Research Paper:
Frequency of *BCR-ABL P190 & P210*, and *JAK2* Mutations in Myeloproliferative Patients in Iran

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ABSTRACT

Background: Hematologic malignancies can be divided into two categories: myeloid and lymphoid, each of which is further divided into acute and chronic. Chronic myeloid diseases consist of Myelodysplasia Syndrome (MDS), Myeloproliferative Neoplasms (MPNs), and a rare group of MDS/MPN. According to genetic studies, the prevalence of some mutations related to cell division is higher in these patients. Given that gene analysis is an available way to diagnose the disease quickly, treat it on time, and help promote effective drugs, this study aims to investigate the frequency of *JAK2*, *BCR-ABL P190*, and *BCR-ABL P210* mutations in patients with neoplasms who refer to Shahid Mostafa Khomeini Hospital, Tehran, Iran, between March 2017 and September 2020.

Materials and Methods: The blood samples of the patients were analyzed by PCR for the expression of *BCR-ABL P190*, *BCR-ABL P210*, and *JAK2* genes to investigate any potential relationship between malfunctioned genes and the existing medical condition.

Results: The mean age of the study patients was 60.5±14.7 years. Of whom 28 (53.8%) were male, and the other 24 (46.2%) were female. Also, 29 patients (55.8%) had Essential Thrombocythemia (ET), 37 patients (71.2%) had at least one of the studied mutations, 21 patients (40.4%) had *JAK2* mutation, and 16 patients (31.8%) had *BCR-ABL* mutation. There was no significant relationship between gender and any of the studied variables. Type of disease had a significant relationship with the studied mutations.

Conclusion: All CML cases in this study had at least one of three types of mutations. The type of medical condition was significantly associated with the studied mutations, for example, CML patients had fewer JAK2 and more BCR-ABL gene mutations. gender was not significantly related to any factor in the study. There was no mortality during the study. All the patients were alive, and most of them (88.5%) were in remission.

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1. Introduction

Hematologic malignancies are divided into two categories: myeloid and lymphoid, each of which is further classified as acute and chronic [1]. Chronic myeloid diseases consist of three categories of Myelodysplasia Syndrome (MDS), Myeloproliferative Neoplasms (MPNs), and MDS/MPN. Myeloproliferative neoplasms include Polycythemia Vera (PV), Essential Thrombocythemia (ET), Chronic Myeloid Leukemia (CML), Primary Myelofibrosis (PMF), chronic neutrophilic leukemia, and less common cases such as chronic eosinophilic leukemia. In this classification, the first four diseases mentioned are considered classic myeloproliferative diseases (Figure 1) [2].

Myelodysplastic Syndrome (MDS) is an abnormality in the bone marrow structure (especially bone marrow hyperplasia, but hypoplasia could be seen either in less often occasions [3] that quantitatively and qualitatively contributes to the decrease in blood cell production [4]. On the contrary, Myeloproliferative Neoplasms (MPNs) usually exhibit an increase in the terminal myeloid cell in peripheral blood [5]. Some of the symptoms that can be seen in MPNs are fatigue (the most common symptom), pruritus, abdominal discomfort, night sweats, itching, bone pain, fever, and weight loss (the least common symptom) [6]. However, most patients have no symptoms unless they get the MPNs complications. Although the incidence of MPNs is more in females, the incidence and complications of PV are more in men [7].

Mutations in genes play an essential role in myeloproliferative diseases. In PV, ET, and PMF, mutations are found in the *JAK2*, *MPL*, *CBL*, *TET2*, *CALR*, or *RBBP6* genes, but no specific gene, such as CML, was found in any of these three diseases [1, 8]. These three diseases are also called *BCR-ABL1*-negative MPNs or Philadelphia chromosome-negative MPNs because this mutation is specific to CML. Albeit in some studies, JAK2 mutation is seen in 100% of PV cases, it is not specific for PV, and its frequency in ET and PMF is about 60%-65% [9]. In 2% of PV cases, 10%-15% of PMF and ET cases, no known gene mutations are found, and they had a poor prognosis [10, 11].

A study in the US in 2005 and 2006 on 848 death among MPNs patients showed that the most mortality is related to complications, especially cardiovascular diseases such as thrombosis or hemorrhage. Just about 3% of the death has no reason and directly is associated with the neoplasia [12].

The mutation of *JAK2 V617F* follows the transfer of G nucleotide to T in the Janus kinase 2 gene, and instead of phenylalanine, valine is formed at position 617 (V617F) [13, 14]. *JAK2* gene mutations are found in almost all patients with PV and approximately 50% of patients with ET or PMF [15]. In general, *BCR-ABL1* (Philadelphia chromosome) is specific for CML, but *BCR-ABL1* and *JAK2* gene mutations have also been observed. Philadelphia chromosome t(9;22) is present in 95% of cases of CML diseases. In *BCR-ABL* mutation, the active tyrosine kinase protein is located in the cytoplasm and causes cell proliferation. This event will result in the transfer of 30 segments of the *c-ABL* proto-oncogene to chromosome 9 instead of the 50 segments of *BCR* on chromosome 22 [16]. Displacement in *BCR-ABL* in about 95% of cases is in the form of breaking the M-*BCR* position (cluster major) in the *BCRL* intron in exon 13 (b2) but can be in other positions such as M-*BCR* in exon 14 (b3) in which the broken fragment enters the intron (*ABL* a2) and these two forms of b2a2 and b3a2. Depending on the amount of fusion protein, they are assigned a number, such as 210 kD fusion protein, which is the most common and is found in b2a2 and b3a2. Other numbers include (*P190* in e1a2) and *P230* in *BCR-ABL* [17].

Gene testing is an accessible and efficient approach to diagnose the disease before clinical symptoms appear. It provides timely treatment and helps develop and promote more effective treatment modalities [18]. The prevalence of these mutations varies in different societies, and knowing their prevalence helps prescribe drugs and estimate the success rate of treatments for the disease. This study aims to investigate the frequency of mutations in *BCR-ABL P190*, *BCR-ABL P210*, and *JAK2* genes in patients with four classic types of myeloproliferative disease.

2. Materials and Methods

This cross-sectional and descriptive-analytical study was performed from March 2017 until September 2020 in Shahid Mostafa Khomeini Hospital. During this study, all patients who were diagnosed with myeloproliferative diseases were included in the study. The inclusion criteria were 1) patients with erythrocytosis, 2) patients with thrombocytosis, and 3) patients with splenomegaly. It should be noted that the inclusion criteria are based on the definition of myeloproliferative diseases and should last at least 2 consecutive weeks in the patient. Infections and other differential diagnoses based on history, associated clinical signs, and the disease course should also be ruled out. As needed and according to the clinical protocol to confirm the diagnosis and distinguishing the

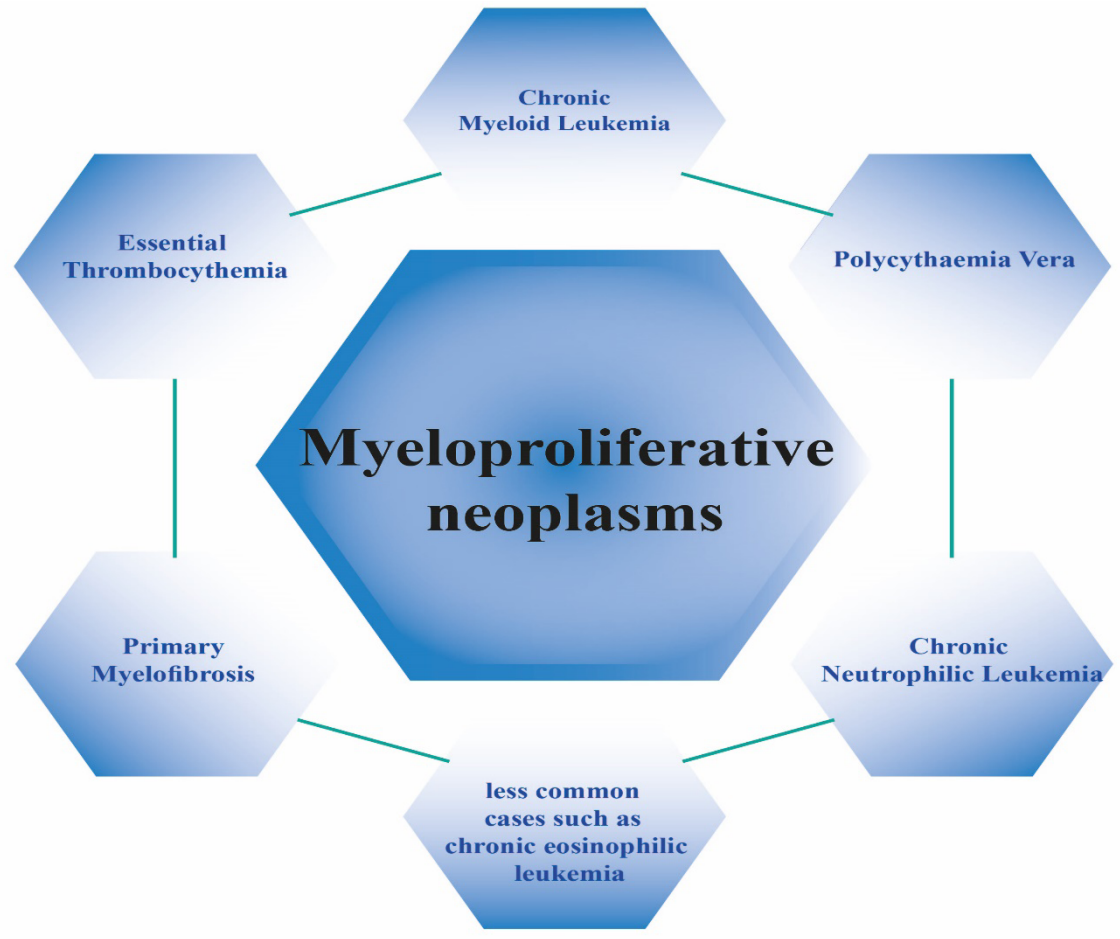

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Figure 1. Myeloproliferative neoplasms including Polycythemia Vera (Pv), Essential Thrombocythemia (ET), Chronic Myeloid Leukemia (CML), Primary Myelofibrosis (PMF), Chronic neutrophilic leukemia, and less common cases such as chronic eosinophilic leukemia

In this classification, the first four diseases mentioned are considered classic myeloproliferative diseases.

type of illness, patient information such as fever, general weakness, fullness, and history of pulmonary thromboembolism or deep vein thrombosis were obtained.

Following the study, blood tests (including Complete Blood Count differential identifies (CBC diff) and uric acid), abdominal and pelvic ultrasound emphasizing spleen size, and Chest x-Ray (CXR) were requested and performed for the patient. Then, blood samples were subjected to Polymerase Chain Reaction (PCR) analysis to detect *BCR-ABL P190* and *BCR-ABL P210* and quantify *JAK2*. The whole process of sampling and study on the sample was performed in the transplant center of Mostafa Khomeini Hospital, and no intervention was performed on patients.

Because the disease is rare, all records of myeloproliferative patients in Shahid Mostafa Khomeini Hospital between 2017 and 2020 were used for the study. Based on the findings of previous works, *JAK2* mutation in various diseases has been reported from 50% to 100%. Assuming 50% mutation (P=0.5), at least 43 specimens are needed to find the 15% difference (d=0.15) with 95% accuracy (z=1.96).

Results were presented as mean and standard deviation as well as frequency percentage. The collected data were analyzed in SPSS version 22 using The Chi-square, t test, and other required tests. A P value of less than 0.05 was considered statistically significant.

3. Results

A total of 52 patients who passed the inclusion criteria were assessed for the frequency of *BCR-ABL P190*, *BCR-ABL P210*, and *JAK2* mutations. The average age of participants was 60.5 y with a standard deviation of 14.7 y with the highest frequency in the age range of

Table 1. The Patients' demographic information (n=52)

Demographic Variables		No. (%)
Gender	Men	28 (53.8%)
	Women	24 (46.2%)
Age, y	20-40	7
	40-60	26
	60-80	16
	80-100	5
	Average	60.59
Medical condition	Essential Thrombocythemia (ET)	29 (55.8%)
	Men	14
	Women	15
	Chronic Myeloid Leukemia (CML)	17 (32.7%)
	Men	9
	Women	8
	Primary Myelofibrosis (PMF)	5 (9.6%)
	Men	4
	Women	1
	Polycythemia Vera (PV)	1 (1.9%)
	Men	1
	Women	0
Frequency of mutations	One or more positive mutation(s)	37 (71.2%)
	No mutations	15 (28.8)
	JAK2 positive	21 (40.4%)
	ET patients	15
	CML patients	2
	PMF patients	3
	PV patients	1
	***BCR-ABL P190* positive**	2 (3.8%)
	ET patients	0
	CML patients	2
	PM patients	0
	PV patients	0
	***BCR-ABL P210* positive**	14 (26.9%)
	ET patients	1
	CML patients	13
	PM patients	0
	PC patients	0

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ET: Essential Thrombocythemia; CML: Chronic Myeloid Leukemia; PV: Polycythemia Vera; PMF, Primary Myelofibrosis.

Table 2. Association between different study variables, according to the statistical analysis

Variables		P
Gender	Medical condition	0.457
	Presence of mutation	0.238
	Type of mutation	0.858
	JAK2	0.862
	BCR-ABL	0.330
Medical condition	Presence of mutation	0.011*
	JAK2	0.022*
	BCR-ABL	0.0001*

*P≤0.05.

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40-60 y. About 53.8% of the participants were male, and 46.2% were female. During the study, all patients were alive, and no fatalities were recorded. Demographic information of the patients, including their medical condition and the frequency of mutations, is presented in Table 1. More than half of the patients (29 cases, 14 men and 15 women) were diagnosed with ET, while PV was observed in only one patient who was a man. There were 17 and 5 cases with CML and PMF, respectively. About 71.2% of patients had one of the *JAK2*, *BCR-ABL P190*, or *BCR-ABL P210* mutations, of whom 37 were positive for *JAK2*, 14 for *BCR-ABL P210*, and 2 for *BCR-ABL P190*. Also, 15 out of 21 patients whose *JAK2* mutation was positive had ET, 2 of them CML and 3 cases PMF. The only man with PV has a positive *JAK2* mutation test. Also, 16 patients' *BCR-ABL1* tests were positive. Two of them were *P190*, and the others were *P210*. Table 1 presents more details, including the frequency of mutations by disease.

Using statistical tests, the relationship between study variables was investigated. The Chi-square test was used to examine the relationship between variables. As per the results, the patient's gender has no significant relationship with the existing medical condition. The same result was observed for the relationship between gender and the presence and type of mutation. However, the mutation and its type seem to significantly affect the patient's medical condition (P=0.011). The type of disease had a significant relationship with the studied mutations, such that people with CML had fewer *JAK2* gene mutations and more *BCR-ABL* gene mutation. It should be noted that all CML cases studied had one of the three mentioned types of mutations. Table 2 presents more details.

Besides, 46 people (about 88.5%) in the study responded to treatment, and their cancer went into remission. The others (6 cases) started to get their treatment. All patients were alive during the study.

4. Discussion

MPN is an abnormality in the function of blood stem cells that quantitatively contributes to the increase in blood cell production with poor quality. It seems that abnormality in the gene's structure plays an essential role in developing myeloproliferative diseases. Therefore, this study investigated three mutations of *JAK2*, *BCR-ABL P190*, and *BCR-ABL P210* in patients with myeloproliferative disease.

In this study, 52 patients with one of the myeloproliferative diseases who referred to Shahid Mostafa Khomeini Hospital in Tehran between March 2017 and September 2020 were studied and examined for mutations in these three genes. An attempt was made to make no difference between the genders in terms of the number of patients. Among the participants, ET was the most common medical condition with 55.8% prevalence, followed by CML with 32.7%. In 71.2% of cases, one of the mutations was present. The highest mutation was related to *JAK2* with 56.7%, followed by *BCR-ABL P210* with 37.8% and *BCR-ABL P190* with 5.4%.

Regarding the relationships between the research variables, gender was not significantly related to any factor in the study. The type of medical condition was significantly associated with the studied mutations, such that CML patients had fewer *JAK2* and more *BCR-ABL* gene mutations. It should also be noted that all CML cases studied in this study had one of three types of mutations.

This study shows that mutations in *JAK2*, *BCR-ABL P190*, and *BCR-ABL P210* genes play a decisive role in the incidence of the disease that directly affects the consequent treatment approach in the patients. So far, limited studies have investigated the association between these genes and myeloproliferative neoplasms, and this study, despite its limitations, tried to shed light on this potential association. Gene mutation is well established to be connected with the development and progression of various cancers; thus, studying the genes involved in cancer initiation and propagation is necessary and practical for further clinical interventions.

5. Conclusion

There were 2 cases whose *BCR-ABL* mutation tests were negative although they had CML, and also there was a patient who had ET; despite that, the *BCR-ABL* mutation test was positive. So the sensitivity and specificity of the Philadelphia chromosome test are not 100% for CML disorder. There was no mortality during the study. All the patients were alive, and most of them (88.5%) were in remission.

Ethical Considerations

Compliance with ethical guidelines

This study was supported by the Research Ethics Committee of Shahed University of Medical Sciences (Code: IR.SHAHED.REC.1399.025).

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Authors' contributions

Conceptualization, investigation, writing – original draft, writing – review & editing, resources, supervision, validation, data curation: All authors; Methodology: Saleheh Zarian, Tooba Ghazanfari; Funding acquisition: Jalal edin Shams; Softaware: Saleheh Zarian; Formal analysis: Saleheh Zarian, Tooba Ghazanfari.

Conflicts of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. These interests include employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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