ASSESSMENT OF THE CHRONIC MYELOPROLIFERATIVE DISORDERS IN PATIENTS WITH CML JAK2V617F MUTATION FREQUENCY OF TABRIZ IN IRAN BY ARMS-PCR TECHNIQUE

Shahin Asadi* and Zeinab Gholami

Tabriz University of Medical Sciences in Iran, Stem Cells Research Center of Hematology and Oncology Cancer Hospital.

ABSTRACT

Background: Chronic myeloproliferative disorders (MPDs) are a heterogeneous group of diseases in which a clonal disorder of hematopoietic stem cells leads to an increase in the level of production in one or more of the blood cell. JAK2V617F recently acquired mutations in many patients with chronic myeloproliferative disorders (MPDs) is described by changing G to T mutation at nucleotide 1849 in exon 12 of the JAK2 gene is located on chromosome 9 identified, which leads to substitution of the amino acid phenylalanine instead of valine at position 617 of the protein JAK2 is the purpose of this study to evaluate the frequency of these mutations in patients with MPDs was. Materials and Methods: In this study, 117 patients were evaluated MPD. In patients identify mutations by ARMS-PCR method. This method is a fast and easy test. Moreover, it is possible to distinguish between homozygous and heterozygous individuals with Mutation provides JAK2V617F. Patient information was obtained by questionnaire, records and sampling were carried out with the consent of the patient. Three patients were also sequencing. Results: Mutations in 6/86% (30/26) of patients with polycythemia vera, 6/46% (15/07) of patients with essential thrombocytopenia, 5/61% (08/13) of patients with primary myelofibrosis and 14% (34/4) patients with chronic myeloid leukemia were identified. Polycythemia vera patients have a mutation, the white blood cells were significantly higher (p = 0.03). In addition, 16 of 26 patients with polycythemia vera JAK2-positive women with mutations associated with sex Showed.in other groups, no significant differences were found. The mutation was confirmed by sequencing. Conclusion: Our results are compatible with other studies and the highest
prevalence of the mutation in polycythemia vera and lowest in chronic myeloid leukemia is identify. Discovery this mutation in the differential diagnosis, prognosis and prediction of response to treatment is useful opportunity to amend the criteria for diagnosis and provides a classification of disease.

KEYWORDS: mutation, JAK2 chronic myeloid neoplasms, CML ARMS-PCR technique.

INTRODUCTION
Chronic myeloproliferative disorders (MPD) are a heterogeneous group of diseases in which a clonal disorder of hematopoietic stem cells leads to an increase in the level of production in one or more of the blood cells. William Domeshek in 1951, the phenotypic similarities between chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytopenia (ET) and idiopathic myelofibrosis (PMF) and specify them as chronic myeloproliferative diseases classified. In 1960, the CML is a specific cytogenetic markers (Bcr-Abl) with Philadelphia chromosome (9:22) t specify a new category was created based on molecular markers and thus the classical MPD + BCR-ABL, the (f) the BCR-ABL CML PV, ET and PMF was classified. In early 1974, it was found that a population of erythroid precursors in vitro in the absence of erythropoietin in PV can grow and this test for early diagnosis of PV was used forms of secondary erythrocytosis. Endogenous erythroid colony formation However (EEC) No specific PV and in some patients ET, PMF and CML and in patients with thrombotic diseases such as were chiari syndrome seen.

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Onset of MPN

Acquisition of the V617F JAK2 mutation

Uncontrolled cell proliferation

Predisposing JAK2 variants

No acquisition

Jak 2: molecular pathway mutations in the cells of myeloproliferative disorders.
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In addition, there are frequent protests at the beginning of erythrocytosis of polycythemia vera (myeloproliferative diseases are the most common type) and should be differentiated from other types of erythrocytosis.\textsuperscript{[1]} In 2005, the relationship between diseases PV, ET and PMF acquired by identifying a mutation in JAK2 tyrosine kinase found more.\textsuperscript{[3]} This mutation of G to T in nucleotide 1849 in exon 12 of the JAK2 gene is located on chromosome 9\textsuperscript{[1-8]} identified which leads to the substitution of the amino acid phenylalanine instead of valine at position 617 of the protein JAK2 is\textsuperscript{[4-8]} Family proteins JAK2, due to the effects of hematopoietic cytokines such as Trombopoetin (TPO), erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF) by phosphorylation of cytoplasmic factors such as STAT (signal transducers and activators of transcription), and mutations lead the continuous activation of JAK2 in the absence of cytokines. The mutation is a risk factor for the development of MPD JAK2V617F considered.\textsuperscript{[9]} Since the discovery of this mutation, so the diagnosis, prognosis and prediction of response to therapy is beneficial.\textsuperscript{[10]} Kralovics reported that patients with mutations JAK2V617F longer a disease with a higher percentage of complications (myelofibrosis, thrombosis and bleeding) are.\textsuperscript{[7]} Because mutations in a small portion of the population is a sensitive method for detecting granulocytic is required.\textsuperscript{[11]} So far, several techniques used to identify this mutation, including: DNA-PCR-Sequencing Genomic, RT-PCR, ARMS-PCR, Allele -Specific PCR, PCR-Restriction Analysis and Real-Time PCR\textsuperscript{12}. In this study, using ARMS-PCR method has been tried for a way to differentiate reactive states Chronic myeloproliferative diseases found.

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\includegraphics[width=\textwidth]{schematic_view.png}
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Schematic view of myeloproliferative disorders.
MATERIALS AND METHODS

After obtaining informed consent, blood samples of 117 patients and 100 normal controls were used. After separation of white blood cells in peripheral blood, DNA was extracted from white blood cells by salting out. Then, primers were designed for mutation JAK2V617F the FISH technique and clinical data from medical records of patients used in this study was extracted. Primer in Table 1.

Table 1: ARMS-PCR primers used in the technique for abnormalities CML-MPN.

<table>
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<tr>
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<th>Primer Sequence</th>
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<tr>
<td>FO</td>
<td>5’- TCC TCA GAA CGT TGA TGG CAG-3’</td>
</tr>
<tr>
<td>RO</td>
<td>5’- ATT GCT TTC CTT TTT CAC AAG AT-3’</td>
</tr>
<tr>
<td>FWt</td>
<td>5’- GCA TTT GGT TTT AAA TTA TGG AGT ATA TG -3’</td>
</tr>
<tr>
<td>RMt</td>
<td>5’- GTT TTA CTT ACT CTC GTC TCC ACA AAA-3’</td>
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PCR conditions were as follows: 94°C for 6 minutes, 40 cycles, denaturation at 94°C for 40 seconds, annealing at 56°C for 45 seconds and elongation at 72°C for 45 second, one cycle at 72°C for 10 minutes. Primers (FO) Forward Outer and (RO) Reverse Outer gene JAK2, a band of 463 bp and primers (FWt) Forward wild-type and (RO) Reverse Outer one allele wild-type to multiply and produce a band of 229 bp and primers (FO) Forward Outer and (RMt) Reverse mutant allele can cause a band of 279 bp. To confirm the presence of mutations and the accuracy of the number of positive samples were sequenced (Figure 1).
Figure 1; Band banding patterns for protein and nucleotide sequencing for mutations JAK2V617F.

RESULTS

In this study, 117 patients with diseases Chronic myeloproliferative study the expression of JAK2 V617F were all they were examined by ARMS-PCR procedure. Results such as CBC and cytogenetics, morphological diagnosis, early diagnosis, and more than 117 medical records of patients were extracted samples 25 samples are not available due to incomplete records and medical history and therefore incompatible with the criteria WHO (despite ARMS test and answered all of them were negative for all samples) were excluded. The data of 92 patients were entered into SPSS and statistical analysis was performed using Mann-Whitney Test. In addition, 100 healthy controls were evaluated for presence of mutations, which are mutations were negative. In 117 patients, mean age 61 years, age range 16 to 86 years were az 117 patients and 26 patients were female and 91 male patients. The 34 CML, 15 patients ET, 13 of PMF and PV 30 with polycythemia vera were from 30 patients, 26 patients had a mutation (86%). In addition, mutations in idiopathic myelofibrosis 8 patients...
out of 13 patients (61%), 7 patients out of 15 patients with primary thrombocythemia (46%) and 4 patients out of 34 patients with chronic myeloid leukemia (14%) were found (Figure 1).

21 patients (10 patients with PV, 3 patients ET, 5 patients and 3 patients with PMF CML) of the 52 patients were male (40%) and 24 patients (16 patients with PV, 4 patients ET, 3 patients with PMF and a patient CML) of 40 patients Women (60%) had a mutation (Diagram 1). 47% of patients with splenomegaly and 53% had no splenomegaly.

Diagram 1: Curve mutations in PMF, CML, PV.

In patients with polycythemia vera the 30 patients, 26 patients (86%) had mutations. Mutation-positive patients had higher mean age. 26 mutation-positive patients, 16 patients were female mutation associated with sex shows. In addition, patients with a mutation rate of white blood cells were higher than patients without mutations (p = 0.03). But the major difference observed in the other side of the 26 patients JAK2 Not From positive, 17 patients with splenomegaly Were. more cases were patients without cytogenetic data could show the relationship between the prevalence of the mutation with cytogenetic abnormalities Saw. From 26 patients, only 2 patients the rest were heterozygous and homozygous mutation with a mutation. Because the number of patients with homozygous was so low we did not do statistical analysis separately for the group. It should be noted that a patient identified as polycythemia vera patients with JAK2 mutation positive with Philadelphia chromosome abnormalities and so far only one case has been reported Cambier case report in 2008.\textsuperscript{[13]}

In patients with primary thrombocythemia, idiopathic myelofibrosis and chronic myeloid
leukemia patients JAK2V617F significant difference between positive and negative attention that this mutation in CML patients Not With very rarely report data on these 4 patients in a separate list mentioned. Identified as CML patients based on the patient's medical records and laboratory findings was available.

Figure 2: Diagram assessment JAK2V617F mutation in polycythemia vera patients.

Figure 3: nucleotide sequence of polycythemia vera with control.
Campbell reported\textsuperscript{[24]} idiopathic myelofibrosis patients JAK2 positive, white blood cell count and neutrophil higher mutation-negative patients were, but spleen size, platelet count and hemoglobin levels did not show significant differences between the two groups. The results obtained in this study, no significant differences between the two groups showed no mutation positive and negative even in the WBC count. patients, 16 positive relationship between the mutation and older Were there also found in the detection of JAK2 mutation. This relationship between age and the effects of aging on the expression of genetic instability.

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\textbf{Schematic of how to activate the mutation in myeloproliferative disorders.}
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**DISCUSSION**

Classic diagnosis of MPD patients negative BCR-ABL, based on clinical criteria and laboratory tests with newer development is undergoing fundamental changes. The recent discovery of the JAK2 V617F mutation opportunity to improve diagnostic criteria and classification of diseases has provided. This high prevalence of JAK2 V617F mutation in MPD patients is confirmed. It should be noted that a small number of cases, the mutation PV PV is not in need of other criteria for diagnosis. However, the identification of mutations, can differentiate between patients with a real MPD and those who have a secondary erythrocytosis or thrombocytosis created. The discovery of JAK2 V617F mutation is not only important but also the presence of the mutation in MPD reclassification clinical results, particularly in thrombotic complications and changes to the positive and negative JAK2 leukemia patients affected. In this study, the prevalence of chronic myeloproliferative disorders, mutations JAK2V617F in 117 patients with ARMS-PCR method was evaluated. The highest prevalence of the mutation in polycythemia vera patients (86\%) were found.
Thrombocythemia, idiopathic myelofibrosis in patients with primary and 46% and 62%, respectively amounts to were With addition of 34 CML patients, 4 patients (14%) Oral Reading mutation show a similar study by Jones et al. (14) by ARMS-PCR was performed in 2005, the prevalence of the mutation in polycythemia vera patients, 81% (72/58) in primary thrombocythemia 41% (59/24) and 43% idiopathic myelofibrosis (35/15) was reportedBecause relatively insensitive mutation detection techniques such as Direct Sequencing Jones mutations in any patient group is From CML (n = 17) did not find that in the published articles stating that the number of samples analyzed is small and can not be The presence of mutations in a small number of patients denied the switch study of 34 patients with CML, 4 patients (14%) had mutations were From the number (three men and one woman), three cases of Philadelphia positive and one new case was negative. Studies by a number of researchers, some of the JAK2 mutation at the same time and in patients treated with imatinib Philadelphia chromosome has shown. In a study by Campbell and colleagues[17] on 806 patients with ET, 414 patients (4/53%) were positive and 362 patients (6/46%) reported that patients with JAK2 positive and negative were This group a significant increase in hemoglobin (p<0.0001), neutrophil count (p<0.0001) and Granulopoiesis bone marrow, vein thrombosis and a higher proportion of deformation is more systemic than those without the mutation are made. In addition, it was found that patients treated with hydroxyurea more positive than negative mutation are the critical study data analysis, no significant difference in the rate of patients with ET RBC, WBC, PLT, HCT and Hb was observed. To justify this, it can be stated that in comparison with Campbell much smaller number of samples (15 samples), respectively.

As well as the prevalence of the mutation in PV patients was 86% which is comparable to the findings of James (86%) 5 [and Jelinek (86%)] 18 [and Jones (81%)] 14 [is. The highest prevalence by Lippert (97%) assay reaction (qPCR) allele-specific quantitative polymerase chain] 19 [and the lowest 65% by Kralovics] 7 [using microsatellite mapping and DNA sequencing was obtained. In this study, Levine and colleagues[6] an important link between the allele and females in PV patients proved that the study confirms these findings. In which 26 patients with polycythemia have the mutation, 16 patients (55%) female patients diagnosed as suffering wers. in one of polycythemia vera (RBC: 8.18, Hb: 16.6, HCT: 40.2) Philadelphia chromosome positive. Cases of acute myeloid leukemia by the polycythemia vera] Amin20 [and Mirza] 21 [reported. In addition, Cambier and his colleagues.[13] In an article in 2008 in a patient case report of two separate myeloproliferative disorders
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(polycythemia vera JAK2 V617F-positive chronic myeloid leukemia and Philadelphia positive) reported. However, mutations in more than half of the cases we have identified idiopathic myelofibrosis in accordance with previous. According to estimates 23, 22, 7 [the prevalence of the mutation in this disorder.

CONCLUSION
Clinical and diagnostic importance is the detection of mutations JAK2V617F. It is clear that the high prevalence of the mutation in polycythemia vera patients, PV can increase the detection of secondary erythrocytosis help. This mutation can be identified for further investigation, such as red blood cell mass (Red Cell Mass) and bone marrow biopsy. In addition, in patients with thrombocytosis, the use of mutation analysis may identify patients with a disorder of stem cell JAK2V617F help. The discovery of imatinib for the treatment of CML based on identification by doctor Brian Draker BCR-ABL tyrosine kinase, which happened almost two decades Aftad. in the future hope that the discovery of JAK2 mutations lead to the development of specific pharmacological inhibitors similar to the ability of PV, ET and PMF Although there are questions that require careful evaluation. First, the drug produced mutant gene rather than having activity against wild-type JAK2 preferences may not cause a significant hematological poisoning?.

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REFERENCES


