https://doi.org/10.29309/TPMJ/2024.31.08.8322

The Professional Medical Journal
www.theprofesional.com
2024, Volume, 31 Issue, 08

ORIGINAL ARTICLE

Impact of JAK2 V617F mutation on disease profile and prognostic risk factors in primary myelofibrosis (MPN) - A tertiary care experience.

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Article Citation: Arif M, Khokhar A, Mohsin S, Naveed A, Ammar A, Mustafa G. Impact of JAK2 V617F mutation on disease profile and prognostic risk factors in primary myelofibrosis (MPN) - A tertiary care experience. Professional Med J 2024; 31(08):1255-1261. https://doi.org/10.29309/TPMJ/2024.31.08.8322

ABSTRACT… Objective: To evaluate the association between JAK2V617F mutation status, PMF disease characteristics, and prognostic features as assessed by the DIPSS scoring system. We analyzed clinical and laboratory data from a cohort of PMF patients to determine the influence of JAK2 mutation on disease presentation and progression. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology and Oncology, King Edward Medical University/Mayo Hospital, Lahore. **Period:** December 2022 to December 2023. **Methods:** Total 27 patients with PMF were enrolled in the study by non-probability consecutive sampling technique. Patients were diagnosed according to WHO 2016 criteria. CBC and serum LDH were performed by automated hematology and chemistry analyzers respectively. Real-time PCR was used to identify the JAK2 mutations. Risk stratification was done by DIPSS criteria. Data was analyzed by SPSS 28. Mean and standard deviation was calculated. Student 'T' test was used for comparison of mean between two groups with and without JAK2V617F mutations. **Results:** Out of 27 patients 66.6% were males and median age was 59 years. Splenomegaly was the dominant clinical feature accounting for 51.4% with mean splenic span of 17.8±2.91cm. Mean LDH levels was 872.5±324. JAK2 positive expression was found to be significantly correlated with increased splenic span and raised LDH levels with P-value of <0.05. Risk stratification showed 11% patients were in high-risk group. **Conclusion:** Marked enlargement of spleen and raised in serum LDH levels indicate that patients in our settings had clinically advanced stage of the disease. Apart from this manifestation of JAK2 V617F mutated patients indicated a more aggressive phenotype of the disease.

Key words: Dynamic International Prognostic Scoring System, Janus-Kinase, Lactate Dehydrogenase, Primary Myelofibrosis, Polymerase Chain Reaction.

INTRODUCTION

Primary Myelofibrosis (PMF) is a clonal myeloproliferative neoplasm (MPN) which has negative Philadelphia chromosome and BCR ABL1 gene. It is usually a disease of elderly in fifth and sixth decade of life. PMF disorder is featured by rapidly progressing clinical course of the patients and a reduced life expectancy. Heterogeneity of this clonal disorder is most commonly characterized by cell count proliferation and increase in blood cell counts of variable degree with progressive change to Cytopenic phenotype and transfusion dependency for survival without proper treatment exhibiting presence of constitutional symptoms, enlargement of spleen with megakaryocyte proliferation, atypia and marked fibrosis in bone marrow. Presence of extra medullary hematopoiesis and capricious mutational changes are also associated with disorder.[1] PMF is inter-related with two other disorders named as essential thrombocythemia (ET) and polycythemia vera (PV) in both morphological and molecular perspectives. The incidence of PMF is low with the reported rate of 0.4 to 1.5 cases per 100,000 in USA. According to the database of well-documented cases of Mayo Clinic, it was found that males are most effected group than females with ratio of 1.6 while median age was found to be 57 years.[2]

From the molecular perspective, myeloproliferative clonal disorders such as PMF is characterized by driver mutations in JAK2 (Janus-Kinase-2), CALR (Calreticulin) or MPL (myeloproliferative leukemia

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Article received on: 10/04/2024
Accepted for publication: 19/06/2024

virus) genes which are activated by JAK-STAT signaling pathway that eventually leads to clonal myeloproliferation derived by stem cells, which is prone to leukemic phase in case of PMF as well as fibrotic transformation in case of other MPN.[3] Most constitutional signs and symptoms associated with PMF are fever, fatigue and night sweats, while other symptoms and signs include weight loss and splenomegaly. About 15% to 30% cases are found to be asymptomatic upon clinical presentation.[4] Apart from ET and PV, PMF is exclusively characterized by the presence of more intense bone marrow pathology along with stromal changes in splenic tissue, among which the most common are fibrosis of reticulin or collagen fibers and neoangiogenesis.[5]

Currently, for PMF criteria of diagnosis is based on the 2016 WHO-criteria and more recently similar 2022 WHO criteria, which included an amalgamated assessment of both the clinical as well as laboratory features. Typical features of PMF are leukoerythroblastosis which includes existence of immature granulocytes, nucleated red blood cells in the peripheral blood along with characteristic dacrocytes, however in exceptional cases such as pre-fibrotic PMF, there is cellular proliferation of white blood cells, platelets and overt leukoerythroblastic picture might not be represented. Manifestation of bone marrow fibrosis in PMF is associated with the driver mutations in JAK2, MPL or CALR.[6]

Causes of mortality in the patients of PMF include leukemic progression that happens in almost 20% of patients. Other reasons may include different comorbidities such as cardiovascular events, thrombocytosis and adverse effects of Cytopenias such as bleeding or different types of infections.[7] Currently, the only definitive treatment option that can prolong the life expectancy or in few cases, cure the disease in the patients of PMF is the Allogeneic hematopoietic stem cell transplant (AHSCT).[8] JAK2 inhibitors provide symptomatic relief in PMF patients by reducing size of spleen resulting in lesser transfusion dependency.

Risk stratification for prognostic purposes in PMF which is also known as contemporary prognostic modeling started in 2009 with the introduction of the International Prognostic Scoring System (IPSS). The system of IPSS was designed for the initial diagnosis of patients of PMF. For the evaluation of survival chances, five predictors of independent importance are applied in this scoring system. These predictors include age more than 65 years, ≥1% circulating blasts, existence of constitutional symptoms of PMF. Biochemical marker such as serum LDH is an independent prognostic factor used in diagnostic criteria of PMF. The presence of adverse factors is given score as 0, 1, 2, and ≥ 3 that is further defined as the presence of low, intermediate (Intermediate-1 and Intermediate-2) and high-risk disease, respectively. In regard to the disease severity levels gradual decrease in the life expectancy of the patients and the corresponding median survivals of the diseased individuals were reported at 11.3, 7.9, 4, and 2.3 years, respectively. Another well-known prognostic factor like levels of WBC count, more than 25 x 10^9 /L, have also been added into algorithms for risk stratification, but its association with the progressive clinical course and mortality risk is not fully understood.

Dynamic prognostic model (DIPSS) was then developed by the IWG-MRT that can be applied at any stage of disease course and not just at the time of diagnosis by using the same prognostic variables of IPSS. Characterization of risk stratification was also modified as low with 0 adverse points, intermediate-1 with 1 or 2 points, intermediate-2 with the presence of 3 or 4 points, and high which represents 5 or 6 adverse point.[9] The main objective of the current study was to determine the association of different clinical and laboratory parameters in the patients with and without JAK2 mutations. Risk stratification of the patients was done with the DIPSS score using online calculator.

There is a significant dearth of research on PMF in Pakistan. Existing global data may not accurately reflect the disease presentation, progression, and response to treatment in our local population. Pakistan's diverse population may harbor unique genetic factors influencing PMF susceptibility and disease course. Identifying these genetic

variations can lead to personalized treatment strategies.

Understanding the prevalence and burden of PMF in Pakistan is crucial for developing effective public health interventions and support systems for patients and their families. In this study, we aim to address the impact of JAK2 mutation in Pakistani patients presenting with PMF in a tertiary care center and assess its correlation with clinicopathological profile.

METHODS

The current study was cross sectional study done at Pathology department and Oncology department of King Edward Medical University/ Mayo Hospital, Lahore over the period of one year from December 2022 to December 2023 and Non-probability consecutive sampling technique was used for the collection of samples. Ethical approval (UHS/REG-19/ERC/2379) was taken from the Institutional Review Board of UHS, King Edward Medical College/Mayo hospital, Lahore. A total of 27 patients with PMF were enrolled in the study. PMF was diagnosed according to WHO 2016 criteria which requires the fulfillment of 3 major criteria along with any of the 2 minor criteria.[10]

Major criteria include manifestation of proliferation of megakaryocytes and atypia with or without the presence of fibrosis while increase in the bone marrow cellularity must be present with the changes in the megakaryocytes. Other two criteria include; not meeting the criteria of WHO 2016 for other types of myeloid neoplasms and JAK2V617F demonstration or in the absence of genetic mutation of JAK2, bone marrow fibrosis was not found to be associated with any other inflammatory or infectious disease.

Minor criteria include 4 features such as presence of anemia, raised level of lactate dehydrogenase (LDH) in the serum, leukoerythroblastic blood picture and enlargement of spleen. Complete blood count (CBC) was performed by automated hematology analyzer DxH 900.

Bone marrow exam was done by taking informed consent from patients. Specimens of bone marrow aspiration and trephine biopsy were taken by commercially available adult size bone marrow biopsy needle as per the standard protocol. Smears prepared from peripheral blood and bone marrow samples stained by Wright-Giemsa and H&E stain, were examined by expert hematologists. Reticulin stain was used to guage bone marrow fibrosis. Biochemical analysis of lactate dehydrogenase was done by using Atellica 1M 1300 (Germany) which worked on the principal of Chemiluminescense immunoassay. Identification of BCR-ABL-1 translocation was done by Real-time PCR. Mutational analysis of JAK2 V617F was done by Polymerase chain reaction (PCR) using a sequence specific primer to the JAK2 target.

Data Analysis

Data was analyzed by using SPSS 28 software. The demographic data, clinical features of the patients and laboratory parameters were analyzed by using descriptive analysis. Results were presented as mean ±SD for the quantitative variables while qualitative variables were expressed as percentages and frequency. For the comparison of mean, Student 'T' test was used. p-value of equal to or less than <0.05 was considered as statistically significant.

RESULTS

A total of 27 confirmed cases of PMF were included in the study. Out of 27 patients, 18 (66.6%) were males while 9 (33.3%) were females with the male to female ratio of 2:1. The mean age was 58.04 years (range 38-73) years with the median age of 59 years. Age distribution in both genders shown in Figure-1.

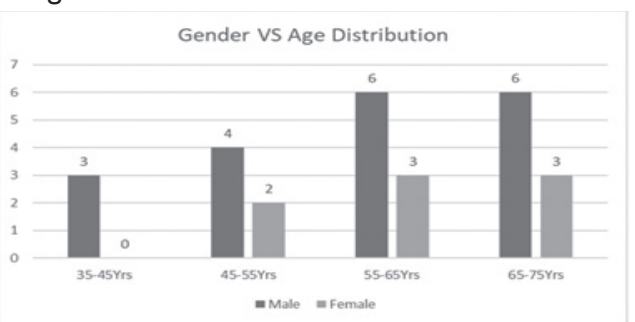

Figure-1. Frequency of different age groups in both genders.

Majority patients (70.4%) were symptomatic and manifested with constitutional symptoms. Symptomatic patients presented with 2 or 3 symptoms. Weight loss was the most common complaint which was present in (51.9%, n=14) patients. Frequency of other symptoms such as fever and night sweats accounted for (40.7%, n=11) each.

Upon physical examination, splenomegaly was the dominant finding which was present in 81.4% (n=22) of patients. Mean splenic span was found to be 17.8±2.91cm. The mean hemoglobin level was 9.25±1.83g/dl while mean total leucocyte count was 13.4±14.8x10^9/L while mean platelet count was 206±320x10^9/L. Overall 44.4% patients were anemic with Hb less than 10g/dl. Thrombocytosis and thrombocytopenia were observed in 29.6 and 33.3% patients respectively. The mean LDH level was 872.5±324 U/L.

JAK2V617F mutation was found to be positive in 77.8% (n=21) patients while 22.2% (n=6) were negative. The comparative analysis of JAK2 positive and JAK2 negative patients presented in Table-I.

Parameters	JAK2 Positive Mean (SD) n=21	JAK2 Negative Mean(SD) n=6	P-Value
Age (Years)	59.9(8.40)	51.3(12.6)	0.05*
Spleen span (cm)	18.98(2.18)	13.9(0.91)	0.0009*
Hemoglobin (g/dl	9.40(1.65)	8.8(2.49)	0.4
TLC count (x 10^9/L)	11.03(14.5)	22.2(13.68)	0.5
Platelets count (x 10^9/L)	234(357.3)	108(83.4)	0.4
Serum LDH levels (U/L)	953.7(296.6)	588.3(269.2)	0.01*

Table-I. Comparative analysis of JAK2 positive and JAK 2 negative patients

Comparative analysis reveals that significant association of JAK2V617F mutation positive expression with splenomegaly and raised serum LDH levels with a p-value of 0.0009 and 0.01 respectively as shown in Table 1. p-value for the comparison of age between JAK2 positive and JAK2 negative group was also significant with a p-value of 0.05. No correlation was noted between JAK2V617F mutation and WBC count as well as Hemoglobin.

Risk stratification was done by DIPSS criteria. More than 50% patients fall in the Intermediate Risk group which reveals moderate risk of disease progression in our patients implying manageable to aggressive disease course.

Representation of risk stratification shown in Figure-2:

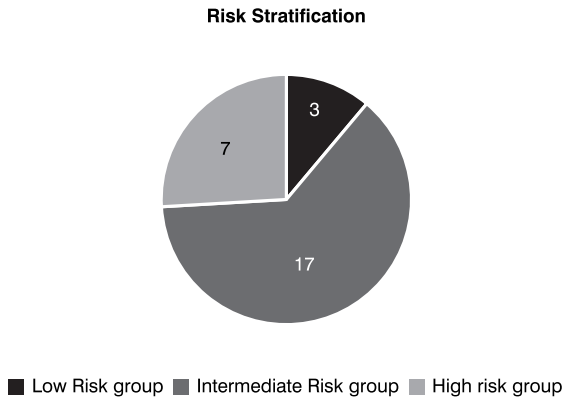

Figure-2. Risk Stratification of patients with Myelofibrosis

DISCUSSION

PMF is a rare myeloproliferative disorder with relatively poor prognosis as compared to other clonal MPN disorders. Dysregulation of the Janus Kinase 2 (JAK2) signal transducer along with activation of signaling pathway of transcription is considered as a distinctive feature of PMF which accounts for almost 50% of patients with PMF.[10]

In the present study, JAK2 mutation was observed in77.8% of the Myelofibrosis identified cases. Positivity of JAK2 in PMF patients has been studied in different ethnic groups ranging from very low as 15% to very high as 76%.[14] A report from regional study in India also reported the presence of JAK2 mutation in 58.8% of patients which was comparable with our results.[15] Positivity of JAK2 in PMF is variable across the globe. Different frequencies of JAK2 in PMF were reported from different countries such as in Egypt (46%), Romania (53.4%), China (40%), United

Kingdom (60%), Taiwan (50%) while 54% positivity ratio was reported in Italian people.[4,16,17] A very interesting finding was reported from Thailand in which 100% positivity of JAK-2 was reported in the patients of PMF. This very high prevalence of JAK2 in Thai people could be attributed to small sample size of only six patients.[18]

In the current study, comparison of different clinical and laboratory parameters was done in the patients of PMF with and without JAK 2 mutations. Firstly, the study shows that PMF is a disease of elderly individuals with a median age of 59 years. Where only 22% individuals were under the age of 50 years when they are initially diagnosed with PMF. This is in accordance with the study conducted in 2020 and 2009 in which the median age of the patients was 68 and 64 years respectively at the time of diagnosis.[11,12] Our study shows that JAK2V617F mutation in PMF is associated with advancing age, higher HB level, raised serum LDH, corresponding to global studies while JAK2V617F negative PMF patients are relatively of younger age group, have higher WBC counts and Thrombocytopenia and lower HB levels is in contrast with other studies.[22]

Males were the most affected group as compared to females. Similar results were reported in Spain.[3] Our study also identified the main clinical parameters associated with PMF. About 70% patients were symptomatic and presented with constitutional symptoms among which weight loss was more commonly identified and accounted for 51% of total cases. Various other studies reported the similar results.[11,13]

Upon clinical examination of the patients, splenomegaly was the most common manifestation which was observed in 81.4% of patients. A study in 2020 also reported the feature of palpable splenomegaly which was present in 80% of the patients of primary myelofibrosis.[11] Anemia was present in 44% of patients with less than 10g/dl Hb value. Another study reported the 39% frequency of anemia in the patients of PMF which in concurrent with our results.[11]

Expression of JAK2 mutation clearly separates the disease spectrum in two main categories and correlation of different clinical and laboratory parameters was compared among two groups. Significant correlation was observed between splenomegaly and JAK2 mutation ($P=0.0009$). Compared with the previous studies, we came across with the concurrent findings as reported in two different studies, one from Pakistan and other from China.[19,20] In our study, raised levels of serum lactate dehydrogenase was also significantly correlated with JAK2 mutation. When compared, mean value of serum LDH in JAK2 positive group was 953.7 ± 296.6 U/L, while in JAK2 negative group mean value of serum LDH was 588.2 ± 269.2 U/L with a p-value of 0.01. Similar results were reported by Larsen et al from Denmark which showed strong association of JAK2 mutation with increased LDH levels.[21]

However, in conflict to our results, various studies reported the correlation of JAK2 mutation with TLC count and HB levels. While in our study, no correlation was established between these two parameters with JAK2 mutational expression. This variation may be attributed to the limited data, small sample size, high burden of anemia due to various other causes in our population, unique genetic diversity, delayed presentation, ignorance of general practices of health and avoidance of medical consultation.[19]

CONCLUSION

In conclusion, most of our findings are in accordance to the studies which were reported from different parts of the world. Marked enlargement of spleen and raised in serum LDH levels indicate that the patients in our settings had clinically advancing stage of the disease. Apart from this manifestation of JAK2 V617F mutated patients indicated a more aggressive phenotype of the disease. We support the recommendation that in the initial evaluation of bone marrow fibrosis, mutational screening for JAK2V617F and DIPSS prognostic scoring should be incorporated. By conducting more research on genetic mutation profile of PMF especially by exploring JAK2 negative cases for further mutational testing in Pakistani patients, we can gain valuable insights into the disease course of Pakistani patients.

Early risk stratification in our PMF patients can improve diagnosis, prognosis, treatment and overall survival rate which ultimately can enhance the quality of life for affected individuals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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2	Abbas Khokhar	Data collection, Manuscript writing.	
3	Shahida Mohsin	Data interpretation, Article review.	
4	Asif Naveed	Statistical analysis, Drafting and final approval.	
5	Ali Ammar	Analysis and interpretation of data.	
6	Ghulam Mustafa	Literature search, Article review.	