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Prevalence of MPL (W515K/L) Mutations in Patients with Negative-JAK2 (V617F) Myeloproliferative Neoplasm in North-East of Iran

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ABSTRACT

Background and Objective: Janus kinase 2 (JAK2) and Myeloproliferative Leukemia (MPL) mutations are confirmatory indicators for Myeloproliferative Neoplasm (MPN). The current study was performed to determine the frequency of MPL mutation in MPN patients without JAK2 mutation, in order to assign MPL mutation frequency in North-East of Iran.

Methods: Total of 105 negative JAK2 cases including 5 Myeloproliferative Disorders (MPD), 15 Polycytemia Vera (PV) and 15 Essential Thrombocytosis (ET) who referred to Qaem Medical Center were assigned to this study. ARMS-PCR was carried out for measuring MPL mutations.

Results: A significant difference was observed between MPL mutant and non-mutant groups from overview of MPL mutation (P=0.00001). From the total studied population, 14.28% were ET cases and 4.71% of them had splenomegaly. About 66.66% had thrombocytosis and 33.33% of all the individuals had leukocytosis according to WHO criteria, and 4.76% of non-MPL mutant individuals had splenomegaly (P=1).

This mutation was reported in 4-6% of ET and PMF individuals. In this research, 4.76 % of studied individuals had MPL (W515L/K) mutation, which were diagnosed with ET.

Conclusion: Generally, the presence of JAK2 and MPL mutations are the most important criteria for MPN diagnosis. The obtained frequency of MPL mutation was similar to previous studies. Despite the high frequency of JAK2 and Philadelphia abnormality, MPL mutation was rare in myeloprolifrative disorders. Further studies are suggested to investigate its prognostic effects for these diseases.

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Introduction

Myeloproliferative neoplasms (MPNs) are defined as diseases which occur due to unlimited reproduction of mature blood cells. It is associated with hematopoietic stem cells changes due to deregulation of signaling pathways (1, 2). Moreover, some of the current myeloproliferative disorders such as Essential Thrombocythemia (ET), Polycythemia Vera (PV), Primary Myelofibrosis (PMF) and Chronic Myeloid Leukemia (CML) (2) are accompanied with thrombotic and hemorrhagic complications (3); they may be transformed to acute leukemia in 4-6% of cases (2).

Some molecular changes such as translocations and mutations are known as infinitive markers for MPN diagnosis. The Janus kinase 2 (JAK2) mutations are confirming indicators of MPN approved by the World Health Organization (WHO) (4); It is a member of Jak Tyrosine kinase which is an important factor in cell proliferation. This mutation was detected in 2005 and introduced for classical MPN categorization, which is as important as the Philadelphia chromosome abnormality. Jak2 (V617F) is located in exon 14 of Jak2

gene. It can be applied for negative BCR-ABL MPN detection (5). This marker was positive in 50-60% of ET and PMF and 90% of PV patients (5, 6). Generally, proliferative disorders are acquired; however, some cases are hereditary such as heredity thrombocytosis (HT) (7).

In addition to JAk2 (V617F), autosomal recessive MPL (Myeloproliferative Leukemia) mutation, affects normal regulation of hematopoietic genes. This oncogenic gene belongs to hematopoietic family (8), which is situated in 1p34 position and encodes thrombopoetin receptor. For the first time, MPL (W515L/K) mutation was discovered in PMF negative BCR-ABL patients in 2006 (9). Furthermore, the most current type of MPL mutations in PMF and ET cases occurred in Tryptophan 515 (10). Different types of MPL mutations were MPL515L, MPL515K, MPL515R and MPL515A in which, Leucin, Lysine, Argenine and Alanine are the substitutes of Tryptophan in the situation of 515 MPL gene (11). In Iranian population, the frequency of MPL mutation was 1.7% among Philadelphia negative myeloproliferative disorders (12). Although several studies reported the frequency and prevalence of MPLW515L/K mutations in MPN patients; there were no report in the cited field for North-East of Iran. Consequently, this research was performed to determine the frequency of MPL mutation in MPN patients with no JAK2 mutation.

Materials and Methods

This study was performed in Cancer Molecular Pathology Research Center, Qaem Hospital, Mashhad University of Medical Sciences. One hundred and five patients were entered in this prospective case–control study; they were suspected of having ET, PV and Myeloproliferative disorder (MPD). They were negative from point of JAK2 mutation. All studied individuals were evaluated for MPN. Medical histories and laboratory reports of the studied subjects were examined and two Hemato-pathologists reviewed the archived slides from 2013 to 2015. The disease was confirmed or rejected by molecular studies, slide evaluation and clinical information. Five cases were MPD, 15 were PV and 15 were ET. All data were obtained from recorded background files; these histories included hematologic indices, clinical signs such as lymphadenopathy, hepatomegaly, splenomegaly and complete blood counts (CBC). Since data were extracted from available evidence files in the research center, no separate sampling was done from patients in this study, and archived samples were used for the test.

DNA was extracted as follows; after acquiring medical history, 2 ml of blood was taken and transferred to Ethylenediaminetetraacetic acid (EDTA-K2) tubes as anticoagulant. Extraction was carried out by QIAamp DNA Mini Kit (Qiagen, Germany) and conforming ficoll gradient centrifugation method. The extracted DNA concentration was evaluated by Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo, Finland). The approved DNA concentration range was 100-200 ng/µl. The samples with concentrations lower than 20 ng/µl were excluded from this research.

Moreover, Amplification Refractory Mutation–Polymerase Chain Reaction (ARMS-PCR) for JAK2 and MPL mutations were carried out conforming prior disseminated methodologies (13). Mismatches were contained to maximize the distinction between wild-type and mutant alleles. Amplifications were performed for JAK2 (V617F) in 20 µl reaction volumes comprising almost 100 ng of genomic DNA, 10 mM dNTPs, 50 mM Magnesium chloride (MgCl2), 1 unit Hot Start Taq polymerase (Takara, Japon), 2.5 µl 10X buffer (Thermo, Finland), and 10 pmol of each forward and reverse primers. The PCR reaction was conducted using specific primers for JAK2: Forward Outer: 5′-TCC TCA GAA CGT TGA TGG CAG-3′, Reverse Outer 5′-ATT GCT TTC CTT TTT CAC AAG AT-3′, Forward wild-type-specific 5′-GCA TTT GGT TTT AAA TTA TGG AGT ATA TG-3′ and Reverse mutant-specific 5′-GTT TTA CTT ACT CTC GTC TCC ACA AAA-3. Thirty-four cycles of PCR were performed with denaturing at 94°C for 30 sec, annealing at 60°C for 45 sec and elongation at 72°C for 45 sec.

In addition, MPL (W515K/L) PCR was carried out in the same situation as JAK2. The amount of 200

nmol/L for primer Fo5′-GCC TGG ATC TCC TTG GTG AC-3′and Ro 5′-GAG GTG ACG TGC AGG AAG TG-3′, 400 nmol/L for primer Riwt 5′-CTG TAG TGT GCA GGA AAC TGT CA-3′and 800 nmol/L for primer FiL 5′-GCC TGC TGCTGC TGA GAT T-3′ or FiK 5′-GCC TGC TGCTGC TGA GTA A-3′ were used. PCR amplification was done by denaturation at 94°C for 5 min, pursued by 40 amplification cycles of 94°C for 30 sec, 63°C for 30 sec and 72°C for 30 sec with an ultimate extension at 72°C for 4 min in the Veriti 96 well thermocyclers (Applied Biosystems, USA). The PCR products were electrophoresed on 2% agarose gel for 30 min (Figure1) (13).

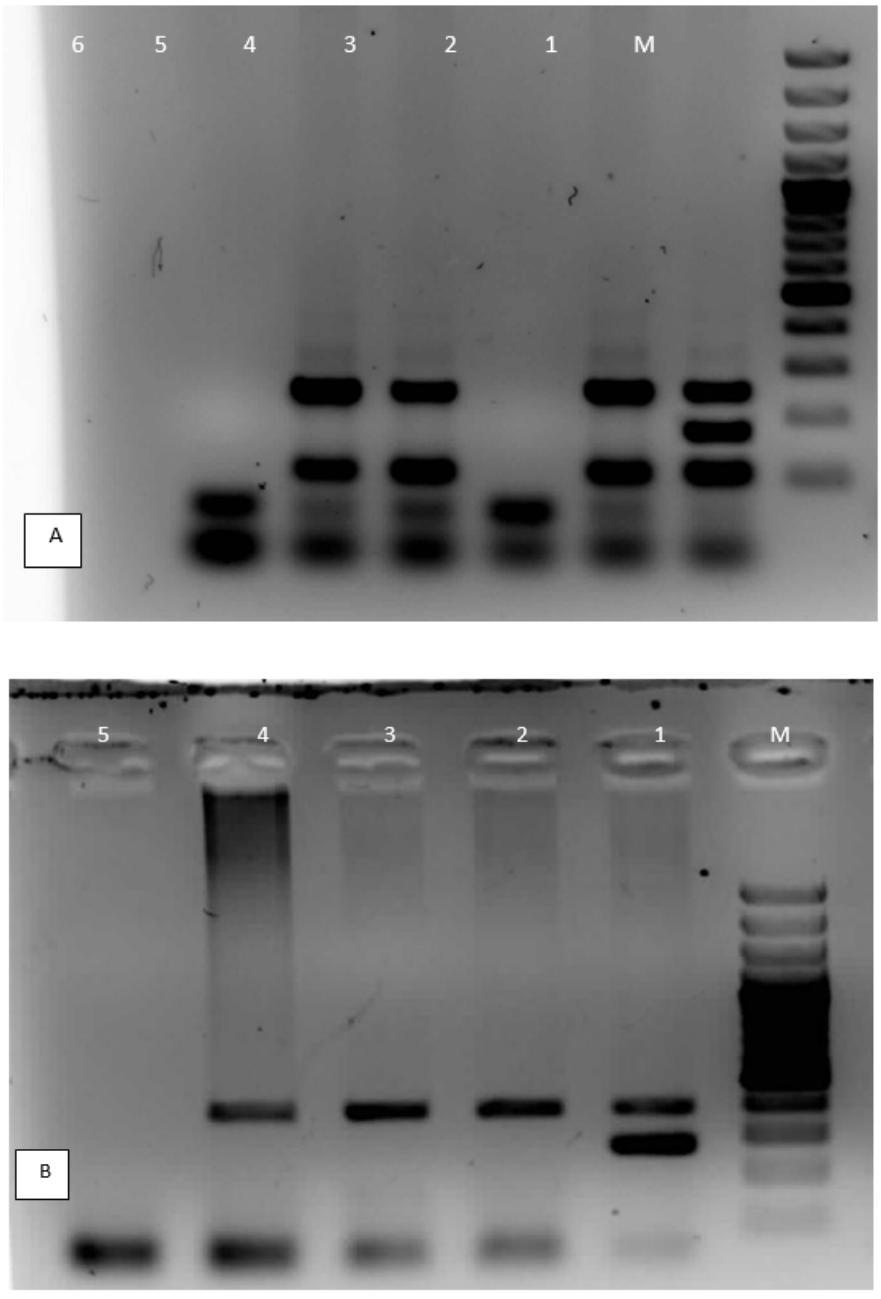

Figure 1. A) Electrophoresis of amplified PCR products for MPLW515L/K mutation. Right to Left: Lane 1: positive control, Lanes 2, 4 and 5: Normal cases, Lanes 3 and 6: Negative Control of MPLW515L/K mutations, M: marker. B) Electrophoresis of amplified PCR products for JAK2 V617F mutation. Right to Left: Lane 1: positive control, Lanes 2 and 3: Negative control, M: marker.

Results

One hundred and five JAk2 negative cases that referred to Qaem Medical Center were entered in this study. About 66.66 % (70) were male and 33.33% (35) were female. Five (4.76%) out of 105 from the studied population were MPL mutant. Moreover, a significant difference was seen between MPL mutated and non-mutated groups from overview of MPL mutation (*P*=0.00001). The mean ages of all studied individuals were 50.8±19.57. Female and male mean ages were 52.5±23.75 and 50.07±18.85, respectively. From the total studied population, 14.28% were ET and 4.71% of them had splenomegaly. In addition, 66.66% had thrombocytosis and 33.33% of whole individuals had leukocytosis according to WHO criteria. Thrombocytosis and leukocytosis were reported in all mutated cases. Moreover, 40.00% and 65.00% of non-mutant group members had leukocytosis (*P*=0.013) and thrombocytosis, respectively (*P*=0.16). Platelet count was meaningfully higher in both groups in comparison with normal individuals (*P*<0.05). Leukocyte and erythrocyte counts were higher and lower in MPL mutated group according to the standard population, respectively. No splenomegaly was reported in MPL mutant group. About 4.76% of non-MPL mutant individuals had splenomegaly (*P*=1). Statistical analysis of hematologic parameters by Mann-withney test are displayed in Table 1.

Exact fisher test was applied to distinguish among the statistical analysis of some myeloproliferative disease containing PV, ET and MPD. Related information is described in Table 2.

Table 1. Statistical analysis of hematologic parameters

variable	MPL Mutant group	Non-mutant group	*P -value*
Age	32.00±1.58	51.78±19.39	0.06
RBC	4.08±0.09	5.64±1.27	0.001
WBC	13.60±0.42	9.90±3.59	0.008
Plt	1690.00±0.01	695.50±397.85	0.0001
Hb	12.20±0.20	15.17±2.95	0.02

RBC: Red Blood Cell, WBC: White Blood Cell, Plt: Platelet, Hb: Hemoglobin

Table 2. Myeloprolifrative data of the studied individuals.

Disease	Mutant group	Non-mutant group	*P-value*
PV	0 (0%)	15 (100%)	0.6
ET	5 (33.33%)	10 (66.66%)	0.6
MPD	0 (0%)	5 (100%)	0.99

PV: polycythemia Vera, ET: Essential thrombocythemia, MPD: Myeloproliferative Disease.

Discussion

MPL is an oncogene which belongs to the hematopoietic family. Different MPL mutations affect normal hematopoiesis mechanisms, thus, it can be reported in many hematopoietic diseases. More than 95 MPL mutations have been discovered, which affect the treatment process and intervention (14, 15). JAK2 is only positive in 50% of ET and PMF patients; MPL is another helpful marker for MPN suspicious cases (11). Generally, JAK2 and MPL mutations are the most important criteria for MPN diagnosis (16).

This study was designed to assign MPL mutation frequency in North-East of Iran. This mutation was reported in 4-6% of ET and PMF individuals. Molecular mechanism of negative JAK2 and MPL were vague and these two mentioned mutations were fruit-

ful for disease process assessment (2). Other molecular markers such as TET2, ASXL1, IDH1, IDH2 and c-CBL have been suggested for MPN evaluation, but their prognostic values were undetermined (2, 4). This mutation has been observed in ET and PMF cases but not in other disorders such as CML, PV, AML and Myelodysplastic syndrome (MDS) (3). However, Akpınar et al. reported MPL W515L/K in 4.8% of negative JAK2 cases (17). Furthermore, in an investigation to determine the frequency of different mutations in Chinese primary Myelofibrosis subjects, Xia et al. revealed that 6.7% of their studied cases, who were negative for Jak2V617F, carried MPLW515L/K mutation (18). However, compared to the mentioned studies, in the current research a total of 4.76 % of the studied individuals had MPL (W515L/K) mutation (19), which did not have JAK2 mutation. Thrombocytosis and leukocytosis were apperceived in 100% of MPL positive cases in our study. Siemiątkowska reported that MPL mutation was associated with thrombocytosis, which confirmed this research finding (20).

Conclusion

Despite high frequency of JAK2 and Philadelphia abnormalities, MPL mutation is rare in myeloproliferative disorders. Our findings were similar to other studies regarding the incidence of MPL mutation. However, our suggestion is to conduct more studies in larger population, patients follow up and mutant cases survival rate determination.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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