Evaluation of Common Genetic Disorders in Myeloproliferative Neoplasms

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Abstract

Introduction: The myeloproliferative neoplasms (MPNs) are a heterogeneous group of diseases characterized by excessive production of blood cells by hematopoietic precursors. Typically, they include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF), and chronic myeloid leukemia (CML). Philadelphia chromosome is the final diagnostic test for CML. Recently, *JAK2* mutation introduced as a diagnostic marker for other MPNs. The aim of this study is evaluation of Philadelphia chromosome in CML patients and *JAK2* mutation in MPNs patients that had been referred to a hematology/oncology clinic in Kermanshah between 2010-2011.

Material and methods: In this study we evaluated common genetic disorders in 124 MPNs patients. Expression of *B2A2 BCR-ABL* mRNA in peripheral blood leucocytes was detected by a reverse transcriptase polymerase chain reaction (RT-PCR) for CML patients. Also, we used AS-RT-PCR method for the detection of the *JAK2* mutation for all of 124 patients.

Results: We found 93.7% CML patients (60/64) with positive Philadelphia chromosome. Also, 85% PV patients (17/20), 46.6% ET patients (14/30) and 40% IMF patients (4/10) had *JAK2* mutation. Notably, we found a CML patient with positive Philadelphia chromosome and *JAK2* mutation.

Conclusion: Diagnosis of MPNs is often complex and expensive but, *JAK2* mutation is a sensitive test, relatively cost-effective for proving clonality in MPNs. Also, more studies are required to determine the exact frequency and prognostic role of the *JAK2* mutation in Philadelphia positive CML patients.

Key words: myeloproliferative neoplasms, *JAK2* mutation, Philadelphia chromosome.

Introduction

The myeloproliferative neoplasms (MPNs) are a heterogeneous group of diseases characterized by excessive production of blood cells by hematopoietic precursors. In addition to thrombotic and hemorrhagic complications, leukemic transformation can occur.(1) Typically, they include 4 clinical entities: polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF), and chronic myeloid leukemia (CML), plus rarer subtypes such as chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES), and chronic eosinophilic leukemia (CEL). These diseases overlap with myelodysplastic/ myeloproliferative diseases (MDS/MPDs) such as atypical CML (aCML) and chronic

myelomonocytic leukemia (CMML), in which proliferation is accompanied by dysplastic features or ineffective hematopoiesis in other lineages.(2, 3)
The molecular basis for CML involves a fusion protein encoded by a chimeric hybrid *Bcr-Abl* gene (Philadelphia chromosome) as the result of a recurrent chromosome translocation *t(9: 22)*. This chimeric protein leads to a constitutive activation of the *Abl* tyrosine kinas by oligomerization through *Bcr*.(4)
The recurrent *JAK2V617F* mutation was identified independently by a candidate gene approach and by high-throughput DNA sequencing of the functional domains of 85 tyrosine kinases in PV, IMF, and ET blood samples. According to the previous studies, the single acquired point mutation *V617F* (1849G>T) in *JAK2* occurs in 50–97% of patients with IMF, ET and PV.(5-9) *JAK2* is a cytoplasmic tyrosine kinas with a key role in signal transduction from multiple haemopoietic growth factor receptors and so is an especially attractive candidate gene.(10, 11)
Philadelphia chromosome makes definitively diagnose for CML. By contrast, The *JAK2* mutation has never been identified in a patient with Philadelphia positive CML(12) but recently, some cases of Philadelphia positive CML with concomitant *JAK2* mutation have been reported.(13-21)
The aim of this study is evaluation of Philadelphia chromosome in CML patients and *JAK2* mutation in MPNs patients in Kermanshah province of Iran.

Material and Methods
We evaluated MPNs patients refer to a hematology/oncology clinic in Kermanshah between 2010-2011. Diagnosis of MPNs was performed according WHO guides and without consider genetic disorders.
We took 10 ml blood sample with EDTA from every patient and Peripheral blood cells were purified then DNA extracted. Expression of *B2A2 Bcr-Abl* mRNA (chimer protein 210 KD) was detected by a reverse transcriptase polymerase chain reaction (RT-PCR) in peripheral blood leucocytes.(22) Also, we used AS-RT-PCR method for the detection of the *JAK2-V617F* mutation. Total RNAs were isolated by using the TRIZOL reagent. First, strand cDNAs were prepared by reverse transcription of total RNAs with random primers. The AS-RT-PCR program was as follows: an initial denaturation step at 95°C for 5 minute, followed by 40 cycles of 40 second. At 94°C, 40 second. At 56°C, 45 second. At 72°C, and a final extension step of 10 minute at 72°C. The mutant specific forward amplified a 295-bp product from the mutated allele, whereas the internal control forward primer should have given a 488-bp product from both the mutant and wild-type alleles. A total of 10 µl from the PCR product were electrophoresed on 3% standard agarose gels (Sigma, Germany) at 80 V for 15 minute. The fragments were visualized by ethidium bromide under UV transilluminator.(21) Statistical significance was assumed at the "*P"* less than *0.05* level. The SPSS software package version 11.5 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis.

Results
We investigated 124 patients (including 62 male and 62 female) with MPNs that refer to our clinic: 64 patients with CML, 30 patients with ET, 20 patients with PV and 10 patients with IMF. Ranging age was from 16 to 82 years old.
We found 93.7% CML patients (60/64) with positive Philadelphia chromosome. Also, 85% PV patients (17/20), 46.6% ET patients (14/30) and 40% IMF patients (4/10) had *JAK2* mutation. There is significantly different between *JAK2* mutation and older age in MPNs patients (*P<0.01*). Also, between specific MPNs subtypes, PV patients with *JAK2* mutation displayed a significantly higher mean age (*P*<0.05). No association was found in ET and IMF patients (Table- 2). Further, mutation in females was higher than males but there are no significant differences between *JAK2* mutant and gender (*P>0.05*).
Notably, we found an 82-year-old male CML patient with positive Philadelphia chromosome and *JAK2* mutation that we reported him previously.(21) The clinical symptom of the patient was suspected ET but after diagnosis of *Bcr-Abl* positive and shift to left of myeloid cells in peripheral blood smear was observed, chronic myeloid leukemia (CML) was confirmed.

Discussion
In this study, we investigated 124 MPNs patients for *JAK2* mutation including 64 patients with CML that we investigate Philadelphia chromosome. *JAK2* mutation exists in two forms that are homozygous and heterozygous. For detection of these forms, should get granulocytes. In this study we used Buffy coat and because of this layer have lymphocytes too (mutation not occur in these cells), we couldn't detect homozygous or heterozygous of this mutation.

Table- 1: AS-RT-PCR primers (MWG Biotech, Germany).

Forward: 5´- GAA GAT TTG ATA TTT AAT GAA AGC CTT - 3´
Reverse: 5´- GTA ATA CTA ATG CCA GGA TCA CTA AGT T - 3´
Mutant: 5´- AGC ATT TGG TTT TAA ATT ATG GAG TAT ATT - 3´

Table- 2: JAK2 mutation in MPNs patients.

	PV		ET		IMF		CML	
	***JAK2* positive**	***JAK2* negative**	***JAK2* positive**	***JAK2* negative**	***JAK2* positive**	***JAK2* negative**	***JAK2* positive**	***JAK2* negative**
Number (%)	17 (85)	3 (15)	14 (46.6)	16 (53.3)	4 (40)	6 (60)	1 (1.5)	63 (98.4)
Age	53.19 ± 10.07	39 ± 23.66	53 ± 9.81	41 ± 12.49	48.91 ± 15.83	45 ± 19.57	82	40 ± 21.83
Sex (M/F)	8/9	2/1	6/8	7/9	2/2	4/2	1/0	32/31

MPNs: Myeloproliferative neoplasms, PV: Polycythemia vera, ET: Essential thrombosis, IMF: Idiopathic myelofibrosis, CML: Chronic myeloid leukemia, M: Male, F: Female

According to WHO guides, first test for diagnosis of PV in suspected patients is evaluating of *JAK2* mutation.(23) Evaluating of low level Erythropoietin is the second test for these patients that who have negative *JAK2* mutation can be identified.(24, 25) In all of our PV cases, Erythropoietin had lower level than normal. Positive *JAK2* mutation and low level of Erythropoietin are the final tests for PV and don't need to other invasive evaluations such as bone marrow aspiration.(23) According to our results, 85% PV patients had positive *JAK2* mutation. In Christine study, the prevalence of *JAK2* mutation in PV patients was 81% (58/72).(26) Also, result of Baxter *et al.*(5) study showed 97% patients with PV had *JAK2* mutation. Further, results of James 86%,(6) Jelinek 86%,(12) and Jones studies 81%,(9) show similar results in compare to our study.

Bone marrow fibrosis occurs in many malignant and non-malignant disorders such as metastatic carcinoma, CML, HCL (Hairy Cell Leukemia), some infections and etc.(27, 28) So, differential diagnosis for IMF from other fibrosis disorders is too hard. Result of previous studies show *JAK2* mutation not occurs in other fibrosis disorders,(29, 30) so, investigation of this mutation is very useful for differential diagnosis for IMF. According to this study, *JAK2* mutation was observed in 40% IMF patients. In support to our results, other studies indicate 35-55 IMF patients with *JAK2* mutation.(5-8) Of course, because of *JAK2* mutation occurs in maximum half of IMF patients, this mutation is not single test for diagnosis.

We found 46.6% ET patients with positive *JAK2* mutation. In support to our results, other studies show about 50% ET patients have *JAK2* mutation.(5-9, 23, 27) Before Identification of this mutation, there wasn't any marker that shows colonal nature of ET. So, differential diagnosis from other disorders with thrombocytosis was too hard. Exists of *JAK2* mutation in patients with thrombocytosis is very helpful for ET diagnosis.(5, 9) Also, Exists of this mutation in ET patients provides valuable prognostic information. These patients have more sensitivity to Hydroxyurea (HU) compared to non mutant patients and need lower amounts of this drug for their treatment.(31, 32)

Even though the *JAK2* mutation has been excluded in a large cohort of *Bcr-Abl* patients,(12) we found one Philadelphia positive CML patients with *JAK2* mutation. Kramer *et al.* were the first group that reported *JAK2* mutation combined with Philadelphia chromosome in 2007.(13) After them, some cases had been reported *JAK2* mutation combined with Philadelphia positive CML patients.(14- 21) More studies are required to determine the exact frequency and prognostic role of the *JAK2* mutation in Philadelphia positive CML patients.

Further, we found 93.7% CML patients with positive Philadelphia chromosome. This chromosomal disorder present in more than 90% of CML patients. The aberration results from a reciprocal translocation between chromosome 9 and 22, creating a *Bcr-Abl* fusion gene.(33)

In summary, diagnosis of MPNs is often complex and expensive but, *JAK2* mutation is a sensitive test, relatively cost-effective for proving clonality in MPNs. It also helps in excluding a large number of secondary causes. 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. In compare to other studies, our results show similar frequency of *JAK2* mutation in MPNs. Further, as regards number of reports that described the coexistence of the *JAK2* mutation in patients with Philadelphia positive CML increase in recently, we recommend that both Philadelphia chromosome and *JAK2* mutation are tested.

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