

FREQUENCY OF JAK2 MUTATION IN BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN KHYBER PAKHRUNKHWA

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ABSTRACT

Foundation: The “BCR-ABL Negative Classic Myeloproliferative Neoplasms” incorporate Polycythaemia Vera (PV), Essential Thrombocythaemia (ET) and Primary Myelofibrosis (PMF). These three issues share a few clinical and research facility highlights including JAK2 V617F transformations.

Objective: To decide the recurrence of JAK2 V617F change in patients with BCR-ABL negative myeloproliferative neoplasm (MPNs) in Khyber Pakhtunkhwa.

Study Design: Descriptive cross-sectional.

Spot and Duration of Study: Hematology Department, Institute of fundamental clinical sciences (IBMS), Khyber Medical University, Peshawar, from October 2016 to September 2017.

Strategy: Sixty-six analyzed patients of MPN were remembered for the investigation. Blood tests of all patients were screened for G-T point transformation (V617F) in the JAK2 quality on chromosome 9 by an allele explicit polymerase chain response (PCR).

Results: JAK2 V617F change was found in 50 (75.75%) out of 66 patients with MPN. Among them, 45 (68.18%) were male and 21 (31.82%) were female. They went in age from 20-80 years with mean period of 57.3 ± 13.3 years for male and 56.3 ± 16.6 for female.

End: JAK2 V617F transformation screening in the fringe blood by allele explicit PCR can be joined into the convincing assessment of patients suspected to have MPN, as this change is available in lion’s share of such patients.

Catchphrases: MPNs, PV, ET, PMF, JAK2 transformation, PCR
Key Words: MPNs, PV, ET, PMF, JAK2 mutation, PCR

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a gathering of clonally determined immature microorganism issues, portrayed by distorted hematopoietic expansion and endurance of at least one cell lines of the myeloid birthplace¹. The phenotypic similitudes among constant myeloid leukemia (CML), Polycythaemia Vera (PV), Essential Thrombocythaemia (ET) and Primary Myelofibrosis (PMF) were featured by William Dameshek in 1951, and assembled them under the rubric of myeloproliferative issues (MPDs)². In 1960, CML turned into the primary disease to be related with a particular cytogenetic marker, the Philadelphia chromosome (Ph1), which in this manner was appeared to hold a corresponding chromosomal movement t(9;22)(q34;q11)^{3,4}. These fundamental perceptions eventually prompted the recognizable proof of causal change

(BCR-ABL) in CML^{5,6}. Appropriately the “exemplary” MPDs are currently ordered into BCR-ABL(+) (CML) and BCR-ABL(-) (PV, ET and PMF)⁷. The cardinal highlights of the three fundamental myeloproliferative issues are an expanded red-cell mass in PV, a high platelet include in ET, and bone marrow fibrosis in PMF. These three problems share numerous qualities, including marrow hypercellularity, an inclination to apoplexy and drain, and a danger of leukemic change in the long haul. The yearly frequencies of both PV and ET are 1 to 3 cases for every 100,000 populace; PMF is more uncommon⁸.

An obtained, increase of capacity change in (JAK2 V617F) is available in a large portion of the patients with BCR-ABL (-) myeloproliferative neoplasms. JAK2 encodes for cytoplasmic tyrosine protein kinases (PTKs), that intercede flagging downstream of cytokine receptors⁹. Enactment of the JAK-cytokine receptor complex outcomes in enlistment and JAK-interceded phosphorylation of substrate atoms including STAT proteins whose resulting atomic movement incites target quality record¹⁰. JAK-STAT flagging has pleiotropic impacts on cell expansion, cell endurance and invulnerable reactions¹¹. JAK2 V617F is a G to T physical transformation of JAK2 quality, in exon 14 of the chromosome 9p24, bringing about replacement of valine to phenylalanine at

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codon 617¹². The relationship of JAK2 with Philadelphia negative MPNs was first revealed in 2005¹³. JAK2 V617F transformation causes cytokine-autonomous actuation of JAK-STAT, PI3K, and AKT pathways¹³.

The World Health Organization (WHO) analytic rules for polycythemia vera, basic thrombocythaemia and essential myelofibrosis, were amended after the revelation of JAK2 change in 2008¹⁴. Different investigations have exhibited that this transformation is available in around 95%, 60% and half instances of PV, ET and PMF separately¹⁵. Assurance of JAK2 transformation is possibly useful in the analysis of MPNs, in light of the fact that it has a critical bearing on the clinical result of these patients, likewise changing treatment technique.

Keeping in view the noteworthiness of this test in setting up the determination of MPNs, the current examination was planned with an expect to assess the function of JAK2 V617F transformation in the analysis of MPNs by evaluating the recurrence of JAK2 change in such patients.

METHODOLOGY

The investigation was directed at the branch of Hematology, Institute of Basic Medical Sciences (IBMS) Khyber Medical University (KMU) Peshawar, from October 2016 to September 2017. Study was started after underwriting obtained from ASARB and moral leading body of KMU. Educated assent were taken from the patients preceding their consideration in the investigation. It was an unmistakable cross sectional investigation and included 66 patients of BCR-ABL negative MPNs. Blood tests were gathered in Ethylene diamine tetra acetic acid derivation (EDTA) tubes at appointed symptomatic focuses and clinics (Hayatabad Medical Complex, Institute of Radiotherapy and Nuclear Medicine (IRNUM) and Blood Diseases Clinic, Peshawar) via prepared phlebotomists applying standard methods. The gathered blood tests were moved to hematology lab IBMS for primer examinations, preparing and capacity. A total blood check (CBC) was performed on blood tests, utilizing Sysmex pochH-100i® Automated Hematology Analyzer. DNA was segregated from all blood tests utilizing manual strategy for DNA extraction. PCR intensification of the JAK2 V617F change was finished by utilizing allele explicit groundworks. PCR enhancement of the JAK2 V617F transformation was finished with a typical converse groundwork (5'- CTGAATAGTCCTACAGT-GTTTTTCAGTTTCA) and a forward explicit preliminary (5'- AGCATTGGTTTTAAAT TATGGAGTATATT). Information was dissected utilizing Statistical Package for the Social Sciences (SPSS) variant 23. Quantitative factors like Age, Hb, TLC, Platelets, and PCV (Hct) were given mean \pm SD. Frequencies and rates were registered for introduction of subjective factors like JAK2 change and sexual orientation.

RESULTS

A sum of 66 analyzed patients of BCR-ABL negative MPN were remembered for the current examination. All the patients were examined dependent on age and sexual orientation conveyance. They went in age from 20-80 years with mean time of 57.3 ± 13.3 years for male and 56.3 ± 16.6 for female. Out of these 66 patients, 45 (68.18%) were male while 21 (31.82%) were female with a male to female proportion of 2.1:1. The mean age and sexual orientation dispersion of study populace as for the conclusion is appeared in table-1. Hematological boundaries of the examination members are appeared in table 2. JAK2 V617F transformation was discovered positive in 90.62%, 63.15% and 60% of patients with PV, ET and PMF separately, by allele explicit PCR (Table-3). Hematological boundaries were discovered higher in JAK2 positive patients with exemption of platelets check which was low (Table-4). Table 1. Age and Gender wise distribution of the study population

DISCUSSION

Myeloproliferative neoplasms (MPNs) are clonally determined immature microorganism issues of haematopoiesis, portrayed by expansion of at least one practically develop cell lines of the myeloid cause. The three traditional BCR-ABL negative MPNs are PV, ET and PMF¹⁴. Up to this point there was no particular hereditary or sub-atomic marker for the determination of MPN and they were analyzed based on preset models. A portion of the tests utilized in these models are costly, not effectively accessible and furthermore need affectability and particularity¹⁵. Revelation of the JAK2 V617F change in 2005 has empowered us to comprehend the sub-atomic and cell premise of MPN¹⁶. Ordinary JAK2 is a tyrosine kinase, joined to the cytokine receptor cytosolic area, assumes a vital function in the transduction of signs from various development factor receptors (Epo-R, Tpo-R, GM-CSF-R, IL-3 receptor)¹⁷. JAK2 change delivers that, this kinase stays dynamic even without development components and cytokines incitement, causing constitutive expansion of develop cells.

The current examination learned a few significant realities in patients with PV, ET and PMF, having a place with Khyber Pakhtunkhwa region of Pakistan. As far as we could possibly know, it is the primary investigation from this area, deciding the greatness of JAK2 change in these issues. The middle age at introduction for each of the three conditions in our examination is tantamount to that depicted in the writing (Table-1)^{8,18}. Sex proportions for PV and PMF in this examination are 1.9:1 and 2.75:1 separately, which is as per recently distributed public and worldwide investigations (Table-1)^{19,20}. Female dominance in ET (female to male proportion of 2:1) has been accounted for in a few investigations which in differentiations the consequences of the current examination (male to female proportion of 2.1:1)²¹. It is, in

	PV	ET	PMF	Total
Number of Patients (%)	32 (48.49%)	19 (28.79%)	15 (22.72%)	66 (100%)
Male	21 (65.62%)	13 (68.42%)	11 (73.34%)	45 (68.18%)
Female	11 (34.38%)	06 (31.58%)	04 (26.66%)	21 (31.82%)
Age in Years (Mean ± SD)				
Male	52.5±12.6	61.4±8.3	59.5±19.8	
Female	64±14.8	53.6±15.9	55.6±14.8	

Table 2. Haematological Parameters

Diagnosis	Sex	Haemoglobin	Haematocrit	MCV	WBC	Platelets
		(g/dl)	(%)	(fl)	(x109/L)	(x109/L)
PV	Male	19.2±2.0	56.2±5.8	75.4±17.1	20.6±13	470±182
	Female	18.1±2.2	53.8±6.5	79.1±12.8	22±13.8	568±224
ET	Male	11.8±1.9	38.01±6.2	83.7±3.5	16.7±10.5	1358±781
	Female	13.0±1.4	39.6±4.5	84.8±8.4	11.8±3.9	986±446
PMF	Male	12.6±2.0	35.7±7.2	76.1±14.3	20.3±17	646±623
	Female	11.6±2.4	34.7±6.0	74.2±12.2	18.3±15.4	570±480

Table 3. JAK2 V617F Mutation Status with respect to diagnosis

JAK2 Mutation	PV	ET	PMF	Total
Positive	29 (90.62%)	12 (63.15%)	09 (60%)	50 (75.75%)
Negative	03 (09.38%)	07 (36.85%)	06 (40%)	16 (24.25%)

Table 4. Haematological Parameters with respect to JAK2 mutation status

	JAK2 V617F Positive	JAK2 V617F Negative
Number of patients	50 (75.75%)	16 (24.25%)
Haemoglobin (g/dL)	15.5±3.2	13.3±3.0
Haematocrit (%)	48.4±10.5	41.5±8.8
White Blood Cells (x109/L)	19.4±12.3	10.1±2.9
Platelets (x109/L)	716±485	876±535

any case, recommended that discoveries from current examination be affirmed in bigger gathering of patients.

JAK2 V617F change is the principal hereditary marker that is straightforwardly connected with the pathogenesis of Philadelphia negative MPN. For a similar explanation JAK2 transformation has been incorporated as a fundamental part (significant standards) in the 2008 WHO indicative models for PV, ET and PMF¹⁴. Distinguishing proof of these substantial changes in the JAK-STAT (signal transducer and activator of record) flagging pathway gives an occasion to create focused

on treatments at the atomic level for MPN patients. The members of the current examination were read for JAK2 V617F transformation. Out of 32 PV patients, JAK2 transformation was positive in 29 (90.62%), while, just 03 (9.38%) patients didn't convey this change. These are the patients who should be screened for JAK2 exon 12 transformations as this change is available in 5% of the JAK2 V617F negative PV patients. The said change was distinguished in 63.15% and 60.00% ET and PMF patients, separately. The predominance of JAK2 V617F change in this examination is practically identical to accessible writing (Table-3)^{15,20}. Hematological boundaries were discovered higher in JAK2 positive when contrasted with JAK2 negative patients aside from the platelet tally which was low (Table-4). These discoveries are upheld by those of an investigation led on 58 MPN patients of Pakistani beginning²².

The consequences of this examination recommend that the presence of the JAK2 change in patients with doubt of myeloproliferative neoplasms can help us in building up the analysis and ensuing administration.

CONCLUSION

Peripheral blood mutation screening for JAK2 V617F by allele specific PCR can be incorporated into the conclusive evaluation of patients suspected to have MPN, as this mutation is present in majority of such

patients.

REFERENCES

1. Tefferi A, Gilliland DG. Oncogenes in myeloproliferative disorders. *Cell cycle*. 2007;6(5):550-66.
2. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood*. 1951;6(4):372-5.
3. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. *Journal of the National Cancer Institute*. 1960;25(1):85-109.
4. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243(5405):290.
5. Teyssier J, Bartram C, Deville J, Potron G, Pigeon F. c-abl Oncogene and chromosome 22" bcr" juxtaposition in chronic myelogenous leukemia. *N Engl J Med*. 1985;312(21):1393-4.
6. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med*. 2001;344(14):1038-42.
7. Tefferi A, Gilliland G. Classification of chronic myeloid disorders: from Dameshek towards a semi-molecular system. *Best Practice & Research Clinical Haematology*. 2006;19(3):365-85.
8. Kutti J, Ridell B. Epidemiology of the myeloproliferative disorders: essential thrombocythaemia, polycythaemia vera and idiopathic myelofibrosis. *Pathol Biol*. 2001;49(2):164-6.
9. Rane SG, Reddy EP. Janus kinases: components of multiple signaling pathways. *Oncogene*. 2000;19(49):5662-79.
10. Benekli M, Baer MR, Baumann H, Wetzler M. Signal transducer and activator of transcription proteins in leukemias. *Blood*. 2003;101(8):2940-54.
11. Kisseleva T, Bhattacharya S, Braunstein J, Schindler C. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene*. 2002;285(1-2):1-24.
12. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *The Lancet*. 2005;365(9464):1054-61.
13. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer cell*. 2005;7(4):387-97.
14. Tefferi A, Vardiman J. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2008;22(1):14-22.
15. Campbell PJ, Green AR. The myeloproliferative disorders. *N Engl J Med*. 2006;355(23):2452-66.
16. Kralovics R, Passamonti F, Buser AS, Teo S-S, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352(17):1779-90.
17. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia*. 2010;24(6):1128.
18. Visani G, Finelli C, Castelli U, Petti M, Ricci P, Vianelli N, et al. Myelofibrosis with myeloid metaplasia: clinical and haematological parameters predicting survival in a series of 133 patients. *Br J Haematol*. 1990;75(1):4-9.
19. Khwaja A. The role of Janus kinases in haemopoiesis and haematological malignancy. *Br J Haematol*. 2006;134(4):366-84.
20. Suhaib A, Jaleel A, Rehan Ahmed L, Tamoor BH, Syed Kazim A, Tanveer Zia Q. JAK2 mutation and myeloproliferative disorders in Pakistan. *Pak J Pathol*. 2008;19(3):73-7.
21. Al-Sayes FM, Gauthaman K. Essential Thrombocythemia: Current Molecular and Therapeutic Insights. *Saudi J Int Med*. 2015;5(1):5-10.
22. Shaikh MS, Shaikh MU, Adil SN, Khurshid M, Ahmed ZA. Clinico-pathological profile and outcomes of patients with polycythaemia vera, essential thrombocythaemia and idiopathic myelofibrosis: a tertiary care center experience from southern Pakistan. *JAMC*. 2016;28(2):293-7.