ASSESSMENT OF JAK2V617F MUTATION FREQUENCY IN PATIENTS WITH POLYCYTHEMIA VERA CITY OF TABRIZ IN IRAN

Shahin Asadi*, Elham Alizadeh Milani, Ali Nazirzadeh©, Vahid Ghorbani

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

ABSTRACT

JAK2V617F mutation in the diagnosis and classification of myeloproliferative neoplasms has found wide application. In this study, JAK2V617F mutation frequency in patients with polycythemia vera Tabriz city were investigated. In this study, blood samples from 87 patients with polycythemia vera JAK2V617F mutation was investigated. DNA samples with proteinase k obtained from peripheral blood was extracted from buffy coat. After quality control, to assess the JAK2 mutation status of ARMS-PCR polymerase chain reaction with allele-specific primers were used. PCR products were electrophoresed on polyacrylamide gel electrophoresis and ethidium bromide staining were evaluated. Results: JAK2V617F mutation in 78.3% (58 patients) was observed myeloproliferative neoplasms. 58 patients with polycythemia vera, essential thrombocytemia, and 11 of 18 patients with primary myelofibrosis. Conclusion: This study showed that JAK2V617F mutation using allele-specific AST in patients with myeloproliferative neoplasms, speed and accuracy of diagnosis and the subsequent fate of patients with neoplasms Myeloproliferative promotes.

KEYWORDS: myeloproliferative neoplasms, mutation JAK2V617F, reaction ARMS-PCR.

INTRODUCTION

Polycythemia, which is also known as polycythaemia or polyglobulia a myeloproliferative blood disease in which the bone marrow makes too needed to produce red blood cells. The disease may also increase the production of platelets and white blood cells.
Myeloproliferative disorder, a proliferative disorder that is associated with leukemia and Eosinophils known. 95% of the normal range of hemoglobin at birth between 13.7 to 20.1 grams per cent. If more than 22 grams of hemoglobin in the first week of infection indicate that the baby is polycythemia. Polycythemia and hyperviscosity causes decreased blood flow to the brain. About 2 months after birth to 10 to 11 grams of hemoglobin per cent drop that to say that the physiological decline. Physiological loss due to conversion of hemoglobin to hemoglobin A, hemoglobin F. After reduction of oxygen from hemoglobin F will cause congestion in utero and at birth. The amount of hemoglobin depends on the age, sex and geographic area. Hyperemia of various types such as hyperemia Family (Familia Polycythemias), Hyperemia relative (Relative Polycythemias), hyperemia secondary (Secondary Polycythemias) and hyperemia Vera (Vera Polycythemias) is. The hyperemia hyperemia Aloe Vera extreme serves as a hematopoietic stem cell red blood cell mass are automatically generated. Myeloproliferative neoplasms (MPN) is a heterogeneous group of diseases in which a clonal disorder of hematopoietic stem cells leads to an increase in the production of blood cells in one or more.[1]

William Domeshek in 1951, phenotypic similarities between chronic myeloid leukemia, CML, polycythemia vera, essential thrombocythemia (ET) and primary myelofibrosis (PMF) and specify them as chronic myeloproliferative disorders (MPD) classified. In 1960, the CML is a specific cytogenetic marker (BCR-ABL) in Philadelphia chromosome or t (9; 22) and specify a new category was created based on molecular markers and thus the BCR-ABL in CML and classic MPD BCR-ABL, the PV, ET and PMF classified.[2] The classification of myeloid neoplasms was published by the World Health Organization in 2008, myeloproliferative disease chronic myeloproliferative neoplasms were called. The identification of common molecular abnormalities such as JAK2 mutation in this group of diseases, or neoplastic clonal nature are identified and therefore replaced the word neoplasm patients.[3]
Schematic view of a slide under a microscope for abnormalities in the blood samples of myeloproliferative.

In early 1974 it became clear that a sub-population of the PV erythroid precursors in vitro in the absence of the hormone erythropoietin can develop and test for the early detection of PV forms of secondary erythrocytosis was used. Also, there are frequent protests in front of the PV erythrocytosis and should be distinguished from other types.\(^4\) Hormone erythropoietin gene is located on chromosome 9 region kidney cortex. These genes or transcription factor HIF-1 (hypoxia inducible factor), which is composed of two alpha and beta chains. HIF factor secreted in response to hypoxia. Erythropoietin receptor (EPOR) on the cytoplasmic side of the field is positive or negative. Which is positive for the proliferation of precursors of red blood cells with enzymes Janus kinase 2 (JAK2) is in contact. JAK2 phosphorylation causes a marked positive message through a signaling pathway JAK2 / Stat5 is. The role of erythropoietin receptor negative phosphatase causes a negative message via Phosphorylated out the medium is the message.
Schematic view of the molecular pathway myeloproliferative disorders.

Acquired G→T mutation at nucleotide 1849 JAK2V617F looking into gene JAK2 exon 12, leading to the displacement caused by phenylalanine amino acid valine at position 617 (V617F) the JAK2 protein.

Schematic view of the molecular genetic mutation Zhanoz enzyme kinase 2.

JAK kinases 4 domain (Domain) were formed:
1-second N terminal FERM called for interaction with cytokine receptors is required.
2-second SH2
3-kinase-like domain (JH2)
4-terminal kinase C (JH1)

V617F mutation in the kinase-like domain (JH2) has been created.\textsuperscript{[5]} Thus, the discovery of this mutation is useful in determining the prognosis and prediction of treatment response.\textsuperscript{[6]} Because mutations in a small proportion of the population-CSF is a sensitive method for detection is required. Several techniques have been used to identify this mutation.

Genomic DNA-PCR-Sequencing, RT-PCR, ARMS-PCR, Allele-Specific PCR, PCR-Restriction Analysis, Real-Time PCR.

Schematic view of the nucleotide sequence mutation JAK2V617F.
MATERIALS AND METHODS
After obtaining informed consent, peripheral blood samples from 87 patients with newly diagnosed or are being treated with myeloproliferative neoplasm and 50 normal control subjects admitted to the hospital holding purify the blood and cancer genetics judge Tabatabai Tabriz by ARMS-PCR method were studied. Clinical and laboratory data of the patients were extracted from medical records. Polycythemia vera patients, including 58 patients, 18 patients and 11 patients had primary Thrombocytopenia primary myelofibrosis. 37 male and 50 were female. The mean age of patients with at least 58 years of age 19 years and maximum 69 years.

Extraction of DNA
Every person with 5 ml of venous blood was collected in tubes containing EDTA anticoagulant and DNA was extracted by proteinase k of whole blood. To do so, briefly centrifuged and the precipitate blood cells to lyse the cells, distilled water was added. The cells were then M Tris buffer and SDS (10%) and proteinase K (20 mg per mL) was added and after overnight incubation, 6 M sodium chloride solution was added and after centrifugation the cells, 70% ethanol was added. After the final centrifugation to precipitate DNA distilled water added and absorbance was measured at a wavelength of 260 nm. Using the absorbance of 260; 280 OD and 1.7 to obtain a high number of DNA purity was sure.

View a diagram of how the mutation on different days of disease recurrence.
Reaction test ARMS-PCR

This method detects changes in a single base under the PCR conditions are ideal. This technique is suitable for detecting the movement of a single G → T mutation in the JAK2. 4-primer ARMS-PCR technique uses a primer Forward outer (FO), a primer Reverse outer (RO), a primer Forward wild-type specific (FWT), a primer Reverse mutant- specific. PCR reactions were performed in a final volume of 25 microliter and 40 cycles. For each reaction, the amount of genomic DNA, primers final concentration of 25 ng and FO, RO and 0.5 microliter FWT three RMT concentration was equal to 1 microliter. PCR products were electrophoresed on 3% agarose gel and stained with ethidium bromide and the presence or absence of mutations were studied. FO and RO JAK2 gene primers are a 463bp band. A wild-type allele of the FWT and RO primers and amplification primers to produce a band of 229bp and 279bp of FO and RMT allele creates a bond.

Table 1: Primers used in the reaction ARMS-PCR to detect mutations in JAK2.

<table>
<thead>
<tr>
<th>Primer Type</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward outer (F0)</td>
<td>5’-TCCTCAGAACGTTGATGGCAG-3’</td>
</tr>
<tr>
<td>Reverse outer (RO)</td>
<td>5’-ATTGCTTTCCTTTTTTACCAAGAT-3’</td>
</tr>
<tr>
<td>Forward wild-type specific (FWT)</td>
<td>5’GCATTTGTGGTTTTAAATTATGGAGTATATG-3’</td>
</tr>
<tr>
<td>Reverse mutant-specific (RMT)</td>
<td>5’-GTTTTACTTACTCTCCGTCACAAAA-3’</td>
</tr>
</tbody>
</table>

Chen and his colleagues tested the sensitivity of mutation detection jak2 0/05 percent to 0.1 percent reported. Individuals homozygous and heterozygous mutations distinguishing between positive approach makes it possible to detect a key role in the presence or absence of mutations in MPN patients as a screening test is reliable.

View a diagram of the mutation in the hormone erythropoietin bands
Figure 1; A→ shows the mutation in polycythemia vera v 617 f in the membrane.
B→ shows the mutation in polycythemia vera v 617 f in the Lysates.
Figure 2: ARMS-PCR for screening positive for JAK2 mutation JAK2V617F columns 2 and 5 shows a patient.

Table 2: ARMS-PCR reaction temperatures used in order to identify mutations in JAK2V617F.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>6 min</td>
<td>Initial denaturation</td>
</tr>
<tr>
<td>94</td>
<td>40 Seconds</td>
<td>Denaturation</td>
</tr>
<tr>
<td>56</td>
<td>45 Seconds</td>
<td>Connection</td>
</tr>
<tr>
<td>72</td>
<td>45 Seconds</td>
<td>Development</td>
</tr>
<tr>
<td>72</td>
<td>10 min</td>
<td>Final development</td>
</tr>
</tbody>
</table>

The results
In this study, 87 patients with myeloproliferative malignancies, referring to the holding Blood and Cancer Genetics Hospital Tabriz refinery Tabatabai judge the JAK2V617F gene expression were evaluated using ARMS-PCR method. Results of tests such as CBC, cytogenetics, Morphologic diagnosis, early diagnosis, etc. were extracted from medical records. SPSS16 enter patient data and the statistical analysis was performed using Mann-whitney test. In addition, 50 healthy subjects were studied as controls were negative for the
presence of mutations. Polycythemia vera of 58 patients, 54 had mutations (86%). 37 male patients, 26 of 50 patients with mutations, 43 had mutations. Patients with positive mutation rate of mutation-negative patients had higher white blood cells, but there was no significant difference in the overall data. As was the case in most patients without cytogenetic data to study the prevalence of mutations associated with cytogenetic abnormalities are not allowed. Only 7 of 58 patients with mutations in patients with homozygous inheritance pattern and the remaining mutations were heterozygous inheritance pattern. Due to the low number of patients homozygous Statistical analysis was not performed separately for the group.

Table 3: Frequency JAK2 mutation in polycythemia vera patients in Tabriz.

<table>
<thead>
<tr>
<th>p</th>
<th>Negative mutant</th>
<th>Positive mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0/4</td>
<td>54 Number of patients</td>
</tr>
<tr>
<td>50</td>
<td>22/29±39/3 Leukocyte count (on/L. 10^3)</td>
<td></td>
</tr>
<tr>
<td>0/04</td>
<td>54/35±5/108</td>
<td>52/59±7/89 Hematocrit (%)</td>
</tr>
<tr>
<td>0/2</td>
<td>18/5±1/146</td>
<td>17/89±1/768 Hemoglobin Grams on liter</td>
</tr>
</tbody>
</table>

DISCUSSION
Negative MPN diagnosis of BCR-ABL, based on clinical and laboratory criteria that the development of new tests is undergoing fundamental changes. JAK2V617F mutation discovery opportunity to reform the existing diagnostic criteria and classification of disease is provided. This high frequency JAK2V617F mutation in patients with PV, ET and PMF approved. In addition, in this study we examined the mutation Sequencing results were confirmed by this method.

CONCLUSION
JAK2V617F mutation detection and diagnosis of many clinical significance. It is evident that the high frequency of this mutation in PV patients can increase detection of erythrocytosis secondary polycythemia vera help. These mutations can be identified for further studies, such as red blood cell mass and reduce bone marrow biopsy.

ACKNOWLEDGMENTS
This study is the result of a research project approved by the Research Institute of Mother and refinement of Hematology and Oncology Research Center for Cancer Genetics and bone
marrow transplants judge Tabatabai hospital in Tabriz Akin Tabriz University of Medical Sciences is the official kidneys colleagues who have accompanied us on this matter would be appreciated. it is noteworthy that this research project sponsored by the judge Tabatabai University of Medical Sciences and Hospital was performed.

REFERENCES


