Original Article

Frequency of JAK2 and MPL Mutation in BCR/ABL Negative Myelofibrosis in KPK

Ayesha Gul[1], Sheeba Ishtiaq[2], Hina Umair[3], Memoona Rasheed[4]

[1] Senior Lecturer, North West School of Medicine, Peshawar.
[2] Assistant Professor Histopathology, Gulab Devi Chest Hospital, Lahore.
[3] Assistant Professor Physiology, Wah Medical College, Wah Cantt.
[4] Molecular Biologist, Islamabad Medical & Dental College, Islamabad.

Author's Contribution
[1] Conception of study
[1,2,3] Experimentation/Study conduction
[1,2,3] Analysis/Interpretation/Discussion
[1,2,3] Manuscript Writing
[1] Critical Review
[1] Facilitation and Material analysis

Corresponding Author
Dr. Ayesha Gul,
Senior Lecturer,
North West School of Medicine,
Peshawar
Email: ayesha_gul07@hotmail.com

Article Processing
Received: 23/11/2021
Accepted: 03/03/2022

Cite this Article: *Gul, A., Ishtiaq, S., Umair, H., Rasheed, M. Frequency of JAK2 and MPL Mutation in BCR/ABL Negative Myelofibrosis in KPK. Journal of Rawalpindi Medical College. 30 Jun. 2022; 26(2): 266-270.*
DOI: https://doi.org/10.37939/jrmc.v26i2.1845

Conflict of Interest: *Nil*
Funding Source: *Nil*

Access Online:

Abstract

Introduction: BCR-ABL1-negative myeloproliferative disorders are a sub-group of myeloproliferative neoplasms (MPNs) that consist of polycythemia Vera (PV), Essential thrombocythemia (ET), and Primary Myelofibrosis (PMF).

Objective: To determine the frequency of JAK2 (p.V617F) and MPL (p.W515L) mutation in primary myelofibrosis in the KPK province of Pakistan.

Materials & Methods: Fifty patients with PMF were enrolled in the study. JAK2 mutation status was determined using allele-specific primers and MPL mutation was detected by the direct Sanger sequencing technique. The data was analyzed using BioEdit by aligning the sequence data with reference genome hg19 assembly.

Results: Among 50 patients, 41 patients were diagnosed with PMF, while 9 patients had secondary myelofibrosis i.e. Post PV-MF and Post ET-MF. Out of the 41 PMF patients, 2 patients had MPL gene variation, while one of the Post ET –MF had an MPL gene variation. Forty-eight (96%) were positive for JAK2 mutation. Five patients who had MPL mutation also showed JAK 2 mutation. Two of the MPL-positive patients were also positive for JAK2 mutation.

Conclusion: We reported the rate of recurrence of JAK2 mutation in 96% of the cases and MPL exon 10 mutations in 6% of the cases.

Keywords: JAK2, MPL, Myeloproliferative neoplasms, BCR-ABL1, Genetic diagnosis.

Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders that results in an increase in the number of erythrocytes, megakaryocytes, or granulocytes in blood circulation.[1] According to World Health Organization (WHO) 2008 criteria and the 2016 revision, classical MPNs include chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[2,3] Among these classes, CML is BCR-ABL1 positive while PV, PMF, and ET are negative.[4] The clinical profile of the patients includes cytopenia of one or more lineages, myelofibrosis, thrombosis, varying degrees of extramedullary hematopoiesis, and risk of transformation to acute leukemia.[5]

Besides morphological evaluation, genetic diagnosis is a major criterion for MPNs. The major discovery in molecular diagnostics of BCR-ABL1 negative MPN occurred with the identification of p.V617F mutation in the JAK2 gene whose frequency is 95% in PV patients and 50-60% in ET and PMF patients (6–8). There are a few reports in this context from developing countries including Pakistan so the present study aimed to improve the knowledge on the clinical and molecular profile of BCR-ABL1 negative in the population of Khyber Pakhtunkhwa (KPK) province of Pakistan.

Materials and Methods

This cross-sectional study was conducted from 2016 to 2018. The study was commenced after the approval from the AS&RB (Advanced Studies and Research Board) and the Research Ethical Council of Khyber Medical University (KMU). Fifty patients with primary and secondary myelofibrosis due to Post ET and Post PV were recruited from the oncology unit of Hayatabad Medical Complex (HMC) and Institute of Radiotherapy and Nuclear Medicine (IRNUM) hospital after obtaining informed written consent. Clinical information was collected under the supervision of a physician and a Complete blood count; total leukocyte count, bone marrow biopsy, and platelet count were performed for each patient.

Peripheral blood was collected in EDTA tubes and DNA was isolated using the standard salting out method. JAK2 mutation (p.V617F) was accessed by using allele-specific primers (Table 1) and the products were visualized by using 1.5% agarose gel. For analysis of MPL exon 10 mutations, p.W515L, sanger sequencing was performed by using primers designed from the flanking regions (Table 1) and the sequencing data was analyzed by using the BioEdit tool by aligning individual sequences with the reference genome (hg19). Online bioinformatics tools were used (MutationTaster, Provean, SIFT, Polyphen-2) to determine the pathogenicity of the variants identified in the sequencing data. Statistical analysis was performed by using SPSS software version 20.0 (Chicago, IL, USA). A p-value of <0.05 was considered significant.

Table 1: Allele-specific and Sanger sequencing primers

Mutation	*Primer*	*Primer Sequences*
JAK2: p.V617F	JAK2-Ex13 F	AAAGGGACCAAAGCACATTGT
	JAK2-Ex13-R(G)	GTTTTACTTACTCTCGTCTCCACACAC
	JAK2-Ex13-R(T)	GTTTTACTTACTCTCGTCTCCACACAA
MPL: p.W515L	MPL_Ex10 F	CCGAAGTCTGACCCTTTTTG
	MPL_Ex10 R	ACAGAGCGAACCAAGAATGC

Results

A total of 50 individuals were recruited for this study. Out of these 50 patients, 41(82%) were diagnosed with Primary myelofibrosis (PMF), while 9 (18%) patients had SMF. These SMF patients included 6(12%) post-PV cases and 3(6%) post- ET. The patient with PMF (range16-80) had a mean age of 49.17 while patients with SMF (post-PV and post-ET) had a mean value of 58.11 (range 41-70). Among the 50 individuals, 16 (32%) were female while 34 (68%) were male. The patients presented with wide-range symptoms that included fever (72%), pallor (62%), easy bruising (12%), splenomegaly (98%), bleeding gums, and hepatomegaly (6%). The hematological parameters of the study population are presented in Table 2.

Table 2: The hematological parameters of the study population

Diagnosis	*Hb g/dl*	*Hct %*	*MCV Fl*	*MCH pg*	*MCHC g/dl*	*WBC X 109/L*	*Platelet count X 109/L*
PMF (n=41)	8.92±2.6	32.46±7.73	70.55±8.04	22.08±3.2	30.2±2.66	21.2±7.59	273.27±204.6
Post ET (n=3)	11.3±2.4	42.33±2.51	72.8±4.8	72.8±4.8	31.1±1.96	22.5±22.3	265.00±97.00
Post PV (n=6)	11.88±4.8	37.33±10.94	66.95±6.9	22.6±3.6	30.5±3.04	21.4±17.5	272.17±230.04

The allele-specific PCR performed for JAK2 mutation detection showed the presence of a mutation in 96% (n=48) of the sampled individuals. Sequencing data analysis of exon 10 of the MPL gene revealed the presence of a missense variation, p.W515K, in heterozygous state in two affected individuals (M7, M33) (Figure 1). Another heterozygous nonsense variant, c.1545G>A (p.W515*), was also identified in another affected individual (M14) (Figure 1). Both variants were predicted to be a disease caused by mutation taster and are known to be associated with the pathogenicity of myelofibrosis. Out of the 41 patients who had PMF, two patients tested negative for JAK2 mutation. All the patients with SMF tested positive for JAK2 mutation. Table 3 represents the hematological profile of patients positive for MPL gene mutation.

Table 3: Hematological profile of patients positive for MPL gene mutation

ID	*Age/Gender*	*Diagnosis*	*JAK2 Mutation*	*Cytogenetic abnormality*	*HB (g/dl)*	*WBC Count (X 10⁹/L)*	*Platelet count (X 10⁹/L)*
M7	27/M	PMF	Negative	Nil	16.3	16.4	161
M14	48/F	PMF	Positive	Nil	6.4	77.6	134
M33	58/M	POST- ET	Positive	Nil	13.8	13.7	153

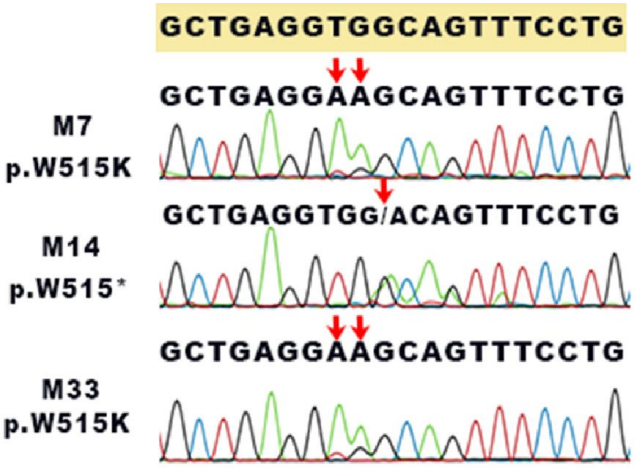

Figure 1: Sequencing data analysis of exon 10 of the MPL gene. Individual M7 and M33 are heterozygous for a missense variation, p.W515K and individual M14 are heterozygous for a nonsense variant p.W515*.

Discussion

Genetic data is an important parameter for the classification of classical BCR-ABL1 negative MPNs. The missense variant in JAK2, p.V617F, is a best-characterized mutation in BCR-ABL1 negative MPNs with a prevalence of >95% in PV, and 50% in ET and PMF.[6-8] It is a gain-of-function mutation that results in constitutive activation of the JAK-STAT pathway which leads to cytokine independent growth.[9] MPL is the second candidate for MPNs and is involved in the downstream signaling pathway that is required for mutant JAK2-mediated transformation of myeloprogenitor cells.[9]

In this study, we recruited 50 individuals of MPNs out of which 82% (n=41) were diagnosed with PMF and 18% (n=9) were diagnosed with SMF (post PV and post-ET). Forty-eight patients tested positive for JAK2 mutation while only three patients (M7, M14, M33) were found to be heterozygous for MPL mutation (p.W515*, p.W515K). All SMF patients were positive for JAK2 while 95% (n=39) of PMF were positive for JAK2 mutation. One of the PMF patients, M7, was negative for JAK2 and positive for the MPL p.W515K variant. The other two patients, M14 and M33, were positive for both JAK2 and heterozygous MPL mutation. The MPL W515 mutations were not found in Post-PV, which was suggestive that in this disease, JAK2 is obvious and is associated with the phenotype of red cell development.

Table 4: Prevalence of JAK2 and MPL mutations in different populations

S. No.	*Population*	*Prevalence of JAK2 Mutation*	*Prevalence of MPL Mutation*	*Reference*
1	China	85% in PV, 58.4% in ET and 65.8% in PMF	1.2% in ET, 2.7% in PMF	12
		-	1% in ET	13
2	France	-	3% of ET and 1% PMF	14
3	Italy	64.7%	4%	15
4	Turkey	43.9% in ET, 54.5% in PMF	2.6% in PMF	16
5	Pakistan	91% in PV, 63% in ET and 60% in PMT	-	17

Mutation screening studies conducted in different populations showed less occurrence of MPL mutation in MPN patients. Studies conducted on Iranian and Egyptian MPN patients didn't reveal any MPL mutation.[10,11] In china, 929 patients were screened for JAK2 and MPL mutations. They reported prevalence of 1.1% (1.2% in ET, 2.7% in PMF) patients for MPL mutation and 67.4% (85% in PV, 58.4% in ET and 65.8% in PMF) of patients for JAK2 mutation.[12] In another study from china, MPL pathological variant was identified in 1% of ET patients.[13] Study conducted in France showed the prevalence of MPL variant in 3% of ET and 1% of PMF patients[14] while the prevalence in Italy and Spain is reported to be 64.7% and 4% for JAK2 and MPL variant respectively in PMF patients.[15] Mutation screening on Turkish MPN patients reported MPL mutation frequency of 2.6% across PMF patients while they didn't find any in ET patients while the JAK2 mutation frequency in ET patients was 43.9%, and in PMF patients it was 54.5%.[16] A study conducted in KPK provide of Pakistan reported the prevalence of JAK2 mutation in 75.75% (n=50) patients among which 91% (n=29) were of PV, and 63% (n=12) were of ET and 60% (n=9) were of PMT.[17] Another study from Pakistan reported positivity for JAK2 p.V617F mutation in 15% (n=16) of the screened patients while JAK2 exon 12 mutation and MPL were found to be negative in all screened patients (n=90).[18] (Table 4) These studies suggest a lower prevalence of MPL gene mutation in PML and our data supports the previous findings.

The driver mutational profile complements the diagnostic approach to MPNs. The effect of the driver mutational profile on diagnosis and the identification of certain somatic mutations provide significant prognostic evidence, mainly in MF. The occurrence of these mutations may promote the diagnosis to a higher-than-expected risk group, which may help in deciding treatment recommendations, predominantly when considering stem cell transplantation. Preferably, a potential assessment of mutational profiles would recognize patterns of new mutations with disease progression and clonal evolution. This measures the effect of disease duration and therapy on the development/evolution of these mutations, and helps clinicians in determining whether or not monitoring allele variance frequency is necessary for all myeloid neoplasm patients.[19]

Conclusion

With help of allele-specific PCR, we were able to identify JAK2 mutation (p.V617F) in 96% (n=48) of the recruited patients out of which 39 were diagnosed with SMF. Heterozygous MPL mutations in exon 10 were identified using sanger sequencing in only 3 patients. The frequency of MPL variant was less in our study population which supports the previous data.

References

1. Skoda RC, Duek A, Grisouard J. Pathogenesis of myeloproliferative neoplasms. Vol. 43, Experimental Hematology. Exp Hematol; 2015. p. 599–608.
2. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Vol. 114, Blood. Blood; 2009. p. 937–51.
3. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Vol. 127, Blood. Blood; 2016. p. 2391–405.
4. Vannucchi AM, Guglielmelli P, Tefferi A. Advances in Understanding and Management of Myeloproliferative Neoplasms. CA Cancer J Clin. 2009 May 1;59(3):171–91.
5. A T. The history of myeloproliferative disorders: before and after Dameshek. Leukemia. 2008;22(1):3–13.
6. James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005 Apr 28;434(7037):1144–8.
7. 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. Lancet. 2005 Mar;365(9464):1054–61.

8. Xia J, Lu MZ, Jiang YQ, Yang GH, Zhuang Y, Sun HL, et al. JAK2 V617F, MPL W515L and JAK2 exon 12 mutations in Chinese patients with primary myelofibrosis. Chinese J Cancer Res. 2012 Mar;24(1):72–6.
9. Vakil E, Tefferi A. BCR-ABL1-negative myeloproliferative neoplasms: A review of molecular biology, diagnosis, and treatment. In: Clinical Lymphoma, Myeloma and Leukemia. Clin Lymphoma Myeloma Leuk; 2011.
10. Ghotaslou A, Nadali F, Chahardouli B, Alizad Ghandforosh N, Rostami SH, Alimoghaddam K, et al. Low frequency of c-MPL gene mutations in Iranian patients with Philadelphia-negative myeloproliferative disorders. Iran J Pediatr Hematol Oncol. 2015;5(1):43–439.
11. Eldeweny S, Ibrahim H, Elsayed G, Samra M. MPL W515 L/K mutations in myeloproliferative neoplasms. Egypt J Med Hum Genet. 2019 Dec 12;20(1):1–7.
12. Lin Y, Liu E, Sun Q, Ma J, Li Q, Cao Z, et al. The prevalence of JAK2, MPL, and CALR mutations in Chinese patients with BCR-ABL1-Negative Myeloproliferative Neoplasms. In: American Journal of Clinical Pathology. Am J Clin Pathol; 2015. p. 165–71.
13. Xu W, Li JY, Xia J, Zhang SJ, Fan L, Qiao C. MPL W515L mutation in Chinese patients with myeloproliferative diseases. Leuk Lymphoma. 2008 May;49(5):955–8.
14. Usseglio F, Beaufils N, Calleja A, Raynaud S, Gabert J. Detection of CALR and MPL Mutations in Low Allelic Burden JAK2 V617F Essential Thrombocythemia. J Mol Diagnostics. 2017 Jan 1;19(1):92–8.
15. Rumi E, Pietra D, Pascutto C, Guglielmelli P, Martínez-Trillos A, Casetti I, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Blood. 2014 Aug 14;124(7):1062–9.
16. Akpinar TS, Hançer VS, Nalçaci M, Diz-Küçükkaya R. Kronik miyeloproliferatif neoplazmlarda MPL W515L/K mutasyonlari. Turkish J Hematol. 2013;30(1):8–12.
17. Hamayun S, Farooq G, Marwat MA, Batool Z, Ali N, Gul S, et al. Frequency of JAK2 mutation in BCR-ABL negative myeloproliferative neoplasms in khyber. 2017;403–6.
18. Latif S, Khan SA, Mahmood A, Mahmood R. Frequency of MPL and JAK2 exon 12 gene mutation in myeloproliferative neoplasms. 2020;70(3):746–50.
19. Patel KP, Newberry KJ, Luthra R, et al. Correlation of mutation profile and response in patients with myelofibrosis treated with ruxolitinib. Blood. 2015;126(6):790-79

.