Frequency of Janus Associated Kinase 2 (V617F) Mutation in Patients of Polycythemia Vera

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ABSTRACT

Objective: To determine the frequency of Janus associated kinase 2 *(JAK2)* mutation in patients of polycythemia vera (PV).

Study Design: Descriptive cross-sectional.

Place and Duration of Study: Haematology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January 2008 to December 2009.

Methodology: Forty-six consecutive patients of PV diagnosed by the conventional haematological criteria were included in the study. Blood samples of all patients were screened for G-T point mutation (V617F) in the *JAK2* gene on chromosome 9 by an allele specific polymerase chain reaction (PCR).

Results: *JAK2* V617F mutation was found in 43 out of 46 patients (93.5%) with PV. Among them, 30 were males (65.2%) and 16 were females (34.8%). Mean TLC in patients with PV was $16.5 \pm 9.1 \times 10^9$/L, mean haemoglobin (Hb) was 17.8 ± 2.0 g/dl, mean platelet count was $531 \pm 261 \times 10^9$/L, mean PCV was 57.9 ± 6.3 l/l, mean MCV was 78.8 ± 11.0 fl and mean MCH was 24.4 ± 4.8 pg.

Conclusion: Peripheral blood mutation screening for *JAK2* V617F can be incorporated into the initial work up of patients suspected to have polycythemia as this mutation is present in majority of such patients.

Key words: Polycythemia. *JAK2* mutation.

INTRODUCTION

Polycythemia vera or primary polycythemia is a chronic, clonal, myeloproliferative neoplasm (MPN) characterized by an absolute increase in the number of red blood cells and in the total blood volume, usually accompanied by leukocytosis, thrombocytosis and splenomegaly.[1]

Polycythemia may be the result of an increase in absolute quantity of red cells or red cell mass (called the absolute polycythemia) or the result of reduced plasma volume (called relative or spurious polycythemia).[2,3] The primary purpose during the evaluation of polycythemia is to determine the presence or absence of PV, because of the prognostic and treatment differences from the secondary causes.[2] Patients with PV are at an increased risk of transformation to myelofibrosis or acute leukemia in addition to the risk of thrombosis, stroke, myocardial infarction and bleeding.[1]

Relative polycythemia is a condition that may not require treatment.[1,4]

Diagnosis of PV was based on the traditional Polycythemia Vera Study Group (PVSG) diagnostic criteria which has stood the test of time, but in some patients it can be misleading.[5] The World Health Organization (WHO) diagnostic criteria for PV, essential thrombocythemia (ET) and primary myelofibrosis (PMF) were revised after the discovery of *JAK2* mutations in 2008 (for example: *JAK2* V617F exon 14 and *JAK2* exon 12 mutations in virtually all patients with PV).[6] Various studies have demonstrated that this mutation is present in more than 95% of the patients with PV and in 50-60% of patients with ET and PMF.[7-11]

Since some of the parameters described by the PVSG, such as the measurement of red cell mass (RCM) and serum Epo levels are expensive and are not easily available in our setup, it is difficult to make a secure diagnosis of PV. Therefore, determination of *JAK2* mutation is potentially helpful in the diagnosis of PV.[12]

Keeping in view the significance of this test in establishing the diagnosis of PV, the present study was designed with an aim to evaluate the role of *JAK2* V617F mutation in the diagnosis of PV by assessing the frequency of *JAK2* mutation in such patients.

METHODOLOGY

The study was conducted at the department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January 2008 to December

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2009. Approval of the study was taken from Ethics Review Board of AFIP. Informed consent were taken from the patients prior to their inclusion in the study. It was a descriptive cross sectional study and included 46 patients of PV diagnosed as per conventional haematological criteria. Blood sample of all the patients were taken in an Ethylene diamine tetra acetate (EDTA) tube and PCR was performed for G-T point mutation (V617F) in the *JAK2* gene on chromosome 9 by using allele specific primers.

JAK2 **mutation analysis:** DNA of each patient was extracted using PUREGENE genomic DNA purification kit by gentra systems (USA), from the fresh peripheral blood taken in EDTA tube, or from the unstained archival bone marrow smears when venous blood was not available. PCR amplification of the *JAK2* V617F mutation was done by a set of three primers. *JAK2* mutant allele was amplified with a common reverse primer (5'-CTGAATAGTCCTACAGTGTTTTCAGTTTCA) and a forward specific primer (5'-AGCATTTGGTTTTAAAT TATGGAGTATATT) producing 203 base pair (bp) amplified product. The common reverse primer and a forward control primer (5'-ATCTATAGTCATGCTGAA AGTAGGAGAAAAG) amplified another 364bp product which served as PCR internal control.

The DNA was amplified in a 20 µl reaction mixture containing 20pM of the common primer and 10pM 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 KCL, 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 $94^{o}C$ for 40 seconds, primer annealing at $58^{o}C$ for 40 seconds and extension at $72^{o}C$ for 1 minute. The PCR amplified products along with 100bp ladder were run on 6% mini polyacrylamide gel electrophoresis (PAGE) at 150V for 40 minutes. The gels were stained by silver nitrate (Figure 1).

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 17. Quantitative variables like Age, Hb, TLC, Platelets, PCV(Hct), MCV and MCH were presented with mean ± SD. Frequencies and percentages were computed for presentation of qualitative variables like *JAK2* mutation, splenomegaly and gender. Data is normally distributed as checked by Kolmogorov-Smirnov and Shapiro-Wilk test.

RESULTS

A total of 46 subjects with PV (Hb > 17.0 g/dl, PCV > 0.52 l/l in males and Hb > 15.0 g/dl, PCV > 0.48 l/l in females) were evaluated in the study. The mean age of the patients was 59.8 ± 14.6 (Mean ± SD) years. Their ages ranged from 23 to 97 years. Most frequent decade was seventh decade with 19 patients (41.3%) followed by sixth decade with 8 patients (17.4%). Among 46 patients, there were 30 males (65.2%) and 16 females (34.8%) with a male to female ratio of 1.9:1. Splenomegaly was present in 32 patients (69.6%) [95% confidence interval (CI) = 56.3 - 82.9%], out of which 28 patients had a palpable spleen while, remaining 4 had a splenic enlargement diagnosed on ultrasonography.

JAK2 V617F mutation was found in 43 out of 46 patients with PV (93.5%) [95% CI = 87 - 100%], detected by an allele specific amplification refractory mutation screening (ARMS) PCR. Statistics for quantitative variables like TLC, Hb, MCV, MCH, Hct and Platelet count in both *JAK2* V617F positive and negative patients are shown in Table I.

DISCUSSION

MPN are clonal, heterogeneous disorders of haematopoiesis arising from transformation in a haemopoietic stem cell and characterized by proliferation of one or more mature functional cell lines such as granulocytes, platelets or erythroid cells. Traditionally MPN have been classified as Philadelphia positive and Philadelphia negative MPN. Philadelphia positive MPN which include chronic myeloid leukemia is defined by its molecular lesion, the BCR-ABL fusion gene, resulting from Philadelphia translocation. The three classical Philadelphia negative MPN are PV, ET and PMF.[7,13] Distinction of PV from secondary polycythemia has remained a complicated task during the evaluation
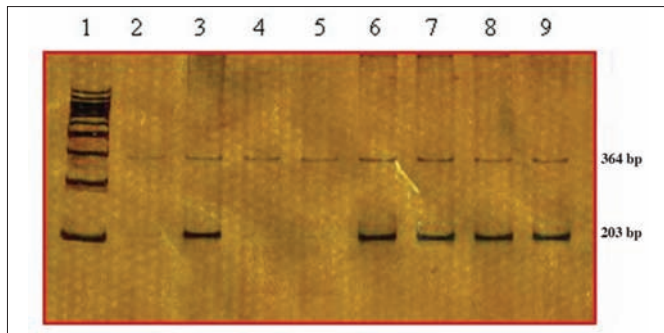
Figure 1: Silver stained page of the *JAK2* mutation analysis.

The PCR internal control is represented by a 364 bp fragment whereas the mutation is represented by a 203 bp fragment. Lane 1 is representative of 100 bp ladder. The lanes 2 and 3 show negative and positive controls respectively. Lanes 4 and 5 show negative results while lanes 6-9 show positive results.

Table I: Haematological parameters for *JAK2* V617F status in PV patients.

JAK2 V617F (status)		TLC (x10⁹/L)	Hb (g/dl)	MCV fl	MCH pg	Hct %	Platelets (x 10⁹/L)
POS	43 (93.5%)	16.6 (±9.2)	17.7 (±2.0)	78.3 (±11.2)	24.0 (±4.7)	58.1 (±6.5)	541 (±264)
NEG	03 (6.5%)	14.6 (±8.4)	19.3 (±.32)	86.1 (±3.9)	29.8 (±1.4)	55.7 (±2.0)	380 (±179)
Total	46	16.5 (±9.1)	17.8 (±2.0)	78.8 (±11.0)	24.4 (±4.8)	57.9 (±6.3)	531 (±261)

of erythrocytosis. Due to differences in clinical profile, disease evolution, prognostic implications and therapeutic options, the significance of differentiating PV from secondary polycythemia cannot be ignored.[14]

Until recently there was no specific genetic or molecular marker for the diagnosis of MPN and the criteria for diagnosis of PV were based on 20 years old standards set by PVSG. Some of the tests used in these criteria are determination of RCM, testing of Epo levels, identification of independent erythroid colonies *in vitro* and cytogenetic analysis of bone marrow. The above mentioned tests are expensive, not easily available and also lack sensitivity and specificity.[7]

Discovery of the *JAK2* V617F mutation in MPN in 2005 has enabled us to understand the molecular and cellular basis of these disorders. Normal *JAK2* protein is a cytoplasmic tyrosine kinase associated with cytoplasmic domain of growth factors and cytokines like Epo, Thrombopoietin, Interleukin-3, Granulocyte colony stimulating factor (CSF) and Granulocyte macrophage-CSF. Mutation in the *JAK2* renders that this kinase remain active even without the growth factors stimulation causing continuous proliferation of mature cells.[7,13]

The principal observation of the study was that th*e JAK2* V617F mutation was present in 43 out of 46 patients (93.5%) with PV. *JAK2* V617F mutation usually occurs in homozygous state in 25-30% of patients with PV.[13,15] Since, WHO 2008 diagnostic criteria for PV includes the presence of *JAK2* V617F or similar clonal mutation irrespective of their homozygous or heterozygous state, such parameter was not included in this study.[6] The results of this study closely match with the results of previous local and international studies.[10,11]

Three patients were negative for *JAK2* V617F mutation. These are the patients who should be screened for *JAK2* exon 12 mutations as this mutation is present in 5% of the *JAK2* V617F negative PV patients. Such patients present mostly with higher Hb, and lower TLC and platelet count than the patients with *JAK2* V617F mutation.[16] *JAK2* V617F mutation is the first genetic marker that is directly associated with the pathogenesis of MPN. For the same reason *JAK2* V617F mutation has been included as an essential component (major criteria) in the 2008 WHO diagnostic criteria for PV, ET and PMF.[6]

The identification of somatic mutations in the JAK-STAT (signal transducer and activator of transcription) signaling pathway in MPN provides an opportunity to develop targeted therapies at the molecular level for such patients. This has guided several groups to develop specific inhibitors of *JAK2* kinase activity.[17] Phase 1 trials with such inhibitors have already been initiated in PMF and post PV/ET myelofibrosis.[17,18] Response to treatment is also affected by *JAK2* V617F

mutation status.[19] For example among patients with ET, those with the V617F mutation are more responsive to hydroxyurea (but not to anagrelide) than are V617F negative patients.[7,20] Given the availability of reliable quantitative assays the depth of response can be directly monitored through quantification of *JAK2* V617F positive cells in peripheral blood.[21]

The results of this study suggest that the presence of the *JAK2* mutation in a patient with polycythemia (PCV > 0.52 l/l in men and > 0.48 l/l in females) can help us in establishing the diagnosis of PV.

CONCLUSION

Peripheral blood mutation screening for *JAK2* V617F by ARMS PCR can be incorporated into the initial evaluation of patients suspected to have polycythemia as this mutation is present in majority of such patients.

REFERENCES

1. Means RT. Polycythemia vera. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, editors. Wintrobe's clinical haematology. 11th ed. Philadelphia: *Lippincott Williams & Wilkins*; 2004.p. 2259-72.

2. Kendall RG. Erythropoietin. *Clin Lab Haem* 2001; **23**: 71-80.

3. Samol J, Littlewood TJ. The efficacy of rHuEPO in cancer related anaemia. *Br J Haematol* 2003; **121**:3-11.

4. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood* 1951; **6**:372-5.

5. Usman M, Bilwani F, Kakepoto GN, Adil SN, Sajid R, Khurshid M. Polycythemia vera and idiopathic erythrocytosis: comparison of clinical and laboratory parameters. *J Pak Med Assoc* 2004; **54**:249-51.

6. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 2008; **22**:14-22.

7. Campbell PJ, Green AR. The myeloproliferative disorders. *N Engl J Med* 2006; **355**:2452-66.

8. Tefferi A. JAK-2 mutation in polycythemia vera-molecular mechanisms and clinical applications. *N Engl J Med* 2007; **356**: 444-5.

9. Larsen TS, Christensen JH, Hasselbalch HC, Pallisgaard N. The *JAK2* V617F mutation involves B-and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. *Br J Haematol* 2007; **136**: 745-51.

10. Ahmed S, Anwar J, Lodhi RA, Hanif TB, Abbas SK, Qureshi TZ. *JAK m*utation and myeloproliferative disorders in Pakistan. *Pak J Pathol* 2008; **19**:73-7.

11. Khawaja A. The role of Janus kinases in haemopoiesis and haematological malignancy. *Br J Haematol* 2006; **134**:366-84. Epub 2006 Jul 5.

12. Chen Q, Lu P, Jones AV, Cross NC, Silver RT, Wang TL. Amplification refractory mutation system, a highly sensitive and simple polymerase chain reaction assay, for the detection of *JAK2* V617F mutation in chronic myeloproliferative disorders. *J Mol Diagn* 2007; **9**:272-6.

13. Levine RL, Gilliland DG. Myeloproliferative disorders. *Blood* 2008; **112**:2190-8.

14. Goldman JM, Melo JV. Chronic myeloid leukemia: advances in biology and new approaches to treatment. *N Engl J Med* 2003; **349**:1451-64.

15. Tefferi A, Lasho TL, Schwager SM, Strand JS, Elliott M, Mesa R, *et al*. The clinical phenotype of wild-type, heterozygous, and homozygous *JAK2*(V617F) in polycythemia vera. *Cancer* 2006; **106**:631-5.

16. Percy MJ, Scott LM, Erber WN, Harrison CN, Reilly JT, Jones FG, *et al*. The frequency of *JAK2* exon 12 mutations in idiopathic erytrhrocytosis patients with low serum erythropoietin levels. *Haematologica* 2007; **92**:1607-14.

17. Pardanani A, Hood J, Lasho T, Levine RL, Martin MB, Noronha G, *et al*. TG101209, a small molecule *JAK2*-selective-kinase inhibitor potently inhibits myeloproliferative disorder-associated JAK2V617F and MPLW515L/K mutations. *Leukemia* 2007; **21**: 1658-68. Epub 2007 May 31.

18. Pardanani A. *JAK2* inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. *Leukemia* 2008; **22**:23-30.

19. Finazzi G, Barbui T. How I treat patients with polycythemia vera. *Blood* 2007; **109**:5104-11.

20. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, *et al*. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. *Blood* 2006; **107**:3339-41.

21. Barosi G, Birgegard G, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, *et al*. Response criteria for essential thrombocythemia and polycythemia vera: result of a European Leukemia Net consensus conference. *Blood* 2009; **113**:4829-33. Epub 2009 Mar 10.

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