Original Article

Evaluation of Jak2 Exon 12 Mutation in Patients with Polycythemia Vera

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Abstract

Background: Polycythemia vera (PV) increases the red blood cells' production. Jak2 Exon 12 mutation as a new molecular test can be helpful for diagnosing of PV patients. In this study, the evaluation of the Jak2 Exon 12 mutation in patients with PV was done. **Materials and Methods:** A total of 120 patients with PV were screened for JAK2 V617F gene mutation using amplification refractory mutation system-polymerase chain reaction and Exon12 JAK2 gene mutation by DNA sequencing. **Results:** There was a significant relationship between the JAK2V617F mutation and hemoglobin, white blood cell, and platelet counts ($P < 0.05$). Two (7.7%) patients (one male and one female) were positive for JAK2 Exon12 mutation (JAK2R541-E543delinsK, JAK2H531Q, and JAK2V 511G). **Conclusions:** The high diversity in the JAK2 Exon12 mutation in studies can be due to using different molecular methods. Further studies are needed to investigate the relationship between JAK2 Exon12 mutation and laboratory parameters.

Keywords: *Jak2 Exon 12, JAK2^{V617F}, polycythemia vera*

Introduction

According to the 2016 World Health Organization (WHO) classification, myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). Among MPNs, the most CML cases have translocation between the long arm of chromosomes 22 and 9 [t (9; 22) (q34; q11)]. The result of this rearrangement is the BCR-ABL gene fusion, but the rest of MPNs are negative for the Philadelphia chromosome.[1,2]

PV is a blood malignancy caused by the disorder of hematopoietic stem cells. This disorder increases the hematopoiesis and the sensitivity of hematopoietic stem cells to growth factors and cytokines which is usually associated with an increase in blood cell production (erythrocytes and subsequent hemoglobin (Hb) levels, as well as increased granulocyte production).[3] Around 75% of Philadelphia-negative MPNs have the JAK2^{V617F} mutation in Exon 14, which approximately 90%–95% of PV and about 60% of essential thrombocytosis (ET) and PMF cases carrying this mutation.[4,5] Therefore, all patients with PV are not diagnosed with the Exon 14 detection method. One of the diagnostic problems in PV is the patients who are negative for JAK2^{V617F} mutations. Finding the new mutations and also the newly discovered biomarkers can help to better diagnosis of these patients.[6-8] The Jak2 Exon 12 mutation is located at the junction of the JH2 and JH3 domains with 12 amino acids and 36 bp. This mutation has been reported to be positive in patients with PV who are JAK2^{V617F} negative.[9] In rare cases, both JAK2^{V617F} and Jak2 Exon 12 mutations have been reported in the patients.[10] The frequency of Jak2 Exon 12 mutation in JAK2^{V617F} negative PV patients is ranged from 2% to 25% in different studies.[11]

All patients with PV are not diagnosed through traditional methods such as clinical and laboratory presentation, bone marrow (BM) smear examination, and molecular tests such as the JAK2^{V617F} mutation. Hence, the new molecular tests such as the Jak2 Exon 12 mutation can be useful in the diagnosis of PV patients. In this study, the Jak2 Exon 12 mutation frequency in patients with PV was evaluated.

Materials and Methods

Study group: Patients and samples

In this study, 120 patients with PV, according to the clinical, laboratory

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findings, such as pruritus, high red blood cell count, and WHO criteria such as Hb levels >18.5 g/dl in male and >16.5 g/dl in female and BM cellularity were included in this study by the hematology specialist. The patients with no laboratory symptoms were excluded from the study. PV patients included 82 males (68.3%) and 38 females (31.6%) (16–87-year-old, median age: 47.50 years). Five milliliters of peripheral blood (PB) samples was collected from each patient in tubes containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant. Then, the mutation of $JAK2^{V617F}$ genes was confirmed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method and Exon12 JAK2 mutation in $JAK2^{V617F}$ negative patients was confirmed by DNA sequencing method. All laboratory parameters in this study, including white blood cell count (WBC), platelet (PLT) count, Hb, and demographic information of patients including age and sex are listed in Table 1. All PB samples were obtained from the Baghaei Hospital of Ahvaz Jundishapur University of Medical Sciences for 1 year with written informed consent from the patients. This study was approved by the Local Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1394.56) and was conducted within 6 months.

DNA extraction and amplification refractory mutation system-polymerase chain reaction for $JAK2^{V617F}$ mutation

Total DNA was extracted from 10^6 isolated cells using Diatom DNA prep 100 based on the manufacturer's instructions. DNA was quantified at 260 nm by spectrophotometry. $JAK2^{V617F}$ mutation was confirmed by ARMS-PCR as follows: 21µl PCR reaction mixture was prepared containing 10 µl red-master mix (2.5 µl 10X buffer, 0.5 µl dNTP, 0.75 µg MgCl2 (50 mM), 0.5 µl Taq polymerase enzyme), 0.5 µl each primer (forward and reverse), 10 µl DW, and 2 µl DNA. The thermal cycling conditions were as follows: 2 min at 95°C for 1 cycle, 30 s at 95°C, 30 s at 60°C, 40 s at 72°C, followed by 30 cycles and 5 min at 72°C performed at 1 cycle. All the samples were run in duplicate to lower handling errors. $JAK2^{V617F}$ primers included JAK2 common 5'-CTGAATAGTCCACAGTGTTTTCAGTTTCA-3'; JAK2 Mutant 5'- CTGAATAGTCCACACAGTGTT TTCAGTT TCA -3'; JAK2 Wild Type 5'- ATCTATAGTCA TGC TGAAAGTAGGAGAAAG -3'. Then, the PCR product was run on 1.5% agarose gel [Figure 1].[12,13]

Table 1: Comparison of the $JAK2^{V617F}$ mutation and laboratory findings in 120 patients with polycythemia vera

$JAK2^{V617F}$	Positive (*n*=94; 78.3%), *n* (%)	Negative (*n*=26; 21.7%), *n* (%)	*P*
Gender (%)			
Male	63 (76.8%)	19 (23.2%)	>0.05
Female	31 (81.6%)	7 (18.4%)	
Age (mean)	53.55	39.04	>0.05
WBC ($\times10^9$L) (mean)	12.54	7.93	<0.05
Hb (g/dL) (mean)	18.03	18.38	<0.05
PLT ($\times10^9$L) (mean)	453.80	229.49	<0.05

WBC: White blood cell, Hb: Hemoglobin, PLT: Platelet

DNA extraction and amplification refractory mutation system-polymerase chain reaction and sequencing for JAK2 Exon12 mutation

After screening for $JAK2^{V617F}$ negative mutations, the samples of these patients were confirmed for detection of the JAK2 Exon12 mutation by DNA sequencing. All the procedures were performed similarly to the $JAK2^{V617F}$ mutation detection except that only nonmutated primers were used for PCR, and the temperature for the second phase of PCR was 62°C due to the lower sub-band. Total DNA was extracted from 10^6 isolated cells using Diatom DNA prep 100 based on the manufacturer's instructions. DNA was quantified at 260 nm by spectrophotometry. JAK2 Exon12 mutation was confirmed by ARMS-PCR as follows: 20µl PCR reaction mixture was prepared containing 10 µl Red-Master Mix (2.5 µl 10X buffer, 0.5 µl dNTP, 0.75 µg MgCl2 (50 mM), 0.5 µl Taq polymerase enzyme), 0.5 µl each primer (forward and reverse), 10 µl DW, and 2 µl DNA. The thermal cycling conditions were as follows: 2 min at 95°C for 1 cycle, 30 s at 95°C, 30 s at 60°C, 40 s at 72°C, followed by 30 cycles and 5 min at 72°C performed at 1 cycle. All the samples were run in duplicate to lower handling errors. JAK2 Exon12 primers included JAK2 Exon12 R: 5'-CCAATGTCACATGAATGTAAATCAA-3'; JAK2 Exon12 F: 5'-TCATTTTACTCCTCTTTGGAGCA-3. Then, the PCR product was run on 1.5% agarose gel.[12,13]

Then, the PCR products were sequenced by 3130XL DNA sequencer. First, the PCR product was cleaned up for purification. The second PCR was performed as follows: 5 µL forward primer and 5 µL reveres primer with 10 µL PCR product with 7 µL Bigdye (determining the color of the nucleotides), 4 µl buffer, and 3 µl distilled water. The second PCR product was cleaned up again for further purification. Then, the second PCR product was added to 5 µL formamide and was incubated in 95° and the second PCR product was sequenced by 3130XL sequencer [Figures 2-4].

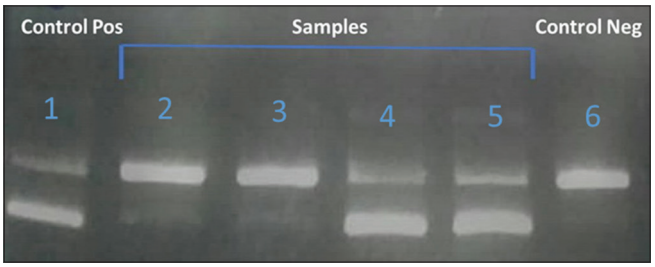

Figure 1: Polymerase chain reaction product on 1.5% agarose gel for JAK2V617F detection of patients with polycythemia vera.1, positive control; 2 and 3, patient's samples with negative result; 4 and 5, patient's samples with positive results; 6, negative control

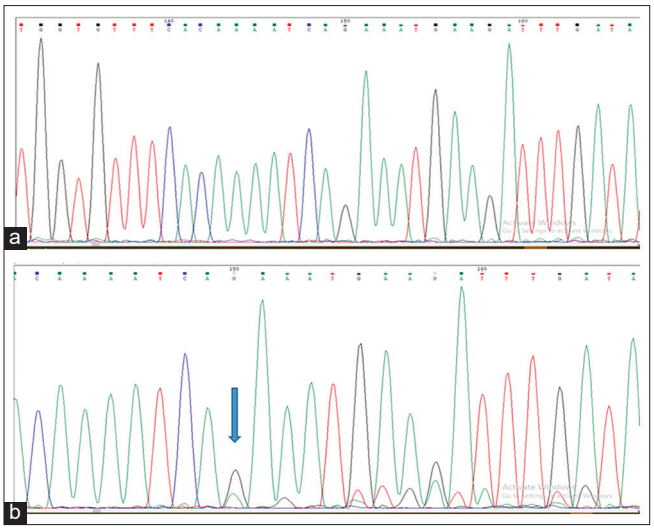

Figure 2: Polymerase chain reaction product of sequencing for JAK2 Exon12 detection of patients with polycythemia vera. (a) JAK2 Exon12 sequencing shows no mutation in a female patient with polycythemia vera. (b) JAK2 Exon12 sequencing shows JAK2R541-E543delinsK mutation in a female patient with polycythemia vera

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 16 (IBM, USA, New York) was used for data analysis. Chi-square and independent *t*-tests were used to analyze the clinical findings of the patients. $P < 0.05$ was considered statistically significant.

Results

Comparison of JAK2^{V617F} mutation with the laboratory and demographic parameters

Among 120 patients with PV, 94 (78.3%) were positive for JAK2^{V617F} mutations, and 26 (21.7%) were negative. There was also a significant relationship between the JAK2^{V617F} mutation and Hb, WBC, and PLT counts ($P < 0.05$). However, no significant relationship was found between JAK2^{V617F} mutation and age, sex ($P > 0.05$) [Table 1].

Comparison of JAK2 Exon12 mutation with the laboratory and demographic parameters

Among 26 patients without JAK2^{V617F} mutation, 2 (7.7%) (one male and one female) were positive for JAK2 Exon12 mutation, and 24 patients (92.3%) were negative. Therefore, the analysis of clinical findings and patient information was not possible due to a low number of positive cases.

Discussion

MPNs are the hematologic disorders created by the extensive proliferation in multinucleated myeloid cells, including CML, PV, ET, and PMF malignancies. The main genetic alteration in CML is the Philadelphia chromosome, which produces the *BCR / ABL1* fusion gene and is the target of imatinib therapy. The molecular basis of other myeloproliferative disorders was unclear before reporting

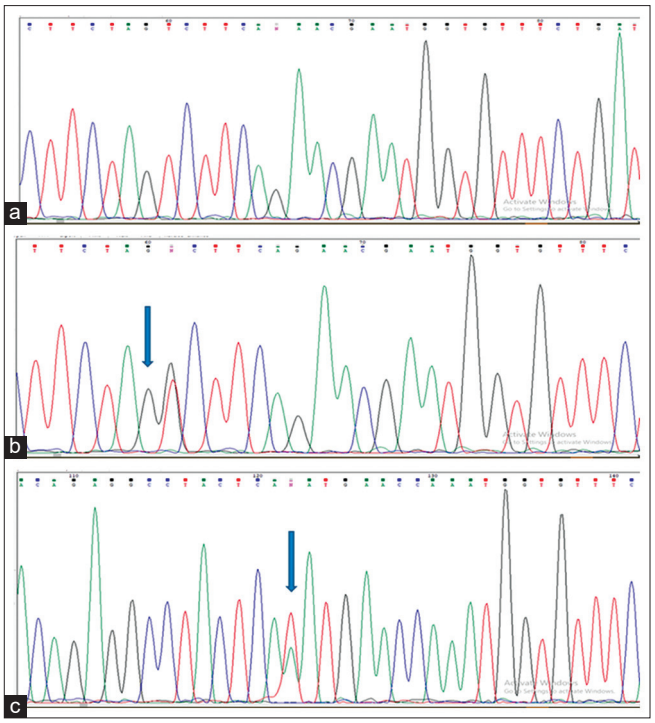

Figure 3: Polymerase chain reaction product of sequencing for JAK2 Exon12 detection of patients with polycythemia vera. (a) JAK2 Exon12 sequencing shows no mutation in a male patient with polycythemia vera. (b) JAK2 Exon12 sequencing shows JAK2V 511 G mutation in a male patient with polycythemia vera. (c) JAK2 Exon12 sequencing shows JAK2H531Q mutation in a male patient with polycythemia vera

the JAK2^{V617F} mutation. Recently, new mutations have been described in exon 12 of the JAK2 gene, which is seen in PV patients with negative JAK2.[14] In general, several mutations in Exon 12 alleles have been reported to date.[15] This group of mutations affects the amino acid residue F537-E543, located in the proximal region of the second JH2 in JAK2, triggering EPO, and causes myeloproliferative changes in the patients.[16] In this study, we evaluated the rate of Jak2 Exon 12 mutation in JAK2 negative patients with PV.

In the present study, out of 120 patients with PV, 94 (78.3%) patients were positive for JAK2^{V617F} mutation and 26 (21.7%) patients were negative for this mutation. To evaluate JAK2 Exon 12 among JAK2^{V617F} negative patients, 2 (7.7%) were positive for JAK2 Exon 12 mutation and 24 (92.3%) patients were negative for this mutation. Several studies to date have investigated the prevalence of Exon 12 mutation in patients with PV. Scott *et al*., in 2007 on 114 patients with PV, identified the JAK2^{V617F} mutation in 110 (96%) patients and the Exon 12 mutation in the 4 remaining patients.[17] Furthermore, in 2007, Pardanani *et al.,* in one study on 220 PV patients, identified JAK2^{V617F} mutation in 214 (97%) and Exon 12 mutation in 5 (2%) of PV patients.[15] Butcher *et al*., as well as Cario *et al*., in 2008, in a study on 62 adult patients and 8 children with PV, found the JAK2^{V617F} mutation in 59 (95%) and 6 (75%) patients and Exon 12 mutation in 3 (5%) and 2 (25%) patients, respectively.[16,18]

Furthermore, in other studies in 2009 in a higher number of patients, Schnittger *et al*. studied on 409 and Bernardi *et al*. on 190 PV patients. The JAK2^{V617F} mutation was negative in both studies, but the Exon 12 mutations were reported 15.9% and 2.6%, respectively.[19,20] Then, in 2010, Siemiatkowska *et al*., Yeh *et al.,* and Zhang *et al*., in separate studies, examined 46, 22, and 89 PV patients and found the JAK2^{V617F} mutation in 41 (89%), 17 (77%), and 73 (82%) patients, respectively. They also observed Exon 12 mutation in 2 (4%), 5 (23%), and 3 (3%) of PV patients, respectively.[14,21,22] In 2007, Passamonti *et al*., in a study on 228 PV patients, reported JAK2^{V617F} mutation prevalence in 320 (95%) patients and Exon 12 in 14 (4%) of PV patients.[15] However, in a recent report, Tefferi *et al*., in 2017, studied 397 patients with PV and reported the JAK2^{V617F} and Exon 12 mutations, 91.6% and 8.3%, respectively.[23] On the other hand, Ibrahim *et al*., in 2019, on 83 patients with PV, showed that the prevalence of JAK2^{V617F} and Exon 12 mutation was 91% and 8.1% in PV patients.[24] In the present study, the Exon 12 mutation prevalence was reported to be 7.7% that was close to other studies.

For the Exon 12 type of mutation and the amino acid involved, one male with JAK2H531Q and JAK2V 511 G mutations and one female with JAK2R541-E543delinsK mutation were observed in the present study. For the first time in 2007, Scott *et al*. reported N542-E543del and F537-K539delinsL mutations in Exon 12.[17] In 2008, Butcher *et al*. reported a JAK2I540 – E543delinsMK mutation in Exon 12. Furthermore, Cario *et al*. in 2008 reported JAK2H538-K539delinsI as a new mutation in Exon 12.[18] Moreover, in 2008, Bernardi *et al*. reported a new I540-N542delinsS mutation in Exon 12 in PV patients.[20] In 2009, Schnittger *et al*. reported D544-L545del, H538DK539LI540S, H538-K539del, and V536-F547dup as four new mutations in Exon 12.[19] Then, in 2010, Yeh *et al*. identified three new variants of N542-E543del, 1 F537-K539delinsL, and 1I540-E543delinsKK mutations in patients with PV.[21] On the other hand, Leszczynska *et al*. in 2016 showed new H538-K539delinsL, E543-D544del, and N542-E543del mutations in Exon 12 of PV patients, which the prevalence of Exon 12 mutation was 4.4% in these patients.[25] In a recent study in 2016, new F537_K539delinsVL and H538_R541delinsLII mutations were observed in Exon 12 of PV patients.[26] However, in 2017, Tefferi *et al*. reported different mutations of H538-K539delins, F537-K539delins, N542-E543delins, E543-D544del, K539L, R541-E543delins, E543delinsMK, and H538-I540del (Ex 242I39 and Ex 374239). Therefore, due to the diversity of mutations in JAK2 Exon12, in several studies, the present study observed JAK2R541-E543delinsK and JAK2V 511G as conventional mutations and JAK2H531Q as a new mutation.

In this study, by examining the relationship between the prevalence of JAK2V617F mutation and demographic parameters, a significant relationship was found between this mutation with Hb, WBC, and PLT parameters ($P < 0.05$). However, no significant relationship was found between the prevalence of JAK2^{V617F} mutation, and age, sex parameters ($P > 0.05$) [Table 1]. Schnittger *et al.* in 2009 showed that there was a significant relationship between the JAK2 Exon12 mutation prevalence and the sex, age of patients, and the JAK2 Exon12 mutation was more common in females and younger age.[19] Then, Yeh *et al*. in 2010 showed that there was a significant relationship between WBC, PLT counts, and JAK2^{V617F} mutation and between erythrocytosis and JAK2 Exon12 mutation in PV patients.[21] Furthermore, Passamonti *et al*., in 2007 found that in patients with JAK2 Exon12 mutation, there was a significant relationship between erythrocytosis, higher Hb, and lower WBC, PLT counts.[15] However, Park *et al*. in 2016 showed a significant relationship between JAK2^{V617F} mutation, and lower WBC, PLT count in PV patients.[26] Tefferi *et al*., in 2017, observed a significant relationship between JAK2^{V617F} mutation and age, WBC, and PLT parameters.[23] On the other hand, Ibrahim *et al*., in 2019, found that higher WBC and PLT counts were significantly associated with JAK2^{V617F} mutation prevalence. They also found a significant relationship between higher Hematocrit (HCT) levels and the JAK2 Exon12 mutation.[24] Therefore, in the present study, as in most studies, there was a significant relationship between Hb, WBC, PLT, and JAK2^{V617F} mutation. However, due to the low JAK2 Exon12 positive cases, it was not possible to investigate its association with the patient's laboratory parameters.

Conclusions

According to the results of the present study and other studies, there was a significant relationship between Hb, WBC, PLT, and JAK2^{V617F} mutation. Furthermore, JAK2 Exon12 mutation was reported in most studies such as our study. The more prevalent cases of this mutation were reported in higher statistical populations in other studies. The large variation in the JAK2 Exon12 mutation types in different ethnicities of the reported studies can be due to the use of different molecular methods. Recent studies have suggested the use of sequencing as a more appropriate method for finding JAK2 Exon12 mutation, so this method was used in the current study to evaluate the prevalence of JAK2 Exon12 mutation. In this study, JAK2R541-E543delinsK was observed as a conventional mutation and JAK2V 511G as a new mutation in PV patients. Although the present study was not statistically comparable with the laboratory parameters of patients due to the limited number of positive cases in JAK2 Exon12 mutation, in most studies, JAK2 Exon12 mutation was significantly associated with higher erythrocytosis, Hb, and HCT. Therefore, further studies and higher statistical populations are needed to investigate the extent, gene diversity, and relationship between the JAK2 Exon12 mutation and the laboratory parameters.

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Conflicts of interest

There are no conflicts of interest.

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