because it is both effective in diagnosing and determining subtypes of patients that respond better to JAK2 inhibitor therapy (43). We have shown that a single acquired point mutation in the JAK2 gene is presented in virtually most PV and PMF and in about half of ET, CML, or patients with MDS-MPN. Therefore, the investigations on the relationship between the JAK2-V617F mutation and the development of disease complications are essential in Iranian patients with MPN.

Conflict of Interest

The authors declared that they have no conflict of interest.

Acknowledgment

We would like to thank the patients who participated in this study.

Funding/Support

This study was supported in part by grant number 1033 from the Pasteur Institute of Iran.

Ethics

All ethical issues have been completely observed by the authors. This study was approved by the Ethics

Review Committee of Pasteur Institute of Iran (Ethical Approval Code: IR.PII.REC.1397.56).

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