

## The Association between Prevalence of JAK2V617F Mutation and Blood Indices in Groups of Patients with Myeloproliferative Neoplasms in Rasul Akram Hospital

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### Abstract

**Introduction:** Myeloproliferative neoplasms (MPNs) are a group of clonal malignant hematologic disease, where the main and common members are; polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocytosis (ET). These group of diseases are able to be transformed into each other.

**Methods:** This cross sectional study conducted the evaluation of JAK2V617F mutation in DNA in peripheral blood of 91 patients with known or suspected diagnosis of MPNs by Real time PCR method and survey of peripheral blood smear.

**Result:** Prevalence of JAK2V617F mutation between patients was %58.2 and PV had most common prevalence ratio between other groups. While mean age of patients was 50/9 yr (for 39.6% male and 60.4% female), three patients (equal of %3.3) were atypical presentation and 2 patients die due to malignant transformation. There were significant differences in age, WBC and PLT (in PV) with prevalence of JAK2V617F mutation. These differences were not significant in other group.

**Discussion:** Current study showed a high rate of association between JAK2V617F mutation in patients with PV, ET, PMF in Iranian patients. Therefore, Peripheral blood mutation screening for JAK2 V617F can be incorporated into the initial evaluation of patients suspected to have chronic myeloproliferative neoplasm and used of this test for determining of association between JAK2V617F mutation, treatment of patients with blood indexes and patients of prognosis.

**Keywords:** JAK2V617F mutation, Myeloproliferative neoplasms, PV, ET, PMF

### Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal malignant hematologic disease. With main and common members of; polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocytosis (ET). (1, 2)

MPNs are due to malignant transformation of multi potential stem cell. (1, 3)

This mutation is a non receptor intracellular tyrosine kinases (TK) with key role in intracellular cytokines. (3) Activation JAK2 mutation can be explained as: erythropoietin– independent erythroid clonal formation and their resistance to apoptosis in-vitro in the absence of erythropoietin and hyper proliferation of other myeloid series. (4, 5, 6) Thus JAK2 mutation is a molecular change for progression of MPNs. (7, 8) We studied prevalence of JAK2V617F mutation in groups of patients with

clinical feature compatible with non CML (Chronic Myeloid Leukemia) in Myeloproliferative neoplasms (MPNs) included: polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocytosis (ET).

### Materials and methods

This research is a cross sectional study, on 91 patients with non CML MPNs.

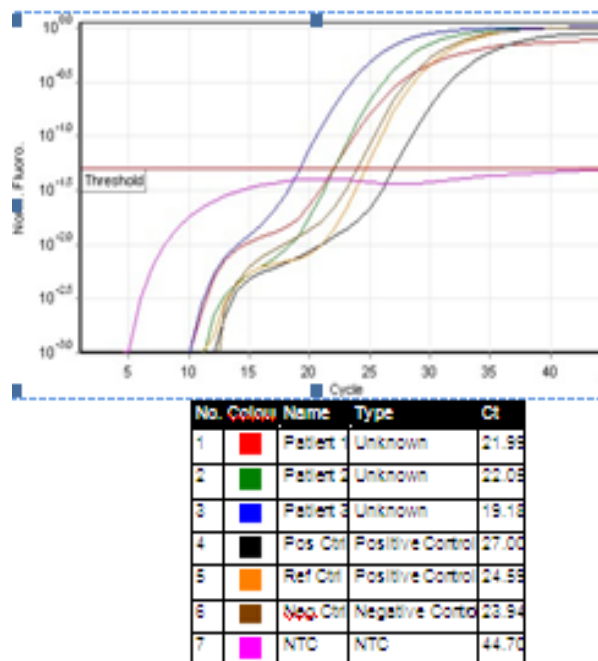
Inclusion criteria:

1. PV: male hemoglobin (Hb) > 18.5g/dl/ Female Hb > 16.5g/dl and /or WBC >  $10.8 \times 10^9/L$
2. ET: PLT >  $450 \times 10^9/L$ , in the absence of reactive thrombocytosis.
3. PMF: Bone marrow aspiration and biopsy compatible with fibrosis and leukoerythroblastic feature in peripheral blood smear and

splenomegally, no feature for: PV, ET and other neoplasms.(9)

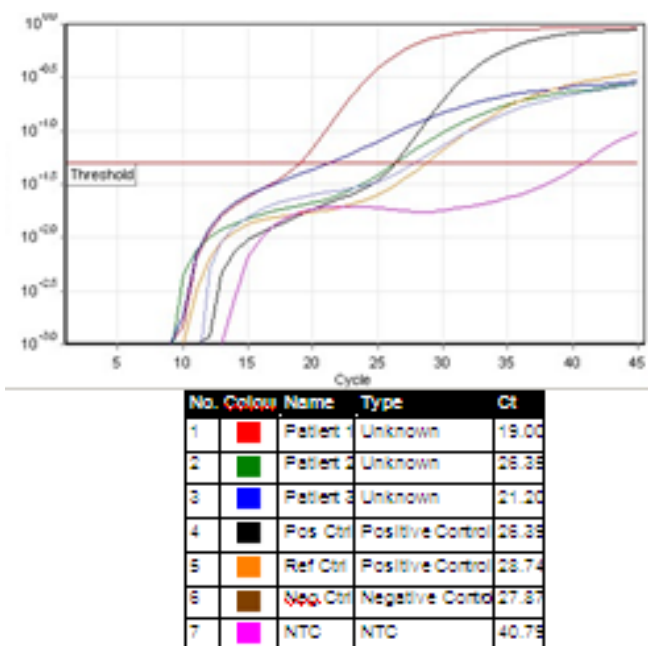
4. Patient with MPNs (new or older)

**JAK2 Normal Allele: Quantitation data for Cycling A Yellow**



**Fig.-1)**

**JAK2V617F Mutant Allele: Quantitation data for Cycling A Green**



**Fig.-2)**

**Figure- 1, 2: Result of diagnosis JAK2V617F mutation with tagmas allelic discrimination method.**

**Table-1-Temperature and cycling in PCR reaction for recognition of JAK2V617F.**

Cycles	Temperature	Time	Stages
1 cycle	95° c	10min	Primary Denaturation
50 cycle s	95°c	15 sec	Denaturation
50 cycles	60°c	60 sec	Connection

Exclusion criteria included:

1. Secondary erythrocytosis
2. Reactive thrombocytosis
3. Myelofibrosis due to other cause (bone marrow metastas, rheumatologic disease, chronic infection.

Five to ten milliliters of fresh peripheral blood collected in EDTA (Ethylene diamine tetracetic) tube and centrifuged with lab net, then WBC from buffy-coat layer to separate for DNA extraction. Lab kit DNA selection was QIA AMP DNA blood mini kit from Qiagen German Company (Table - 1). After cellular lysis DNA was extracted and used for diagnosis of JAK2V617F mutation with real time PCR methods - this method is highly sensitive (100% ) and specify (98- 99% )for detection of multiplication of JAK2V617F gene to do with tagman allelic discrimination method and rotor gene 6000 system (corbet company australia). For confirmation of result we have four control group included: positive control group, referral control (lowest diagnostic level), negative control (DNA without mutation) and control group without DNA pattern. Overall, patients with fluorescent signal equal or higher than referral control approved for positive JAK2V617F mutation in this method. The exist specific prop for type of mutation heterozygote or homozygote: positive result by increasing of signal in real time system canal. With this method complete patient with positive mutation were homozygote (Figures 1, 2)

### Result

In this cross sectional research we assay JAK2V161F mutation in 91pateints, with known or suspected diagnosis of a non CML MPNs. The collected data from patients' and lab test result analyzed by the SPSS-version 16 statistical program by chi- square tests (for comparison of qualitative variables) and Oneway ANOVA (for quantitative variables and comparison between groups). Thirty-six patients were male (%39.6) and 55 were female (60.4%), mean age was 50.9y ranging from 35.2 to 66.6 years. Forty-three (47.3%)of them were with diagnosis PV, 40 (44%) of those ET and 8 (8.8%) of they were PMF. Mean percentage of hematocrit in PV group was 52%± 7.5% and in ET 40.2± 7.6% and in PMF equal to 31.3± 4.8% (Significant difference between three group: P- value= <0.001). JAK2V161F mutation was detected in %58.2 patients and Prevalence of JAK2V617F mutation was 76%, 45% and 68% in PV, ET, PMF respectively. There was significant difference in JAK2V617F mutation Prevalence between three

**Table- 2: Comparison Jak2 mutation status and laboratory feature in patients with non CML, MPN.**

Variables	PV		ET		IMF	
	Jak-2 positive	Jak-2 negative	Jak-2 positive	Jak-2 negative	Jak-2 positive	Jak-2 negative
	33 (76%)	10 (23/4%)	18 (45%)	22 (55%)	5 (62/5%)	3 (37/5%)
*Age(averag) Mean±SD	55/3+14/7	43 +11/1	58/5±17/7	43/3±14/2	48± 11/8	43±11/3
Hct (mean) ±SD	52/7± 8	50/7±5/7	30/3±6/1	41/02±8/7	32/6± 4/8	26/3+5
*WBC(mean)×10 <sup>9</sup> /L	17/2	9/5	10/4	10/9	75/3	7/4
Plt×10 <sup>9</sup> /L	1853	315	1864	381	493	357

P- value refers to the comparison of JAK2V617F mutation positive versus negative subjects. PV: Polycythemia vera; ET: Essential thrombocythemia; IMF: Idiopathic myelofibrosis; P- value<0.009; WBC: White blood cell; Hct: Hematocrit. \*P- value<0.05: Significant.

group (P- value: 0.009) and there was direct correlation between age and leukocytosis and thrombocytosis with positive JAK2V617F mutation in PV patients, (PV: 0.01, 0.008 and 0.010, Respectively), (Table-2).

### Discussion

In this study we assessed prevalence of JAK2V617F mutation on 91 patients in three groups: PV, ET, and PMF.

The main goal of our study was comparison of JAK2V617F mutation prevalence. Accordingly we found a high prevalence of JAK2V617F mutation in patients with PV (76.6%) compared with PMF and ET (68.5% and 45% respectively). The result reported similar to other studies as well, but in comparison with other studies we found high prevalence of JAK2V617F mutation in patients with PMF. A study was done on Iranian patients by Karimzade et al in 2011 in Imam Khomeini hospital on 89 patients, showed the same result- in this study prevalence of JAK2V617F mutation were 86% in PV, 61% in PMF and 53 in ET group and zero percent in CML group,(9) also an Indian study (Sazwal et al.) in 2010 was detected prevalence JAK2V617F between patients with MPD 82% in PV and 52%, 70% in PMF and ET groups (respectively).(10) This should be noted that the frequency of JAK2V617F mutation in PMF was much higher (68%) than what reported in the West (50%),(1) but in our study 8 patients among 91 patients had PMF (low prevalence) hence the associated with these groups was not statistically significant and more confirmation on larger population studies seems to be essential from this region.

As similar to other studies our result shows higher prevalence of JAK2V617F mutation in PV, but the differences observed in this study could be concluded:

1) In present study, applied experimental method for JAK2V617F mutation was Real time PCR method, and determined zygosity. All patients were

homozygote, to note that the frequency of homozygosity was much higher (100%) than that reported in the West (approximately 30% of PV patients and 60% of PMF patients, homozygosity is rare in ET).(1) Since, homozygosity was predominant in our study in all the disease subtypes, it could be possible that as developing country our patients present late in the course of the disease and thus they have a higher allele burden compared to that reported in the West.

2) In other study, applied methods were specific-PCR or AS-PCR and showed little difference in percent frequency in JAK2V617F mutation between our study and other.

3) Higher specific and sensitive ability real time PCR than other methods (Analytical detection 1% JAK2 positive mutation with specificity 98- 99% and sensitive ability 100%).

Present study showed that PV group with positive JAK2V617F mutation have significant relation with higher age, sex, leukocytosis and PLT (PV<0.05), but this correlation is not found in PMF and ET group.

Karimzade et al. study showed similar result in Iranian patients with consideration of high prevalence of JAK2V617F mutation in chronic myeloproliferative neoplasm.(9) In present study two patients (2.2%) with previously diagnose (PV) die due to malignant transformation (progress to ALL), that confirmed with bone marrow aspiration and flow cytometry. Transformation to acute leukemia is uncommon in the absence of exposure to mutagenic agents.(1) Thrombotic (3 patients) or haemorrhage (1 patients) complications was observed in (4.3%) patients that whom were JAK2V617F mutation negative. But in other studies JAK2V617F mutation has been associated with more frequent evolution into secondary myelofibrosis,(11) and higher risk of thrombotic complication.(12)

In summary, our findings support the recommendation that peripheral blood mutation screening for JAK2V617F mutation should be

incorporated into the initial evaluation of patients with suspected chronic myeloproliferative neoplasms. It is a sensitive and simple test, relatively cost-effective. However, since the mutation may be absent in a few cases of PV, ET and PMF, it cannot be used as a single test for rule out or diagnosis and should be done in addition to other tests.

Current study showed a high rate of association between JAK2V617F mutation in patients with PV, ET, PMF in Iranian patients. Therefore, peripheral blood mutation screening for JAK2V617F can be incorporated into the initial evaluation of patients suspected to have chronic myeloproliferative neoplasm and used of this test for determining of association between JAK2V617F mutation, treatment of patients with blood indexes and patients of prognosis recommended.

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