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The Impact of JAK2 V617F, CALR, and MPL Mutations as Molecular Diagnostic Markers of Myeloproliferative Neoplasms in Kurdish Patients. A Single-center Experience

Maysam Basim Najm[1*], Sana Dlawar Jalal[2], Hisham Arif Getta[3]

[1] Department of Biochemistry, College of Medicine, University of Sulaimani, Kurdistan Region-Iraq
[2] Department of Pathology, College of Medicine, University of Sulaimani, Kurdistan Region-Iraq
[3] Department of Pathology, College of Medicine, University of Sulaimani, Kurdistan Region-Iraq

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ABSTRACT

Myeloproliferative neoplasms have a high prevalence and genetic mutations play a role in their occurrence. Determination of these mutations can be valuable in the screening, diagnosis, and treatment of patients. Therefore, this study was conducted to investigate the mutation of JAK2, CALR, and MPL genes as diagnostic and prognostic biomarkers in patients with myeloproliferative neoplasms in the Kurdistan region of Iraq. This case-control study was conducted in 2021 on 223 patients with myeloproliferative neoplasm referred to Hiwa Sulaymaniyah Cancer Hospital. The data were collected from three groups of Polycythemia Vera (PV) patients (70 people), Essential Thrombocythemia (ET) (50 people), and Primary Myelofibrosis (PMF) (103 people) by sampling for JAK2, CALR, and MPL gene mutation tests and demographic and clinical information have been collected through examination. The data were analyzed by SPSS v. 23 software and descriptive and chi-square statistical tests. The study included 223myeloproliferative neoplasms (MPN) patients. JAK2 V617F mutation was detected mostly in PV patients and CALR and MPL mutations in ET and PMF patients and this mutation difference was significant in prognosis and disease diagnosis. An association between JAK 2 mutation and splenomegaly was also demonstrated. Considering the lack of a definitive diagnostic method in myeloproliferative disease, the results of this study showed that molecular studies, including JAK2 V617F, CALR, and MPL mutations and other hematological tests can be useful and effective in the diagnosis of MPN. In addition, it is necessary to pay attention to new diagnostic methods.

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Introduction

Myeloproliferative neoplasms (MPNs) are a diverse category of clonal illnesses characterized by the persistent and excessive generation of mature cells from one or more myeloid lineages (1). Three subgroups have been identified: primary myelofibrosis (PMF), essential thrombocythemia (ET), and polycythemia vera (PV). All three forms tend to develop into acute myeloid leukemia (AML). ET and PV can advance to PMF disease. Thrombotic and hemorrhagic events are more likely to occur and to do so in a way that affects morbidity and mortality (2). The annual incidence of MPNs was reported to be 2.17 cases per hundred thousand people. Also, the US National Cancer Institute estimates that 20,000 people are diagnosed with MPN annually, and there are 295,000 people with it (3). There are various diagnostic methods for early detection and planning for treatment and increasing the survival of patients with MPNs. One of them is the use of molecular diagnostic methods. WHO (2016) diagnostic criteria for myeloproliferative neoplasm include the diagnosis of PV, ET, and PMF associated with MPL/CALR/JAK2 mutations. Some clinical features and tests help diagnose this disease, including bone marrow (BM) biopsy, high hemoglobin, and hematocrit, abnormal level of serum erythropoietin, leukocytosis, increased LDL, and splenomegaly (4). Different studies reported that the determination of some special mutations can be valuable in the screening and diagnosis of different diseases and treatment of patients (5,6). The co-occurrence of these three mutations has been documented. However, they are mutually exclusive (7). Elisa Rumi and Mario Cazzol (8) noted in their study that the co-occurrence of these three mutations was a diagnostic sign for MPN and indicates a good prognosis for therapeutic success. But sometimes, some patients have clinical symptoms similar to MPN, but none of these three mutations have been observed in their genetics. These cases are called "triple negative" (9). Driver mutations in MPN are now formally included in the WHO diagnostic criteria for primary myelofibrosis (58 percent JAK2, 25 percent CALR and 7 percent MPL), PV (98 percent JAK2 mutational frequency), and ET (60 percent JAK2, 22 percent CALR, and 3 percent MPL mutational frequency) (10). Considering that there are genetic differences depending on the geography (11,12), and there is no study on the genetic mutation status of these diseases in the Iraqi region and especially Iraqi Kurdistan, this study aims to use molecular aspects for the diagnosis of MPN according to WHO criteria 2016 that (not only depend on morphological and clinical aspects for diagnosis) but using JAK2, CALR, MPL genes for diagnosis and prognosis.

* Corresponding author. Email: ph.maysam.b@gmail.com

Materials and Methods

Study design and setting

This study is a case-control study conducted in 2021 on patients with myeloproliferative neoplasms referred to Hiwa Cancer Hospital in Sulaymaniyah, Iraq.

Participants

The study included 223 MPN patients, 70 patients with PV, 50 patients with essential ET, and 103 patients with primary myelofibrosis (PMF). All patients were diagnosed before 3 years according to clinical and laboratory data, but without molecular tests as a major diagnostic criterion according to the 2016 World Health Organization (WHO) classification for myeloproliferative neoplasms (4). The sampling method was convenient. Inclusion criteria were: patients with age => 60 years old and all Philadelphia chromosome (Ph) negative MPN that were diagnosed before 3 years. Exclusion criteria were: Patients with age under 60 years old, pregnant females, patients with other malignancies, and other myeloid malignancies were excluded, including chronic myeloid leukemia and MDS, Chronic eosinophilic leukemia.

Procedure

Sample of Collection

A five ml of blood sample was collected in a specific code labeled tube for each patient and directly put in the cool box, then transferred on the same day to a deep freezer at -80 c (Acculab®), USA. In addition to blood sample collection, clinical data were also collected by a comprehensive questionnaire, and laboratory data were also recorded and collected from stored patient information in the cancer center.

Genomic DNA Extraction and Purification

To perform DNA extraction, blood samples were defrosted at room temperature. Each sample was placed on a shaker for 5 minutes, then 200 microliters of each blood sample were placed in a labeled Eppendorf tube under a DNA extraction hood. The DNA extraction and purification were performed using an Automated Nucleic Acid Extraction and Purification System (MAG PURIX®) 12 Evo device (13).

Then the quality and quantity of DNA were checked using a NanoPhotometer (P-class, IMPLEN, Germany). Also, the NanoPhotometer was used to detect the concentration and purity of the DNA sample using 2 microliters of extracted DNA (14).

Real-time PCR and Mutation Analysis

JAK2V617F mutations were assessed using real-time PCR (MIC PCR®) biomolecular system BMS (15) and using gb ONCO ® JAK2 V617F Kit, Generi biotech (16). The CALR mutations (TYPE 1, TYPE 2) were assessed using real-time PCR (Rotor-Gene® Q 5plex HRM) PCR, QIAGEN, and using ipsogen® CALR RGQ PCR Kit, QIAGEN, Germany. Also, MPL W515L/K mutations were assessed using real-time PCR (Rotor-Gene® Q 5plex HRM) PCR, QIAGEN, and using ipsogen® MPL W515L/K MutaScreen, QIAGEN, Germany. The procedure was performed according to the kit manufacturer's instructions. The PCR conditions for the investigation of different mutations were represented in Table 1.

Data analysis

SPSS software Version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The difference between the two groups (positive and negative mutation) was evaluated using a t-test. Also, differences between mutations were evaluated using the Chi-square. P-values less than 0.05 were considered to be significant.

Results

The study included 223 myeloproliferative neoplasms (MPN) patients seen at Hiwa cancer hospital, Sulaimani, Iraq. 70 patients with polycythemia vera (PV), 50 patients with essential thrombocythemia (ET), and 103 patients with primary myelofibrosis (PMF), all patients were diagnosed before 3 years according to clinical and laboratory data, but without molecular tests as major diagnostic criteria according to 2016 WHO classification for myeloproliferative neoplasms.

Evaluation of mutations

Investigation of JAK2 mutation showed that 68 PV patients had the positive mutation and 2 patients had the negative mutation. Also, in ET, 25 patients had a Positive

Table 1. The PCR condition for mutations.

Process	temperature (°C)	Time	Cycle
JAK2V617F mutations			
Initial Denaturation	95 °C	3 minutes	1 cycle
Denaturation	95 °C	10 seconds	50 cycles
Annealing +Elongation +Fluorescence Acquisition	60 °C	20 seconds	
CALR mutations			
Initial Denaturation	95 °C	10 minutes	1 cycle
Denaturation	95 °C	15 seconds	45 cycles
Annealing +Elongation +Fluorescence Acquisition	60 °C	60 seconds	
MPL mutations			
Initial Denaturation	50 °C	2 minutes	1 cycle
Denaturation	95 °C	10 minutes	50 cycles
Annealing +Elongation +Fluorescence Acquisition	60 °C	1 minute	

mutation and 25 patients had a negative mutation, and in PMF, 62 patients had a Positive mutation and 41 patients had a negative mutation, and there was a significant difference in this mutation among the three patient groups (P < 0.001). Investigation of CALR mutation showed that all PV patients had negative mutations. In ET, 23 patients had a positive mutation and 27 patients had a negative mutation, and in PMF, 36 patients had a Positive mutation and 67 patients had a negative mutation, and there was a significant difference in this mutation between the three groups of patients (P < 0.001). Examination of MPL mutation in patients showed that all PV patients had the negative mutation and out of 50 ET patients, 11 patients had a positive mutation and 39 patients had a negative mutation. And in 103 PMF patients, 95 patients had the negative mutation and 8 patients had a positive mutation. There was a significant difference between these three diseases in terms of MPL mutation (P <0.001). The results in PV patients showed that 2 patients had the triple-negative mutation and 68 patients did not have the triple-negative mutation. Also, 2 ET patients had the triple-negative mutation and 48 patients did not have this mutation. Also, only 8 PMF patients had the triple-negative mutation, and 95 people patients did not have the triple-negative mutation (Table. 2).

2 ET patients had the triple-negative mutation and 48 patients did not have this mutation. Also, 8 PMF patients had the triple-negative mutation and 95 patients did not have this mutation.

PV patients

The mean age of PV patients in positive JAK2 mutation was 70.2±5.7 and in negative JAK2 mutation was 71.5±13.4. The mean hemoglobin, HCT, number of leukocytes and platelets in patients with positive JAK2 mutation, and the mean hemoglobin, HCT, and number of leukocytes and platelets in patients with negative JAK2 mutation are shown in Table 3. The results showed that the mean age, hemoglobin, HCT, number of leukocytes, and platelets in JAK2 positive and negative mutation patients did not differ significantly. Also, the number of PV patients with positive JAK2 mutation was 68 and with negative JAK2 mutation was 2.

ET patients

The mean age of ET patients with positive JAK2 mu-

Table 2. Evaluation of the frequency of mutations in three groups (PV, ET, and PMF) of patients.

Mutations		PV	ET	PMF	P-value*
JAK2 V617F	Positive	68 97.1%	25 50.0%	62 60.2%	< 0.001
	Negative	2 2.9%	25 50.0%	41 39.8%	
CALR	Positive	0 0.00%	23 46.0%	36 35.0 %	< 0.001
	Negative	70 100.0%	27 44.00%	67 65.0%	
MPL	Positive	0 0.00%	11 22.0%	8 7.8%	< 0.001
	Negative	70 100.0%	39 78.0%	95 92.2%	
Triple negative	Yes	2 2.90%	2 4.00%	8 7.80%	0.33
	No	68 97.10%	48 96.00%	95 92.20%	

* P-value-based chi-square.

Table 3. Demographic and clinical characteristics of PV patients.

PV patients		P[1]	JAK2 mutation
Age in years	Positive	70.2±5.7*	NS
	Negative	71.5±13.4	
Hemoglobin, g/dL	Positive	20.6±4.8	NS
	Negative	22.8±3.1	
HCT %	Positive	61.9±14.5	NS
	Negative	68.4±9.3	
Leukocyte, *10^9 /L	Positive	14.8±5.9	NS
	Negative	15±3.4	
Platelet Count,*10^9/L	Positive	273±85.7	NS
	Negative	232.5±77.1	

*Mean ± SD, P1: P-value based on T-test, NS: No Significant.

tation was 69.3±5.1 and in negative JAK2 mutation was 67.7±5.6. Also, these two groups did not differ significantly in terms of average age. The mean of hemoglobin, HCT, leukocyte and platelet count in ET patients with positive and negative JAK2 mutation were not significantly different. The number of ET patients with positive JAK2 mutation was 25 and those with negative JAK2 mutation were also 25 Table 4.

The mean age in ET patients with positive CALR mutation was 67.5±5.6 and in negative CALR mutation was 69.4±5.1. Also, these two groups were not significantly different in terms of average age. Examination of mean hemoglobin, HCT, leukocyte, and platelet count in ET patients with positive and negative JAK2 mutation did not show any difference. The number of ET patients with positive CALR mutation was 23 and those with negative CALR mutation were 27 (Table 4).

The mean age of ET patients with positive MPL mutation was 69.27±5.061 and in negative MPL mutation was 68.28±5.52 and the two groups were not significantly different in terms of average age. The mean hemoglobin, HCT, leukocyte and platelet count in ET patients with positive and negative MPL mutations did not differ significantly. The number of ET patients with positive MPL mutation was 11 and with negative MPL mutation was 39 (Table 4).

PMF patients

The results showed that the mean age, hemoglobin, HCT, leukocyte, and circulating blasts were not different in the negative and positive mutation groups. But the average number of platelets in PMF patients with positive JAK2 mutation was 242.6±1.71 and in negative mutation patients, it was 351.8±154.7 and the two groups had a significant difference in the average number of platelets ($P \leq 0.001$). The number of PMF patients with positive JAK2 mutation was 62 and those with negative JAK2 mutation were 41.

The results have shown that the mean variables of age, HCT, leukocyte, and circulating blast were not different in the two groups of negative and positive mutation. The mean percentage of HCT in PMF patients with positive CALR mutation was 30.9±7.4 and in patients with the negative mutation was 34.2±8.1, and the two groups had a significant difference in mean HCT percentage ($P \leq 0.03$). The average number of platelets in PMF patients who had positive CALR mutation was 426.8±2.66 and in patients with the negative mutation, it was 210.5±68.6 and the two groups had a significant difference in terms of the average number of platelets ($P \leq 0.001$). The number of PMF patients with positive CALR mutation was 36 and those with negative CALR mutation were 67.

The mean age, hemoglobin, HCT, leukocyte, circulating blast, and mean survival in PMF patients with positive and negative MPL mutation were not significantly different. The mean number of platelets in PMF patients who had positive MPL mutation was 205.8±100.6 and in negative mutation patients, it was 292.9±123.5. And the two groups had a significant difference in terms of the average number of platelets ($P \leq 0.04$). The number of PMF patients with positive MPL mutation was 8 and with negative MPL mutation was 95 (Table 5).

Clinical and medical problems for patients

PV patient

The clinical findings of PV patients was shown in Table 6. These findings are expressed according to the type of mutation. In PV patients with JACK 2 mutations, 48 patients with splenomegaly, 11 patients with a history of thrombosis, and 4 patients had transfusion needs. There was no significant difference in clinical forms according to JACK2 mutation. In PV patients with CALR mutations, 49 patients with splenomegaly, 11 patients with a history of thrombosis, and 4 patients with transfusion needs. There was no significant difference in clinical forms according to CALR mutation. These cases were seen only in negative mutation patients. In PV patients with MPL mutations, 49 patients with splenomegaly, 11 patients with a history of thrombosis and 4 patients had transfusion needs. There was no significant difference in clinical forms according to MPL mutation. These cases were seen only in negative mutation patients.

ET patient

The clinical findings of ET patients show in Table 7. These findings are expressed according to the type of mutation. In ET patients with JAK 2 mutations, 2 patients with splenomegaly, 20 patients with a history of thrombosis, and 2 patients with transfusion needs. There was no significant difference in splenomegaly and transfusion need

Table 4. Demographic and clinical characteristics of ET patients.

ET patients		JAK2 mutation	CALR mutation	MPL mutation	P^1	P^2	P^3
Age in years	Positive	69.3*± 5.1	67.5±5.6	69.27±5.06	NS	NS	NS
	Negative	67.7± 5.6	69.4±5.1	68.28±5.52			
Hemoglobin, g/dL	Positive	14.7±1.1	15.1±1.0	15.21±0.75	NS	NS	NS
	Negative	15±1.1	14.7±1.1	14.74±1.14			
HCT %	Positive	43.9±3.1	45.1±3.0	45.59±2.2	NS	NS	NS
	Negative	45.1±3.2	43.3±3.3	44.15±3.37			
Leukocyte, *10^9 /L	Positive	9.9± 1.9	10.7±1.6	10.08±2.07	NS	NS	NS
	Negative	10.8±1.5	10.0±1.8	10.40±1.67			
Circulating Blast %	Positive	803±106	818±116.6	729.54±129.48	NS	NS	NS
	Negative	872.6±126	771.4±113.4	810.69±107.16			
Platelet Count *10^9/L	Positive	69.3*± 5.1	67.5±5.6	69.27±5.06	NS	NS	NS
	Negative	67.7± 5.6	69.4±5.1	68.28±5.52			

*Mean ± SD, P-value based on T-test, & NS: No Significant, P^1 JAK2 mutation, P^2 CALR mutation, P^3 MPL mutation.

Table 5. Demographic and clinical characteristics of PMF patients.

PMF patients		JAK2 mutation	CALR mutation	MPL mutation	P[1]	P[2]	P[3]
Age in years	Positive	68.8*±5.2	67.3±5.6	67.3±5.6	NS	NS	NS
	Negative	67.4±5.5	68.8±5.1	68.8±5.1			
Hemoglobin, g/dL	Positive	11.3±2.7	10.3±2.5	10.3±2.5	NS	NS	NS
	Negative	10.6±2.6	11.4±2.7	11.4±2.7			
HCT %	Positive	33.8±8.1	30.9±7.4	30.9±7.4	NS	0.03	NS
	Negative	31.9±7.7	34.2±8.1	34.2±8.1			
Leukocyte, *10^9 /L	Positive	17.9±6.6	17.2±7.3	17.2±7.3	NS	NS	NS
	Negative	16.5±8.0	17.4±7.3	17.4±7.3			
Circulating Blast %	Positive	0.4±0.5	0.4±0.5	0.4±0.5	NS	NS	NS
	Negative	0.5±0.6	0.4±0.5	0.4±0.5			
Platelet Count *10^9/L	Positive	242.6±71.1	426.8±66.2	426.8±66.2	0.001	0.001	0.04
	Negative	351.8±154.7	210.5±68.6	210.5±68.6			
median survival	Positive	8.9±5.1	9.9±9.1	9.9±9.1	NS	NS	NS
	Negative	10.7±8.6	9.4±5.1	9.4±5.1			

*Mean ± SD, P-value based on chi-square, NS: No Significant, P[1]: Significance of JAK2 mutation, P[2]: Significance of CALR mutation, P[3]: Significance of MPL mutation, N: number, %: percent.

Table 6. The clinical findings of PV patients.

PV patient		JAK2 mutation		CALR mutation		MPL mutation		P1	P2	P3
		Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Positive N (%)	Negative N (%)			
Splenomegaly	Yes	48(70.6)	1(50)	0	49(70)	0	49(70)	NS	NS	NS
	No	20(29.4)	1(50)	0	21(30)	0	21(30)			
History of thrombosis	Yes	11(16.2)	0	0	11 (15.7)	0	11(15.7)	NS	NS	NS
	No	57(83.8)	2(100)	0	59 (84.3)	0	59 (84.3)			
Transfusion Need	Yes	4(5.9)	0	0	4(5.7)	0	4(5.7)	NS	NS	NS
	No	64(94.1)	2(100)	0	66 (94.3)	0	66 (94.3)			

*Mean ± SD, P-value based on chi-square, NS: No Significant, P[1]: Significance of JAK2 mutation, P[2] : Significance of CALR mutation, P[3] : Significance of MPL mutation, N :number, %: percent.

Table 7. The clinical findings of ET patients.

MPL mutation		JAK2 mutation		CALR mutation		MPL mutation		P[1]	P[2]	P[3]
		Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Positive N (%)	Negative N (%)			
Splenomegaly	Yes	2(8)	6(24)	4(17.4)	4 (14.8)	2 (18.2)	6(15.4)	NS	NS	NS
	No	23(92)	19(76)	19(82.6)	23(85.2)	9(81.8)	33(84.6)			
History of thrombosis	Yes	20 (80)	3 (12)	4 (17.4)	19 (70.4)	7 (63.6)	16(41)	0.001	0.001	NS
	No	5(20)	22 (88)	19 (82.6)	8(29.6)	4(36.4)	23(59)			
Transfusion Need	Yes	2(8)	3(12)	4(17.4)	1(3.7)	1(9.1)	4(10.3)	NS	NS	NS
	No	23(92)	22(88)	19 (82.6)	26(96.3)	10(90.1)	35(89.7)			

*Mean ± SD, P-value based on chi-square, NS: No Significant, P[1]: Significance of JAK2 mutation, P[2]: Significance of CALR mutation, P[3]: Significance of MPL mutation, N: number, %: percent.

according to JAK2 mutation. But in JAK 2 mutation, in terms of occurrence of history of thrombosis, there was a difference in meaning ($P \leq 0.001$). However, patients with a history of thrombosis and patients without a history of thrombosis had a significant difference in JAK 2 mutation ($P \leq 0.001$).

In ET patients with CALR mutations, 4 patients with splenomegaly, 4 patients with a history of thrombosis, and 4 patients with transfusion needs. There was no significant difference in splenomegaly and transfusion need according to CALR mutation. But in CALR mutation, in terms of the occurrence of history of thrombosis, there was a difference in meaning ($P \leq 0.001$). However, patients who had a history of thrombosis and patients who did not have a history of thrombosis had a significant difference in CALR mutation ($P \leq 0.001$).

In ET patients with MPL mutations, 2 patients with splenomegaly, 7 patients with a history of thrombosis, and 1 patient with transfusion needs. There was no significant difference in clinical data according to MPL mutation. There was no significant difference in clinical data according to MPL mutation.

PMF patient

The clinical findings of PMF patients show in (Table 8). These findings are expressed according to the type

Table 8. The clinical findings of PMF patients .

PMF patient		JAK2 mutation		CALR mutation		MPL mutation		P1	P2	P3
		Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Positive N (%)	Negative N (%)			
Splenomegaly	Yes	5(8.1)	0	0	5(7.5)	2(25)	3(3.2)	NS	NS	0.006
	No	57(91.9)	41(100)	36(100)	62 (92.5)	6(75)	92(96.8)			
History of thrombosis	Yes	25(43.3)	11(26.8)	12(33.3)	24(35.8)	2(25)	34 (35.8)	NS	NS	NS
	No	37(59.7)	30(73.2)	24(66.7)	43(64.2)	6(75)	61(64.2)			
Transfusion Need	Yes	42(67.7)	27(65.9)	27(75)	42(67.7)	4 (50)	65(68.4)	NS	NS	NS
	No	14(34.1)	14(34.1)	9(25)	25(37.3)	4(50)	30(31.6)			

*Mean ± SD, P-value based on chi-square, NS: No Significant, P^1: Significance of JAK2 mutation, P^2: Significance of CALR mutation, P^3: Significance of MPL mutation, N: number, %: percent.

of mutation. In PMF patients with JAK 2 mutations, 5 patients with splenomegaly, 25 patients with a history of thrombosis, and 42 patients with transfusion needs. There was no significant difference in clinical data according to JAK2 mutation. In PMF patients with CALR mutations, no patients with splenomegaly, 12 patients with a history of thrombosis, and 27 patients with transfusion needs. There was no significant difference in clinical data according to CALR mutation. In PMF patients with MPL mutations, 2 patients with splenomegaly, 2 patients with a history of thrombosis and 4 patients with transfusion needs. In MPL mutation, in splenomegaly incidence, there was a difference in meaning ($P \leq 0.006$). Patients with a history of splenomegaly and patients without a history of splenomegaly had a significant difference in MPL mutation ($P \leq 0.006$).

Discussion

In this study, 223 patients diagnosed with three types of myeloproliferative neoplasms, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) were examined in terms of JAK2 V617F, CALR, and MPL mutations. The results showed that three mutations can play an important and key role in prognosis, diagnosis, and treatment for myeloproliferative neoplasm patients.

Based on studies, JAK2 V617F mutation was seen in 95% of PV patients and about 50-60% of ET and PMF patients (12,17–19). In this study, it was shown that the frequency of JAK2 V617F mutation was 97.1% in PV patients, 50% in ET patients, and 60.25%in PMF patients. In the study of Latif *et al*. (20) in Pakistan, the frequency of JAK2 V617F mutation in PV patients was 53%, in ET patients was 24% and in PMF patients was 22%, and the frequency of this mutation was higher in the present study. In the other studies, the frequency of V617F, CALR, and MPL mutations in three groups of PV, ET, and PMF patients and the results of the frequency of mutations in comparison with the present study showed that the frequency of all mutations in all three diseases was higher than the mutations in the current study (21–23).

The JAK2 gene encodes a protein tyrosine kinase that is involved in stimulating cell growth and division, particularly in controlling the production of blood cells in the bone marrow. MPNs are caused by mutations in the JAK2 gene. These mutations change the function of the protein, resulting in the uncontrolled growth of blood cells (24,25). A review of previous studies has shown that other hematological disorders such as chronic myeloid leukemia and multiple myeloma lack this acquired mutation. In addition, this mutation may be associated with myelofibrosis and spleen infections, so this mutation alone cannot be used in the diagnosis of myeloproliferative disease (26) The results have shown that in examining the frequency of JAK2 V617F mutation in three types of myeloproliferative neoplasms, the mutation frequency was higher in PV patients. In this study, the frequency of mutation in PMF was higher than in ET, which was not consistent with most studies, because, in most studies, the frequency of JAK2 mutation in ET was higher than in PMF (27,28).

CLAR mutation was the most common genetic abnormality after JAK2 mutation, which was seen in 20-25% of adults with essential thrombocythemia and 25-30% of adults with primary myelofibrosis. CALR mutation can be one of the main criteria for ET and PMF. CALR mutation may have implications for disease progression and prognosis in individuals with MPN. Studies showed that people with CALR mutations had a longer overall survival rate than people with JAK2 or MPL (myeloproliferative leukemia) mutations. Also, people with this mutation had a lower risk of thrombosis than other essential thrombocythemia people with positive JAK2 mutation. Also, the CALR mutation associated with MPNs was somatic, so it cannot be transmitted to the next generation. In the study of Belcic Mikic et al. (29), which was conducted to investigate CLAR mutations in patients suspected of myeloproliferative neoplasms, the results showed that most CALR mutations are seen in ET patients and this mutation had a significant difference from other mutations in the diagnosis of the disease, which is consistent with According to the results of the present study, the highest CLAR mutation was seen in ET and PMF patients.

Considering that the JAK2 V617F mutation was found in only 50-60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET), the World Health Organization (WHO) had mandated the analysis of mutations to diagnose MPN. For this reason, in the study of S Roy *et al*. (30) That 23 MPN patients were examined for MPN and the results indicated that the CLAR mutation had the necessary sensitivity for the prognosis and diagnosis of MPN disease, which is in line with the results of the present study, which shows the importance of following the WHO guidelines in the diagnosis of the disease is showing.

The reason that most of the patients with ET or MF are negative for JAK2 mutation, other somatic mutations play a role in this field, which can be referred to myeloproliferative leukemia (MPL) gene mutations. The MPL gene is

located on chromosome 1p34 and expresses the thrombopoietin receptor, which plays a role in platelet production along with thrombopoietin. The two mutations W515L and W515K in the MPL gene lead to severe anemia and are seen in 5% of PMF patients, 1% of ET patients, and 10% of Post-ET Myelofibrosis patients. MPN patients who had the disease due to mutations in the MPL gene suffered more severe anemia and need more blood transfusions. Therefore, the diagnosis of these two mutations in PMF and ET patients is important both in terms of diagnostic application and in terms of disease prognosis. Patients with MPD who had a negative JAK2 test were tested for MPL gene mutations for further molecular investigations based on the WHO diagnostic criteria (31).

Mutation analysis in the studied patients showed that PV patients were negative for an MPL mutation, but 22% of ET patients and 7.8% of PMF patients had MPL mutation. In the review conducted by Constantinescu *et al*. (32), it was shown that the studied patients were negative for a JAK2 mutation, but ET and PMF patients had MPL mutation, and these three mutations had a significant difference in the diagnosis and prognosis of the disease, which is consistent with the results of this study. Also, examining the survival of patients had shown that patients with MPL mutation had a worse prognosis and survival than other mutations, which showed the importance and place of this mutation in the diagnosis of the disease.

Examining the demographic and clinical characteristics of PV patients showed that this average age, hemoglobin, HCT%, Leukocyte, and Platelet Count were higher than in the study conducted by RH Zulkeflee *et al.* (33) and there was no significant difference between these patients, while in the mentioned study, there was a significant difference between WBC and Platelet Count.

Also, the examination of ET and PMF patients in terms of demographic and clinical characteristics of patients showed that ET patients did not have significant differences in demographic and clinical variables. In PMF patients, there was a significant difference between positive and negative mutation individuals in terms of HCT% and Platelet Count. Examining these results with the results of other studies showed that the average of demographic and clinical variables in this study was higher than in other studies (34–36).

Splenomegaly is one of the clinical manifestations of MPN disease. It has been shown that splenomegaly was associated with JAK2 mutation and the presence of this mutation caused the size of the spleen to increase. In the present study, it was also shown that splenomegaly is related to JAK2. Splenomegaly was more common in people who have JAK2 (37).

Other clinical manifestations of PMF were a history of thrombosis and transfusion needs. The results showed that patients with JAK2 mutation had more history of thrombosis and transfusion needs, and on the other hand, these problems were more common in PV patients, which is in line with the results of other studies (38,39).

Limitation

Working in a treatment center and the sample size is small.

Conclusion

So far the definitive method for diagnosis of Myeloproliferative disorder has not been discovered in the patients It has been shown that molecular studies including (JAK2 V617F, CALR, and MPL mutations) and other hematological tests help to diagnose the type of MPN and due to the frequency and comparability of these mutations, the standardization of diagnosis and treatment control Prognosis is essential.

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Interest Conflict

The authors have no conflicts of interest to declare.

Author's contributions

All authors passed the criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

Data Availability

The authors guarantee that the data of this research will be provided at the request of other researchers.

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