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JAK2 V617F mutation in Iranian patients with myeloproliferative neoplasms: clinical and laboratory findings

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Aim: The JAK2 V617F mutation has been described as a frequent genetic event among a majority of patients with polycythemia vera, essential thrombocythemia, and myelofibrosis. In this research, we evaluated the prevalence of the JAK2 mutation and its clinical and laboratory correlation in patients with myeloproliferative neoplasms.

Materials and methods: A total of 615 patients with suspected myeloproliferative neoplasms (MPNs) were analyzed for the JAK2 V617F mutation. After DNA extraction, detection of the mutation was done using allele-specific PCR. Positive samples were subsequently analyzed with PCR-RFLP by the restriction endonuclease BsaXI. The patients were also analyzed for hematological indices.

Results: Of 615 patients, 175 (28.4%) patients were positive for the JAK2 V617F mutation, whereas 440 (71.6%) patients were negative. The positive group included 79 (45.1%) patients with essential thrombocythemia, 62 (35.4%) patients with polycythemia vera, 27 (15.4%) patients with primary myelofibrosis, and 7 (4%) patients with unclassified MPNs.

Conclusion: The frequency of the JAK2 mutation in our study is compatible with previous reports. JAK2 V617F mutation screening can be incorporated in the initial evaluation of patients suspected of having MPNs. The relationship between the JAK2 mutation and hematological indices can be used in new diagnostic and therapeutic strategies.

Key words: JAK2, mutation, myeloproliferative neoplasms

1. Introduction

Myeloproliferative neoplasms (MPNs) are a group of hematological neoplasms that share similar molecular and cellular abnormalities. However, these diseases differ in their phenotypes, clinical presentation, and therapy (1–3). MPNs have been previously called MPDs, or myeloproliferative disorders. However, according to the World Health Organization (WHO) classification, MPN is the most up-to-date term for these diseases (4). This reflects the underlying clonal genetic changes that are a salient feature of this group of diseases. According to the WHO classification, MPNs are divided into 2 large groups: those that possess the BCR-ABL1 fusion protein, such as chronic myeloid leukemia (CML), and those that are BCR-ABL1–negative (5), including polycythemia vera (PV), essential thrombocythemia (ET), myelofibrosis (MF), mastocytosis, chronic neutrophilic leukemia, and chronic eosinophilic leukemia. The latter group of MPNs shares elements of pathogenesis and symptomatology that may be related to dysregulated Janus kinase (JAK) signaling (2). The family of tyrosine kinases, termed Janus kinases (JAKs), has a vital role in signal transduction in cells. JAK2 is essential for the proliferation and differentiation of erythroid and megakaryocytic lineages (6,7). JAK2 plays a role in downstream signaling pathways, such as the JAK/signal transducer and activator of transcription (STAT) pathway that is involved in cytokine signaling. JAK2 possesses 7 defined regions of conserved homology, denoted as JAK homology (JH) domains 1–7 (8). JH2 is a pseudokinase domain that early functional studies show has an inhibitory effect on the JAK2 kinase domain (9–11).

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The clinical presentation and laboratory findings of these diseases are variable, but common elements can include splenomegaly, increased risk of thrombosis and/or bleeding, overproduction of one or more of the myeloid cell lines, bone marrow hypercellularity with or without fibrosis, debilitating symptoms, and the potential to progress to secondary acute myelogenous leukemia (12). MPNs are relatively rare conditions. In the United States there are about 95,000 patients with PV, about 80,000 patients with ET, and about 16,000 to 18,500 patients with MF. These diseases usually affect older adults; the average age of onset is 61 (13).

In 2005, 4 independent research groups identified a single acquired mutation in the JAK2 gene on chromosome 9 that had a high incidence of occurrence in patients with PV, ET, or MF (14–17). The point mutation in exon 14 of JAK2 alters codon 617 from a valine to a phenylalanine. This amino acid alteration in the JH2 domain of JAK2 causes a constitutive activation of the tyrosine kinase, which is believed to confer erythropoietin hypersensitivity and erythropoietin-independent survival to the myeloid stem cell (18). This mutation is a gain of function mutation, in that it releases the autoinhibitory action of JH2 and recruits STAT in the complete absence, or in presence of only trace quantities, of hematopoietic growth factors (19–21).

The detection of the JAK2 V617F mutation provides a qualitative diagnostic parameter for the identification of the nonchronic myelogenous leukemia subgroup of MPNs. Different groups reported a variable frequency of JAK2 V617F mutation ranging from 65%–97% for PV, 23%–57% percent for ET, and 35%–%57 percent for MF (14,16,22,23). In this study, we evaluated the prevalence of the JAK2 mutation and its clinical and laboratory correlations in patients with MPNs.

2. Materials and methods

2.1. Patients

Included in this study were 615 patients with suspected MPNs that were referred by physicians for JAK2 V617F mutation analysis. The necessary investigations, including measurements of white blood cell (WBC) count, red blood cell (RBC) count, platelet (Plt) count, WBC differential count, hemoglobin (Hb), hematocrit (Hct), and clinical characteristics, were performed whenever required. Local ethical approval for the study protocol was obtained, and written informed consent was taken from all patients.

2.2. DNA extraction

We took a blood sample (10 mL) from every patient. Granulocytes were separated from peripheral blood, and genomic DNA was extracted using conventional phenol/chloroform protocol after proteinase K digestion following standard published protocols. DNA was quantified using spectrophotometric measurements.

2.3. Analysis of the JAK2 V617F mutation

All DNA samples were genotyped for the JAK2 V617F mutation by an allele-specific (ASO) polymerase chain reaction (PCR), exactly as described by Baxter et al. (14), in which 1 μmol/L of a common reverse primer, 0.5 μmol/L of a forward primer specific for the mutant allele (giving a 203-bp product), and 0.5 μmol/L of another forward primer amplifying a 364-bp product from both mutant and wild type alleles, which also serves as an internal PCR control, were used. Samples that were positive for the mutation were subsequently analyzed via PCR-restriction fragment length polymorphism (PCR-RFLP) with the restriction endonuclease BsaXI (New England Biolabs, Hitchin, UK), which allows for estimation between mutated and wild-type alleles. Successful amplification was confirmed by electrophoresis on an ethidium bromide-impregnated 2% agarose gel. The G-T mutation destroys a BsaXI site present in the wild-type JAK2 sequence. This approach allows both normal and mutant alleles to be visualized and can be distinguished between homozygous and heterozygous mutations.

2.4. Statistical analysis

Comparison of clinical characteristics between cases with and without JAK2 V617F mutation was done using the Mann–Whitney U test (Wilcoxon rank sum test) for WBC and Plt count, the t-test for age and Hb, and the chi-square test for sex, using SPSS 11. $P < 0.05$ was considered statistically significant.

3. Results

The JAK2 V617F mutation was analyzed in 615 patients with suspected MPNs that were referred by physicians for JAK2 V617F mutation analysis. In this group, 175 (28.4%) of the patients were positive for the JAK2 V617F mutation, whereas 440 (71.6%) patients were negative for the JAK2 V617F mutation. The JAK2 V617F mutation-positive group (175 patients) included 79 (45.1%) patients with ET, 62 (35.4%) patients with PV, 27 (15.4%) patients with primary myelofibrosis (PMF), and 7 (4%) patients with unclassified MPNs. The JAK2 V617F mutation-negative group (440 patients) included 94 (21.4%) patients with ET, 61 (13.8%) patients with non-MPN polycythemia, 41 (9.3%) patients with MF, 4 (0.9%) patients with CML, 11 (2.5%) patients with thrombocytopenia, 3 (0.7%) patients with hairy cell leukemia, and 226 (51.4%) normal referred patients. Other patient characteristics, including the sex and age of both positive and negative groups, are shown in Table 1 and Table 2, respectively. The mean ages of JAK2 V617F-positive patients were 59.86 ± 14.53 years (range: 21–88) in males and 57.14 ± 16.08 years (range: 23–85) in females, and those of the JAK2 V617F-negative patients were 43.29 ± 17.74 years (range: 4–91) in males and 48.12 ± 18.96 years (range: 6–82) in females.

Table 1. Characteristics of patients with the JAK2 mutation.

Disease	Number of patients (%)	Male/female, n	Male mean age (range)	Female mean age (range)
ET	79 (45.1)	40/39	60.93 ± 14.48 (22–85)	57.28 ± 16.64 (23–80)
PV	62 (35.4)	31/31	58.82 ± 14.12 (28–88)	56.74 ± 14.64 (23–84)
PMF	27 (15.4)	18/9	61.39 ± 12.43 (38–85)	55.22 ± 18.95 (28–85)
Unclassified MPNs	7 (4)	6/1	55.17 ± 23.58 (21–84)	81
Total	175 (100)	95/80	59.86 ± 14.53 (21–88)	57.14 ± 16.08 (23–85)

Table 2. Characteristics of patients without the JAK2 mutation.

Disease	Number of patients (%)	Male/female, n	Female mean age (range)	Male mean age (range)
ET	94 (21.4)	46/48	48.94 ± 18.93 (14–82)	48.54 ± 19.12 (11–89)
CML	4 (0.9)	1/3	58.33 ± 16.80 (40–73)	70
PMF	41 (9.3)	19/22	57.05 ± 14.03 (77–32)	64.26 ± 18.49 (19–91)
Polycythemia, non-MPN	61 (13.8)	49/12	34.75 ± 18.05 (16–65)	42.41 ± 16.96 (48–65)
Thrombocytopenia	11 (2.5)	6/5	48.4 ± 22.6 (30–76)	49.17 ± 9.75 (32–61)
Hairy cell leukemia	3 (0.7)	2/1	65	49.5 ± 0.71 (49–50)
Normal referred patients	226 (51.4)	171/55	45.85 ± 19.33 (6–81)	39.36 ± 15.72 (7–78)
Total	440 (100)	294/146	48.12 ± 18.96 (6–82)	43.29 ± 17.74 (4–91)

Both positive and negative groups were analyzed for hematological indexes, including WBC count, RBC count, Plt count, WBC differentiation count, Hb, and Hct. The overall presence of the JAK2 V617F mutation in the positive group was associated with a higher WBC count ($P < 0.01$) in JAK2-positive men than JAK2-negative men, whereas there was no statistical difference in women. RBC count, Hb, and Hct were higher in JAK2-positive women than JAK2-negative women ($P < 0.01$), but RBC count, Hb, and Hct did not differ in JAK2-positive and -negative men. Plt count was higher in both JAK2-positive men and women than in the negative group ($P < 0.01$). In a differential WBC count, the neutrophil count was higher in both JAK2-positive men and women than in the negative group ($P < 0.01$). However, the lymphocyte count was lower in both JAK2-positive men and women than in the negative group ($P < 0.01$). Hematological indexes including WBC count, RBC count, Plt count, WBC differentiation count, Hb, and Hct in the positive and negative groups are shown in Tables 3 and 4.

Table 3. Hematological parameters in JAK2-positive and -negative patients.

		WBC	RBC	HB	HCT	Plt	Basophils	Eosinophils	Monocytes	Lymphocytes
Positive patients	ET									
	mean ± SD	11.69 ± 5.47	5.96 ± 3.74	14.40 ± 1.64	44.02 ± 7.72	867.9 ± 332.58	1.41 ± 1.08	3.7 ± 1.97	3.83 ± 2.19	20.57 ± 10.14
	Median	10.2	5.57	14.3	43.3	803	1	3	4	19
	(range)	(5.8–43.8)	(3.54–37.7)	(9.5–17.5)	(2.3–19.2)	(355–2571)	(0–7)	(1–8)	(0–8)	(4–48)
	PMF									
	mean ± SD	18.54 ± 18.36	4.41 ± 1.28	11.99 ± 2.27	37.59 ± 6.45	296.63 ± 158.82	1.56 ± 0.81	3.27 ± 2.98	4.71 ± 6.18	17.22 ± 10.89
	Median	12.6	4.3	12.3	37.8	293	1	3	3.5	14
	(range)	(3.5–85.5)	(2.14–7.72)	(5.5–15.6)	(20–46.6)	(44–677)	(1–4)	(1–15)	(1–32)	(1–42)
	PV									
	mean ± SD	13.21 ± 5.58	7.23 ± 1.15	18.02 ± 1.48	54.59 ± 8.01	515.82 ± 227.98	1.46 ± 1.27	2.95 ± 2.07	3.02 ± 1.62	17.10 ± 8.22
	Median	12.15	6.95	17.8	55.15	512.5	1	3	3	18
	(range)	(4.7–29.4)	(5.27–9.91)	(14.1–21.6)	(2.9–65.8)	(151–1028)	(0–6)	(0–8)	(0–7)	(2–40)
	MPN									
	mean ± SD	8.12 ± 2.41	5.43 ± 0.63	15.3 ± 0.97	45.3 ± 1.11	289.67 ± 87.64	1.33 ± 0.58	2.75 ± 0.96	3.75 ± 1.89	19 ± 7.62
	Median	7.5	5.27	15.25	45.8	261	1	2.5	4.5	16.5
	(range)	(5.6–12.1)	(4.63–6.42)	(13.7–16.6)	(43.3–46.2)	(191–436)	(1–2)	(2–4)	(1–5)	(13–30)
Negative patients	ET									
	mean ± SD	11.48 ± 7.82	5.17 ± 1.75	13.05 ± 2.3	43.75 ± 40.94	874.89 ± 370.26	1.34 ± 0.98	3.14 ± 2.03	4.96 ± 2.58	26.45 ± 12.46
	Median	9.3	4.82	13.35	40.3	776	1	3	5	25
	(range)	(4.3–49)	(2.62–15.97)	(8.6–19.4)	(24.4–432)	(461–2350)	(0–5)	(1–8)	(1–15)	(1–52)
	CML									
	mean ± SD	113.03 ± 36.33	3.99 ± 0.67	10.78 ± 1.26	34.53 ± 3.5	475 ± 417.86	5 ± 6.08	3.5 ± 0.71	6 ± 4.25	7.67 ± 1.53
	Median	112.9	3.97	10.7	33.75	391.5	2	3.5	6	8
	(range)	(71–155)	(3.3–4.73)	(9.6–12.1)	(31.6–39)	(63–1054)	(1–12)	(3–4)	(3–9)	(6–9)
	PMF									
	mean ± SD	15.51 ± 20.53	4.01 ± 1.01	10.56 ± 1.97	33.16 ± 6.17	233.51 ± 169.6	2.5 ± 2.77	3.21 ± 2.83	6.63 ± 7.79	21.35 ± 14.16
	Median	6.4	3.97	10.4	33	247	1	2	5	19
	(range)	(1.2–95.4)	(0.5–15.54)	(6.9–15.7)	(22.1–52.5)	(25–663)	(0–11)	(1–14)	(1–40)	(5–59)
	Polycythemia non-MPN									
	mean ± SD	7.89 ± 3.04	6.74 ± 0.92	18.82 ± 1.82	55.98 ± 4.37	205.18 ± 74.14	0.91 ± 0.53	3.21 ± 2.22	4.16 ± 2.55	34.82 ± 14.36
	Median	7.2	6.5	18.7	54.9	213	1	3	3.5	33.5
	(range)	(2.9–17.7)	(5.55–9.27)	(9.5–23.3)	(49.6–69.3)	(20–403)	(0–2)	(0–10)	(0–11)	(3–93)
	Thrombocytopenia									
	mean ± SD	10.52 ± 15.54	4.96 ± 0.78	14.25 ± 2.32	41.84 ± 5.94	58.55 ± 21.34	1.8 ± 2.39	3.05 ± 2.22	4.16 ± 2.55	34.82 ± 14.36
	Median	5.7	4.61	14.1	39.9	61	1	3	3.5	33.5
	(range)	(2.1–56.3)	(3.61–6.4)	(9.5–17.7)	(32.6–51.3)	(19–96)	(0–6)	(0–10)	(0–11)	(3–93)
	Normal									
	mean ± SD	7.64 ± 2.81	5.53 ± 1.04	15.81 ± 2.04	46.45 ± 4.73	244.94 ± 98.18	0.91 ± 0.60	3.89 ± 8.67	4.89 ± 2.25	32.86 ± 10.91
	Median	7	5.59	16.5	47.7	223	1	2	5	32
	(range)	(2.4–21)	(0.5–15.54)	(7–23.9)	(25.8–60.6)	(57–971)	(0–4)	(0–88)	(0–11)	(1–76)

Table 4. Comparison of hematological parameters between male and female patients with the JAK2 mutation. **: P < 0.01.

	Male JAK2 ^{V617F} (+)	Male JAK2 ^{V617F} (-)	Female JAK2 ^{V617F} (+)	Female JAK2 ^{V617F} (–)
Age, mean ± SD (range)	59.86 ± 14.53 (21–88)	43.29 ± 17.74 (4–91)	57.14 ± 16.08** (23–85)	48.12 ± 18.96 (6–82)
WBC ($\times 10^9$/L), mean ± SD Median (range)	13.51 ± 10.85** 10.5 (4.1–85.5)	9.15 ± 9.29 7.3 (2.1–128)	12-.77 ± 6.32 11.15 (3.5–39)	12.46 ± 18.65 7.85 (1.2–155)
RBC, mean ± SD Median (range)	5.94 ± 1.56 5.89 (2.14–9.91)	5.69 ± 1.29 5.69 (0.5–15.97)	6.4 ± 3.8** 6.15 (3.01–37.7)	4.94 ± 1.42 4.72 (2.05–14.63)
HB (g/dL), mean ± SD Median (range)	15.38 ± 2.97 15.65 (5.5–21.6)	15.92 ± 2.68 16.7 (6.9–21.2)	15.32 ± 2.47** 15.1 (9.7–21.1)	13.27 ± 3.058 13.3 (7.4–23.9)
HCT, mean ± SD Median (range)	46.52 ± 9.67 46.7 (2.9–65.8)	48.27 ± 23.53 48.3 (22.1–432)	47.2 ± 9.82** 46.2 (2.3–79.2)	40.47 ± 8.58 40.05 (24.1–69.3)
Plt ($\times 10^9$/L) mean ± SD Median (range)	605.91 ± 391.254** 532.5 (101–2571)	321 ± 283.87 229 (19–1697)	666.7 ± 299.117** 683.5 (44–1311)	466.72 ± 394.96 354.5 (25–2350)
Lymphocytes, mean ± SD Median (range)	17.31 ± 8.64** 17 (1–42)	31.82 ± 13.72 32 (1–93)	20.24 ± 10.48** 20 (1–48)	27.73 ± 12.16 27.5 (4–60)
Neutrophils, mean ± SD Median (range)	67.26 ± 13.94** 69 (6–88)	56.14 ± 13.43 56 (3–91)	67.31 ± 12.18** 65.5 (18–90)	59.32 ± 13.58 61 (6–89)

4. Discussion

It has been recently shown that the majority of patients with PV, and substantial numbers of patients with PMF and ET or other MPNs, carry a single nucleotide mutation in the JAK2 gene (14–16,23–25). Since determination of the JAK2 point mutation contributes to the diagnosis of these diseases, elucidation of JAK2 V617F mutation is profitable and could be helpful in therapeutic targets, especially when particular chemotherapeutic agents are used in the treatment of other cancers (26,27). Therefore, in this study, the JAK2 V617F mutation was identified in 615 patients with suspected MPNs that were referred by physicians for this mutation analysis.

In this study the JAK2 V617F mutation was identified in all patients with PV (100%), whereas other studies reported different results, including those of Baxter et al. (97%) (14), James et al. (88%) (15), Levine et al. (74%) (16), Kralovics et al. (65%) (23), Jones et al. (81%) (24), Zhao et al. (83%) (17), and Jelinek et al. (86%) (25). The most prevalent JAK2 mutation in PV was reported by Baxter et al. (97%), where they used an allele-specific PCR method, whereas the lowest reported prevalence was from the study of Kralovics et al. (65%), in which they used microsatellite mapping and DNA sequencing methods for JAK2 mutation detection. Our study showed a higher frequency of JAK2 V617F mutation in patients with PV (100%) than that reported in the West. This difference could be attributed to ethnic variation or the sampling population, but it needs confirmation by further research. In this study, patients with PV showed a higher WBC

count ($P < 0.01$). This finding is consistent with previous studies, such as that of Speletas et al. (28), which showed a higher WBC count ($P = 0.02$) in patients with PV.

We have identified the JAK2 V617F mutation in approximately 79 (45.6%) of patients with ET. Similar studies reported different results, including those of Baxter et al. (57%) (14), James et al. (43%) (15), Levine et al. (32%) (16), Kralovics et al. (23%) (23), Jones et al. (41%) (24), and Jelinek et al. (30%) (25). Campbell et al. evaluated 806 patients with ET for JAK2 mutation in 2005 (29). They showed that 414 (53.4%) of the patients were positive for the JAK2 mutation and 362 (46.6%) were negative. They also reported that patients who were positive for the JAK2 mutation had a higher Hb level ($P < 0.0001$) and neutrophil count ($P < 0.0001$) than the negative group. However, our results did not show any statistical discrepancy in Hb level ($P > 0.1$) or WBC count ($P > 0.1$) in the JAK2 mutation positive group as compared to the negative group. This may be related to the difference in the population of patients in our study and the study of Campbell et al.

In this study, the JAK2 V617F mutation was identified in 27 patients with PMF (39.7%); nevertheless, other studies reported different results for this mutation in patients with PMF, including those of Baxter et al. (50%) (14), James et al. (43%) (15), Levine et al. (35%) (16), Kralovics et al. (57%) (23), Jones et al. (43%) (24), Jelinek et al. (95%) (25), and Campbell et al. (29). Recent research has shown the diagnostic and prognostic importance of hematological markers in various diseases (30,31). The PMF patients with the JAK2 positive mutation have a higher WBC and neutrophil count compared to JAK2 negative mutation patients with PMF, but platelet count, spleen size, and Hb level did not show any difference between JAK2-positive and -negative patients with PMF. In our study, patients with PMF showed a higher WBC count ($P = 0.07$) and Hb level ($P = 0.007$) in patients with JAK2 positive mutation as compared to patients with JAK2 negative mutation, but Plt count ($P = 0.12$) did not show any difference between JAK2-positive and -negative patients with MF. Jelinek et al. (25) used a pyrosequencing method for JAK2 mutation analysis in patients with PMF and reported the highest prevalence (95%), unlike Levine et al. (16), who reported the lowest prevalence in PMF patients with JAK2 mutation.

Briefly, this study has presented the importance of peripheral blood mutation screening for JAK2 V617F in the initial evaluation of patients with suspected MPNs. JAK2 mutation analysis is a sensitive and simple test, relatively cost-effective for proving the nature of these diseases. It also helps in eliminating a large number of secondary causes. However, since the mutation may be absent in some cases of MPNs, it cannot be used as a single test for making the diagnosis. It should be done in addition to other hematological or biochemical tests. JAK2 mutation screening can also be used for other indications such as unexplained erythrocythemia, thrombocytosis, and uncommon thrombotic complications. Finally, detection of this mutation may have an important role in the treatment of patients who would respond to JAK2 inhibitor therapy.

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