# FREQUENCY OF MPL AND JAK2 EXON12 GENE MUTATION IN MYELOPROLIFERATIVE NEOPLASMS

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## ABSTRACT

***Objective:*** Detection of MPL and JAK2 exon12 gene mutation in myeloproliferative neoplasms (MPN) in JAK2V617F negative Myeloproliferative Neoplasms.

***Study Design:*** Cross sectional study.

***Place and Duration of Study:*** Department of Haematology, Armed Forces Institute of Pathology, from Jun 2017 to Jun 2018.

***Methodology:*** Total of 90 newly diagnosed JAK2V617F negative myeloproliferative neoplasms patients were enrolled in the study. Clinico-haematologic features were noted. DNA was extracted from bone marrow samples. Molecular analysis was performed for MPL and JAk2 exon 12 gene by Sanger Sequencing. Results were analyzed by using Genetic Analyzer HITACHI 3130.

***Results:*** Out of these 106 patients 86 were males and 20 were females. On mutation analysis of 16 (15%) patients were found positive for JAK2V617F while 90 (84.9%) were JAK2V617F negative. We then studied these 90 JAK2V617F negative myeloproliferative neoplasms patients for other mutations such as MPL gene and JAK2 exon12. On the basis of clinical features, physical examination, Blood complete picture, bone marrow aspiration and trephine biopsy findings 48 (53.3%) were diagnosed as PV, 22 (24.4%) as ET, 20 (22.2%) as PMF. JAK2 exon12 mutation analysis was screened in 48 (54%) patients and remaining 42 (46%) patients, out of which 22 (24.4%) ET patients and 20 (22.2%) were diagnosed as PMF analyzed for MPL gene mutations.

***Conclusion:*** We have found that none of these patients carry JAK2 exon12 and MPL gene mutation.

**Keywords:** Myeloproliferative leukemia protein protoncogene, Myeloproliferative neoplasms, Polycthemia vera.



## INTRODUCTION

Myeloproliferative neoplasms (MPNs) consist of clonal diseases which develop from overproduction of mature blood cells[1]. According to WHO classification (2016) Polycythemia vera (PV), Essential thrombocythemia (ET), and Primary myelofibrosis (PMF) are included in classical non BCR-ABL MPNS[2]. In 2005 point mutation in JAK2 gene was discovered in non BCR-ABL MPNs. JAK2V617F mutation is the predominant mutation found in 90-95% PV, 50-60% ET and 30-40% PMF patients[3]. In JAK2V617F negative PV patients, JAK2 exon12 mutation have been found in 3% cases where as MPL gene mutation was found in 3 to 5% of ET and PMF patients[4].

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MPL gene is located at chromosome 1p34 and encodes for the thrombopoietin (TPO) receptor[5]. MPL exon 10 mutations appear to result in ligand independent activation of the thrombopoietin receptor and its downstream cell signaling pathways[6].

Recently, In JAK2 V617F negative ET and PMF patients MPL gene mutation was first published in 2006. There are two most common MPL mutations such as substitution of w515L (tryptophan to leucine) and w515K (trytophan to lysine) and these mutations were found in 0 to 3% ET and 0 to 10% PMF patients[7] loss of tryptophan induces constitutive activation of MPL gene[8]. The MPL gene encodes the thrombopoietin receptor and located in juxtamembrane region of receptor which mainly causes both cytokine independent growth and hyper TPO sensitivity in all cell lines by activating STAT/ERK/AKT pathway. Identification of this novel mutation

improves the diagnostic approach to MPNs as well as have an important role in presentation of these patients. It was discovered that patients with MPL mutation have increased platelet count and decreased Haemoglobin level as compared to JAK2V617F positive patients.

In 1951, Dameshek and Henthell described the clinical characteristics of Myeloproliferative disorders. JAK2V617F mutation was discovered as novel mutation in MPNS[17]. Recently next generation sequencing allows the discovery of novel molecular findings in JAK2V617F negative patients such as JAK2 exon12, CALR and MPL gene mutation[9].

In 2007 JAK2 exon12 mutation was first discovered in JAK2V617F negative PV patients. There two most common mutations such as insertions and deletions. JAK2 exon12 mutation affects region adjacent to pseudokinase domain[10]. According to literature, JAK2 exon12 is present in 3% of PV patients and they usually presents with idiopathic erythrocytosis or early stage PV and had favourable outcome[10].

Identification of these novel mutations such as MPL, JAK2 exon12, and CALR gene mutation bring revolution in history of MPNs as, these mutations have role in diagnosis as well as prognosis of MPNs.

Studies have shown that it is not uncommon to find MPNs in Pakistan. Due to lack of resources in Pakistan, there is unavailability of molecular analysis facilities. Hence, the data related to MPNs in Pakistan is very limited. We performed this analysis in AFIP to assess the MPL and JAK2 exon 12 mutations frequency in our country. The purpose was also to analyze the clinico-haematologic features of the patients under consideration. The process will aid in prognostic stratification of subjects under consideration, which helps in taking decisions of treatment.

## METHODOLOGY

This cross sectional study was conducted at Haematology Department, Armed forces institute of pathology from June 2017 to June 2018. A total of 106 newly diagnosed MPN patients were enrolled in the study. Sample size was calculated by WHO calculator[11-15]. Bone marrow samples were collected by using non probability convenience sampling technique. After approval of Ethical committee of AFIP and CPSP, (Reference No: FC-HEM16-26/READ-IRB/17/379) informed consent was taken and questionnaire Performa was filled. MPN adult patients were diagnosed according to WHO 2016 diagnostic criteria. Newly diagnosed patients of all ages and both genders were included and MDS-MPNs and patients on treatment were excluded.

### MPL W515 Mutation Analysis

We performed Sanger Sequencing on all peripheral bone marrow DNA samples using standard protocols and conducted bioinformatic analyses to identify somatically acquired mutations. EDTA blood (3ml) was collected. DNA was isolated in molecular lab by using Solgent Genomic DNA preparation kit (column based). PCR was performed on Proflex according to manufacturer's instruction. Each reaction tube contained 2µl DNA. primers 1µl (forward primer 5′-CCGAAGTCTGACCCTTTTTG -3′, reverse primer 5′-ACAGAGCGAACCAAGAATGC -3′). PCR programe used for MPL exon 10 and JAK2 exon12 mutation analysis is shown in table-I. Second Purification done by Beckman coulter purification kit yields final product of 27µl. Results were analyzed by using HITACHI Genetic analyzer 3130.

### JAK2 Exon12 Mutation Analysis

We performed Sanger Sequencing on all peripheral blood/bone marrow samples of DNA using standard protocols and conducted bioinformatic analyses to identify somatically acquired mutations. 3ml EDTA blood was collected. DNA was isolated in molecular lab by using Solgent Genomic DNA preparation kit(column based). PCR program is shown in table-I.

All statistical analysis were performed using SPSS program version 24 the variables like age,

gender, cell count were given. The percentages and mean, medians were calculated for variables.

## RESULTS

A total of 106 patients were diagnosed as MPNs from June 2017 to June 2018 at Armed Forces Institute of Pathology. Out of these, 86 (82%) were males and 20 (18%) were females with M:F ratio of 4.3:1. On mutation analysis, 16 (15%) patients were found positive for JAK2V617F and 90 (84.9%) were negative.

We then studied these 90 JAK2V617F negative MPN patients for other mutations such as MPL gene and JAK2 exon12. On the basis of clinical features, physical examination, Blood complete picture, bone marrow aspiration and trephine biopsy findings 48 (53.3%) were diagnosed as PV, 22 (24.4%) as ET and 20 (22.2%) as PMF JAK2 exon12 mutation analysis was performed in 48 (53.3%) PV patients and remaining patients, 22 (24.4%) ET and 20 (22.2%) PMF were analyzed for MPL gene mutation as shown in fig-1.

We have found that none of these patients carry JAK2 exon12 and MPL gene mutation along with International data as shown in table-II.

**Table-I: Polymerase chain reaction program used for MPL exon 10 and JAK2 exon 12 Mutation analysis.**

| MPL exon 10<br>JAK2 exn 12 | First polymerase chain reaction | Sequencing polymerase chain reaction |
|---|---|---|
| Initial Denaturation | 94°C/01 min | 96°C/05 mins |
| Denaturation | 94°C/50 secs | 96°C/10 secs |
| Annealing | 58°C /1 min | 40°C/1 min |
| Extension | 72°C/1 min 30 secs | 60°C/4 mins |
| Final Extension | 72°C/3 mins | 72°C/3 mins |

**Table-II: Comparison of Armed Forces Institute of Pathology data with International Data.**

**Comparison with International Studies**

| Study | Patients | Disease | Frequency of MPL gene | JAK 2 Exon 12 |
|---|---|---|---|---|
| 1. Taiwanian | 88 | MPN | 0 | 0 |
| 2. Chinese | 343 | " | 3.5% (ET)<br>12.5% (PMF) | - |
| 3. Istanbul | 77 | " | 2% (PMF) | - |
| 4. Chinese | 30 | PMF | 2% | 0 |
| 5. Pakistani (AFIP) | 90 | 48 (PV)<br>42 (ET & PMF) | 0 | 0 |

## DISCUSSION

Genomic landscape of MPN's is rapidly evolving. Appropriate integration of molecular data with clinical and pathological findings are important for correct diagnosis of disease[11,12]. Mutational analysis of these driver mutations such as JAK2 exon12 and MPL gene are indicated in clinically suspected JAK2V617F negative MPN patients[13]. Hence the identification of these novel molecular markers are essential for diagnosis of MPNs, In addition mutational testing can also provide prognostic as well as important clinical correlation[15,16]. WHO 2016 has incorporated MPL and JAK2 exon12 mutations in diagnostic criteria of MPN's[8]. We conducted this study to determine frequency of these mutations in JAK2 V617F –ve MPN's patients in our population[14].

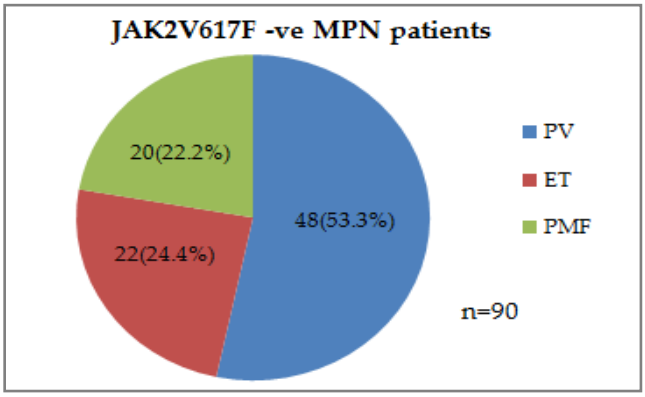


**Figure: Disease frequency.**

Frequencies of MPL and JAK2 exon12 mutations are not consistent in Asian studies . In

our study, 90 JAK2V617F –ve MPN patients were investigated for MPL W515L/K and JAK2 exon mutations but we could not find any of these mutations[12]. Lieu *et al* conducted study among 88 Taiwanese patients with MPNs and could not find any MPL mutation[13-16]. These findings are similar to our results as shown table-I. Another study which was conducted by Ruan *et al* in chinese 343 MPN patients and he found that 3.5% ET and 12.5% of PMF patients had MPL W515K/L mutation[17-19].

Akpinar *et al*[1] conducted study in Istanbul in which he analyzed 77 MPN patients and found that MPL mutations were present in only 2% patients of PMF1.

Another study conducted in chinese population by Xia *et al*[4] concluded that out of 30 PMF patients only 2 JAK2V617F negative patients harboured MPL mutation where as none had JAK2 exon12 mutations.

According to international data, frequency of JAK2 exon12 in JAK2V617F -ve PV patients is estimated around 3% and these patients usually presents with idiopathic erthrocytosis and have favourable outcome[20].

However, as disease biology may be different in different populations, the much lower frequency in our population can be attributed to geographical, racial and ethnic differences. Our data is quite different from international data due to small sample size and our own population characteristics. Larger sample size study should be conducted in Pakistani population to know the exact frequency of these mutations.

## CONCLUSION

Discovery of MPL W515K/L and JAK2 exon12 mutations in JAK2V617F negative MPN patients found to be important molecular diagnostic marker as well as offers potential for target therapies and also affects prognosis of disease[1]. In our study these mutations are not observed in JAK2V617F negative patients which may attribute to our own population characteristics and small sample size. Further studies including large sample size will help in determining exact frequency of these mutations in our population.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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