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FREQUENCY OF JANUS ASSOCIATED KINASE 2 (JAK2) MUTATION IN PATIENTS OF BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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ABSTRACT

Objective: To determine the frequency of Janus associated kinase 2 mutation in the patients of BCR-ABL negative classical myeloproliferative neoplasms.

Study Design: Cross-sectional descriptive study

Place and Duration of Study: Molecular Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from Jul 2011 to Jul 2012.

Patients and Methods: Ninety three consecutive patients of Polycythaemia vera (PV), Essential thrombocythaemia (ET) and Idiopathic myelofibrosis (IMF) diagnosed by the conventional haematological criteria were included in the study. All patients were screened for G-T point mutation (V617F) in the JAK2 gene on chromosome 9 by an allele specific PCR.

Results: Out of the 93 myeloproliferative neoplasm (MPN) patients, 33(35%) had polycythaemia vera, 36(39%) had essential thrombocythaemia and 24(26%) had idiopathic myelofibrosis. JAK2 mutation was seen in 64/93 (69%) patients including 33/33(100%) in PV, 19/36(52.6%) in ET and 12/24(50%) in IMF.

Conclusion: Classical myeloproliferative neoplasms are an important group of heamatological disorder in our country. JAK2 gene mutation is seen in significant proportion of these disorders (69%). JAK2 mutation analysis can be used to differentiate between polycythemia vera and secondary polycythemia in most cases with near certainty, where it was found in 100% of the cases.

Keywords: Polycythemia vera, Essential thrombocythemia, Idiopathic myelofibrosis, myeloproliferative neoplasm, JAK2 mutation.

INTRODUCTION

The concept of myeloproliferative disorders (MPD) was first described by William Dameshek in 1951[1]. In 2001, World Health Organization (WHO) described the term chronic myelo-proliferative diseases (CMPD), which was replaced by term myelo-proliferative neoplasm (MPN) in 2008 by WHO[2]. The classical BCR –ABL negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF)[2,3]. In MPN there is a defect at the stem cell level causing proliferation of one or more haemopoietic components in the bone marrow. These are uncommon haematological malignancies with world-wide incidence of 2–3 per 100,000, 1.5–2 per 100,000 and 0.5-1.5 per 100,000 respectively[4].

At present, the mutation which is found most commonly in classical BCR-ABL negative MPN is JAK2 V617F[5]. In 2008, WHO diagnostic criteria for PV, ET and PMF were revised by incorporating molecular markers (JAK2 and MPL mutations) in the diagnostic criteria and also underscoring the role of histology in differentiating clonal from non-clonal myeloproliferation. JAK2 positively is seen in nearly all patients with PV and approximately fifty percent patients with ET and IMF.

The primary purpose during the evaluation of classical MPN is to determine the presence or absence of PV, ET and IMF because of the prognostic and treatment differences[6]. Patients with PV, ET are also at risk of transformation to myelofibrosis or acute leukemia in addition to thrombosis, stroke, heart attack and bleeding[7].

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Relative polycythemia is a condition that may not require treatment[7].

In March 2005, an acquired point mutation was reported in the Janus associated kinase 2 (JAK2) gene (termed JAK2 V617F) in PV and related MPD (essential thrombocythemia and idiopathic myelofibrosis)[8]. This mutation was found in more than 95% of patients who had PV[9].

The World Health Organization (WHO) diagnostic criteria for PV, essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) were revised after the discovery of JAK2 mutations in 2008 (for example: JAK2 V617F and JAK2 exon 12 mutations in virtually all patients with PV. Mutations in thrombopoietin receptor (MPL W515K/L) have been found in patients with ET and IMF. The frequency of MPL mutation in ET and IMF is approximately one and five percent respectively. This mutation is not found in PV[10]. Mutation in TET2 gene was also found quite often in advanced age MPN patients with or without JAK2 mutation. TET2 gene mutation also had little prognostic significance[11].

The objective of this study was to evaluate the frequency of JAK2 mutation among BCR-ABL negative classical MPN. This would help in rapid and reliable diagnosis of PV, ET, IMF.

PATIENTS AND METHODS

This cross-sectional descriptive study was carried out at Molecular Department of Armed Forces Institute of Pathology (AFIP) from July 2011 to July 2012. The subjects were enrolled from those who were referred to the Department of Haematology, AFIP. All newly diagnosed patients of BCR-ABL negative classical MPN of any age and gender were included in the study. From each patient verbal informed consent was obtained. After obtaining informed consent brief history was taken and clinical examination was performed. After reassuring the patient and taking antiseptic measures, 5 ml of venous blood was drawn from antecubital vein. For anticoagulation ethylenediamine tetra-acetic acid (EDTA) at concentrations of 1.5 ± 0.5 mg/ml was used. DNA was extracted using PUREGENE genomic DNA purification kit by gentra systems (USA).

JAK2 mutation analysis

For the PCR amplification of the JAK2 mutation (V617F), a set of three primers were used. To amplify JAK2 mutant allele a common reverse primer (5'CTGAATAGTCCTACAGTGTTTTCAGTTTC) and a forward specific primer (5'-AGCATTTGGTTTTAAATTATGGAGTATATT) producing 203bp amplified product was used. PCR internal control was another 364bp product which resulted from amplification by the common reverse primer and a forward control primer (5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAAG)[12].

The DNA was amplified in a 20 µl reaction mixture containing 20 pM of the common primer and 10 pM each of the two forward primers, 0.5 units of Taq polymerase (Fermentas Life Sciences, Lithuania), 30 mM of each dNTP, 10 mM Tris HCl (pH 8.3), 50 mM KC*l*, 1.5 mM $MgCl_2$, 100 mg/ml gelatin and 0.1-0.3 µg of genomic DNA. Thermal cycling comprised of 25 cycles of denaturation at 94°C for 40 seconds, primer annealing at 58°C for 40 seconds and extension at 72°C for 1 minute. The PCR amplified products along with 100 bp ladder was run on 6% mini-polyacrylamide gel electrophoresis at 150V for 40 minutes. Silver nitrate was used to stain the gels (fig. 1). In the figure PCR internal control is represented by a 364bp fragment whereas the mutation is represented by a 203bp fragment. The lanes 1 and 2 show negative and positive controls respectively. Lanes 3, 6 and 7 show negative results while lanes 4-5 show positive results.

SPSS version 16.0 was used for analyzing data. Quantitative variables like age, haemoglobin, total leukocyte count , platelets and PCV were described with mean and standard deviation (SD) for JAK2 mutation positive and negative classical MPN. For presentation of qualitative variables like JAK2 mutation and

gender, frequencies and percentages were computed. Comparison of haematological parameters between JAK2 positive and negative cases was done using independent samples't-test /Mann Whitney U test where appropriate. $p<0.05$ was considered statistically significant.

RESULTS

A total of 93 subjects with classical myeloproliferative neoplasms were evaluated in the study, 33(35%) of them were polycythaemia vera, 36 (39%) were essential thrombocythaemia and 24 (26%) were having idiopathic myelofibrosis. Out of the 93 patients of classical MPN, 57 (61.3%) were male and 36 (38.7%) were females. There was male predominance in classical MPN with the male to female ratio 1.5:1.

Table-1: Comparison of haematological parameters of JAK2 positive and negative classical myeloproliferative neoplasms.

Variables	JAK2 Positive	JAK2 Negative	*p*-value
Age(years)	54.28±15.59	41.28±13.75	<0.001
WBC(109/L)	18.77±12.74	14.53±22.26	0.001
Hb(g/dl)	14.88±3.92	11.22±2.65	<0.001
PCV(%)	0.48±0.12	0.36±0.07	<0.001
Platelets(109/L)	802.50±484.85	799.72±616.41	0.86

Values were expressed as mean ± SD

The age distribution among patients classical of MPN was very wide, ranging from 20 to 82 years. Mean age of presentation in JAK2 positive and negative patients of classical MPN was 54.28 ± 15.59 and 41.28 ± 13.75 years respectively.

Overall JAK2 V617F mutation was detected in 64 (69%) out of 93 patients enrolled in study with classical myeloproliferative neoplasms.

Out of 93 subjects splenomegaly was found in 70 (75.2%). Patients found positive for JAK2 V619F mutation had higher splenomegaly (84.3%) than patients who were negative (55.1%) $p=0.003$. JAK2 positive patients of classical MPN presented with high mean age, white blood cell count, haemoglobin and packed cell volume than JAK2 negative patients of classical MPN ($p<0.01$) as shown in table 1.

DISCUSSION

Myeloproliferative neoplasms (MPN) are clonal, heterogeneous disorders of hematopoiesis. They arise from transformation in a haemopoietic stem cell and are characterized by proliferation of one or more mature functional cell lines such as granulocytes, platelets or erythroid cells. All MPN though distinct but are closely related disorders. Traditionally MPN have been classified as Philadelphia positive and Philadelphia negative MPN. Philadelphia positive MPN include chronic myeloid leukemia (CML). CML is defined by its molecular lesion, the BCR-ABL fusion gene, resulting from Philadelphia translocation. There are three classical Philadelphia negative MPN, polycythemia vera (PV), essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF)[13].

Until recently there was no specific molecular or genetic marker for the diagnosis of MPN. The criteria for diagnosis of PV were twenty years old standards which were set by PVSG. Tests which were included in these criteria were determination of RCM, identification of independent erythroid colonies in vitro, testing of erythropoietin levels and cytogenetic analysis of bone marrow. The above mentioned tests are costly, not widely available and also lack sensitivity and specificity[13].

Discovery of the JAK2 V617F mutation in MPN has enabled us to understand the molecular and cellular basis of these disorders. Normal

JAK2 protein is a cytoplasmic tyrosine kinase. It is associated with cytoplasmic domain of growth factors and cytokines like thrombopoietin, erythropoietin, GM-CSF, G-CSF and IL-3. As a result of this mutation, this kinase remains active even without the growth factors stimulation causing continuous proliferation of mature cells. Various studies have reported that this mutation is present in more than 95% of the patients with PV and in 50-60% of patients with ET and IMF[13]. Mutation in exon 12 of JAK2 gene is reported in approximately 5% cases of JAK2 negative PV. JAK2 mutation has also been reported in few healthy elderly individuals, atypical MPN and in lymphoid lineages of some patients with PV [13,14].

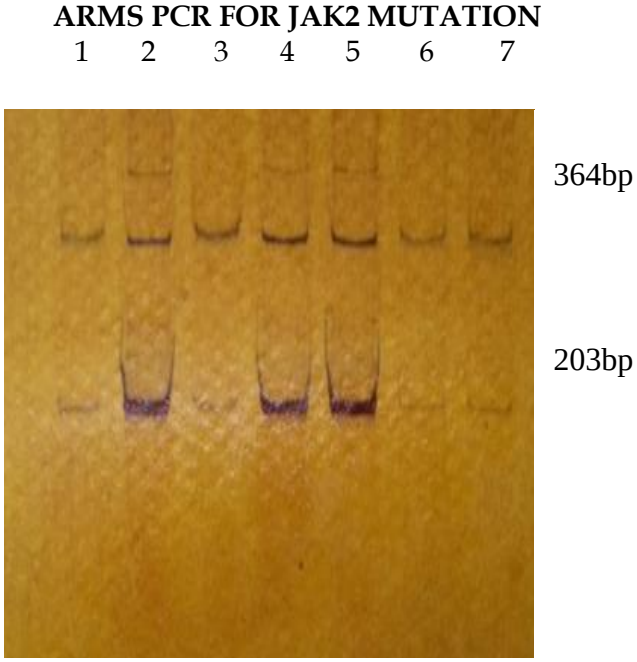

Figure-1: Silver stained PAGE of the JAK2 mutation analysis.

Test for the detection of JAK2 V617F mutation is now widely available. With the advent of this test, the diagnostic workup has become simplified. There are different assays for its detection including allele-specific polymerase chain-reaction (PCR) assay, restriction-enzyme digestion, pyrosequencing and real-time PCR. All these assays are sufficiently sensitive to detect the presence of a heterozygous mutation in as few as 5 to 10% of cells. These assays are useful diagnostic tools since they have low false positive results rate[15].

Keeping in view the high sensitivity and significance of this test in establishing the diagnosis of classical MPN, the present study was designed with an aim to evaluate the frequency and the role of JAK2 V617F mutation in the diagnosis of classical MPN.

The overall male predominance in our study population could be an incidental finding due to under representation of females seeking medical attention in our country[16], as most other studies and literature show an equal gender distribution in PV and IMF, whereas female predominance in ET[7,17]. In PV 16 (48.4%) patients had TLC of > 15 x 10^9/L. In a study conducted by Landolfi et al it was found that patients with a white cell count of > 15 x 109/L have significantly higher risk of thrombosis, mainly myocardial infarction[18].

Splenomegaly was found in 25 (75.7%) of PV patients, whereas 22 (61.1%) and 23 (95.8%) of ET and IMF patients respectively were also having splenomegaly. Patients found positive for JAK2 V619F mutation had higher splenomegaly (84.3%) than patients who were negative (55.1%). An Indian study also showed that incidence of splenomegaly is higher (86%) in patients of classical MPN with JAK2 mutation than patients without JAK2 mutation (58%)[19]. Previously, splenomegaly in the presence of absolute polycythemia was considered to be almost diagnostic of PV[20,21].

The principal observation of the study was that the overall JAK2 V617F mutation was present in 64 (69%) out of 93 patients with classical MPN. The positive and negative cases were clear cut. In this study the allelic specific primers which were used have not only high analytic sensitivity of 0.05 to 0.1% but they also have a high diagnostic accuracy of 99%[22]. All 33 (100%) patients of PV, 19/36 (52.7%) of ET and

12/24 (50%) of IMF were found positive for JAK2 V617F mutation.

JAK2 V617F mutation is usually found in homozygous state in 25-30% of patients having PV[23]. The WHO 2008 diagnostic criteria for PV, ET and IMF includes the presence of JAK2 V617F or similar clonal mutation regardless their homozygous or heterozygous state. This parameter was not included in our study.

The results of this study suggest that determination of JAK2 V617F mutation is a useful diagnostic modality in our country where it is quite cumbersome and also expensive to investigate for secondary causes of polycythaemia and thrombocytosis. PCR based test for JAK2 mutation detection is very reliable in establishing the cause whether it is primary or not.

JAK2 mutation analysis can also be useful in undiagnosed patients with unexplained splenomegaly, venous thrombosis, aquagenic pruritis, myelofibrosis, persistent thrombocytosis, leukoerythroblastosis and extramedullary haematopoiesis[24].

The prognosis of MPN can be predicted by JAK2 mutation analysis. It can also be used for assessing the relationship of gene dose to phenotype and also for monitoring therapy[25]. The major breakthrough after understanding molecular pathology of MPN is the development of JAK2 inhibitors which can block JAK2 STAT signaling pathway.

CONCLUSION

Classical myeloproliferative neoplasms are an important group of heamatological disorder in our country. JAK2 gene mutation is seen in significant proportion of these disorders (69%) and it can be used to differentiate between polycythemia vera and secondary polycythemia in most cases with near certainty, where it was found in 100% of the cases. The utility of serum erythropoietin assay, red cell mass measurement and endogenous erythroid colonies in vitro is limited in our setup because these tests are not widely available and are also costly.

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