JAK2 mutation, a potential cause of increasing Hemoglobin levels in symptomatic aged women

Running title: JAK2 mutation, higher Hemoglobin in symptomatic women

Zeinab Siahmargoie
Iranian blood transfusion Organization, Gorgan branch, Iran, siahmargoie@yahoo.com,
ORCID: 0009-0008-8318-5878
Mohammad taher Hojjati
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran, mthhematology@gmail.com
ORCID: 0000-0002-6592-6467
Hadi Bazzazi
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran, hadi.bazzazi@gmail.com,
ORCID: 0000-0003-4303-5214
Khodaberdi Kalavi
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran, kalavi@goums.ac.ir,
ORCID: 0000-0001-7577-5888
Mana Zakeri
department of medical genetics, faculty of medicine, hormozgan university of medical sciences, bandar abbas, Iran, Mana_zakeri1165@yahoo.com,
ORCID: 0000-0002-5936-957X
Hadi Joshaghani
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran
ORCID: 0009-0001-7764-4125

Correspondence: Mohammad taher Hojjati
Address: paramedicine school, Golestan university of medical sciences, Hirkan Blv, Gorgan, Iran
Tel: +989113562819
Email: mthhematology@gmail.com

Abstract

Background: Polycythemia Vera (PCV) is a type of myeloproliferative neoplasm (MPN) in which the progenitors of the erythroid lineage become overactive and produce large amounts of red blood cells (RBCs). More than 90% of people with PCV have a Janus kinase 2 (JAK2) gene mutation. In this study, we examined the status of possible JAK2 gene mutations in people with higher-than-normal hemoglobin (Hb) levels that physicians introduced to the laboratory.
Methods: In this descriptive cross-sectional study, JAK2 alleles were tested for possible JAK2 mutations in genomic DNA of 72 cases using a TaqMan-specific probe.
Results: Out of 72 patients, 24 were women (33.3%) and 48 (66.6%) were men, among them 39 (54.2%) were negative and 33 cases (45.5%) were positive for JAK2 mutation. The data also showed that 15 out of 24 female patients (62.5%) had positive JAK2 mutation, while in the male patients, 18 out of 48 (37.5%) were positive for JAK2 mutation.
Conclusion: According to our research, investigation of the Jak2 mutation, especially in women who had Hb levels upper than normal, seems to be necessary.
Keywords: Polycythemia Vera (PCV)**,** JAK2 mutations**,** Hemoglobin (Hb), Women

Introduction

Erythrocytosis refers to an increased age-related erythrocyte count and is classified into relative erythrocytosis, which is caused by a reduction in plasma volume (hemoconcentration), or absolute erythrocytosis, which is defined by increased erythrocyte mass (1). Absolute erythrocytosis can be driven by a clonal bone marrow disease, Polycythemia Vera (PV) with the incidence of 0.84%, or be secondary to another disease, which can be caused by many physiologic and/or benign pathologic factors, with an incidence and prevalence of 6%–8% (2). PV is a chronic proliferative neoplasm with clonal stem cell expansion of myeloid lineage, with the amplification of red blood cell (RBC) mass and other myeloid cells, requiring differential investigation for other myeloproliferative disorders (3). In the 2008 World Health Organization (WHO) criteria for polycythemia vera (PV), arbitrary hemoglobin (Hb) thresholds of more than 18.5 g/dL in males and 16.5 g/dL in females were used as a surrogate marker for increased red cell mass (RCM) (4), but in 2016 the new criteria described. The hemoglobin threshold, which was >18.5 g/dL for men and >16.5 g/dL for women, is decreased to >16.5 g/dL for men and >16 g/dL for women. A hematocrit threshold was also included in the criteria, which is >49% for men and >48% for women (5).

The molecular characterization of PV was revolutionized in 2005 by the detection of a somatic mutation, the JAK2 V617F gene. Janus kinase (JAK) is a family of non-receptor tyrosine kinases that are encoded by the gene region localized in 9p24, an acquired point mutation in *JAK2* in exon 12 of the *JAK2* gene (6). JAKs perform their effect by further phosphorylating transcription factors called signal transducer and activator of transcription (STAT), and in erythroid progenitors, the JAK-STAT pathway is particularly important to cell proliferation, cell differentiation, and cell apoptosis regulation, in the hematopoiesis pathway and Hb production (7). The mutation induces a constant hyper-activation of the JAK2 protein, which enhances (Erythropoietin) EPO-independent growth of erythroid progenitors and eventually increases cell proliferation and therefore Hb elevation (8). In this study, we examined people with higher-than-normal hemoglobin levels according to age and gender who were referred by physicians to the laboratory to investigate the JAK2 mutation.

Methods

In this descriptive cross-sectional study, JAK2 mutations in 60 patients who had high hemoglobin according to WHO criteria (5), with initial suspicion of PCV, were investigated by the oncologists and referred to the laboratory. The study was registered at Islamic Azad University, Gorgan branch, with the registration code 162474677. Initially, Whole blood of approximately 4 ml, was collected in EDTA from each patient, and were subjected to complete cell count (CBC) using an automatic analyzer Sysmex KX-21N (sysmex, Japan) for basic tests such as the number of white blood cells (WBC), platelets (PLT), the level of hemoglobin (Hb), and hematocrit (Hct). Then, Demographic information of the patients was recorded, and all patients signed the informed consent form to participate in this study.

Genomic DNA was extracted using the AccuPrep® Genomic DNA Extraction Kit according to the manufacturer's instructions (Bioneer, Korea). The extracted DNA was kept at 4°C until further use. To detect JAK2 Mutation, the portion of the JAK2 V617F gene that acquired the mutation was amplified by allele-specific oligonucleotide (ASO) Polymerase Chain Reaction (PCR). Briefly, the PCR was performed using 20- 200 ng of template DNA, 20 µM master mix, 5 µM forward control primer (FC), 5 µM forward specific primer (FS), 5 µM reverse primer, 5 U/µL Taq enzyme, and distilled water. A list of primers and their nucleotide sequences, and amplification conditions are shown in Tables 1 and 2, respectively.

Table 1. Allele-specific oligonucleotide-polymerase chain reaction primers

Primer	Sequences (5' → 3')
Forward control	ATC TAT AGT CAT GCT GAA AGT AGG AGA AAG
Forward specific	AGC ATT TGG TTT TAA ATT ATG GAG TAT ATT
Reverse primer	CTG AAT AGT CCT ACA GTG TTT TCA GTT TCA

The amplification conditions include initial denaturation at 95°C for 10 min, followed by 14 cycles of denaturation (94°C for 20 sec), annealing (65°C, 60 sec), and extension (72°C for 60 sec). These cycles were followed by a final extension step at 72°C for 5 min. The results were analyzed by Lightcycler96 (Roche, Germany) (Figure 1)

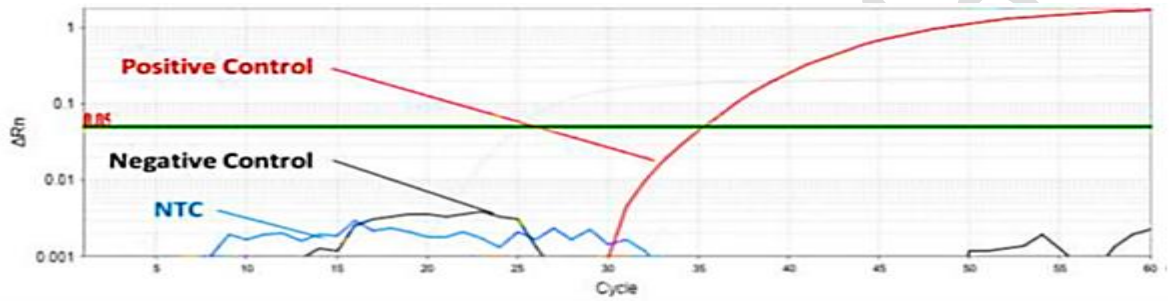

Figure 1. The result of PCR. The green line shows the threshold between Positive and Negative results. The red line revealed a positive result, and the black line shows a negative result for the JAK2 mutation

Results

Out of 72 patients, 24 were women (33.3%) and 48 (66.6%) were men; among them, 39 (54.2%) were negative and 33 (45.5%) were positive for the JAK2 mutation. The data also showed that 15 out of 24 female patients (62.5%) had a positive JAK2 mutation, while in the male patients, 18 out of 48 (37.5%) had the mutation present (p=0.013).

Table 2 shows the information of the patient population by gender without considering the incidence of JAK-2 mutation. The results indicated that there was no significant difference between men and women in any of the investigated parameters. However, the comparison of these parameters without considering the gender and only based on the occurrence of JAK-2 mutation showed that only in the age parameter, there was a significant difference between the two groups; men and women, and with increasing age, the incidence of this disease increases (p=0.024) (Table 3)

Table 2. Patient population information without considering JAK-2 mutation incidence

Gender		Age	WBC	RBC	Hb	PLT*1000
Female	Mean	44.13	9.711	5.378	14.125	629
(No: 24)	SD	17.578	4.4536	1.7982	4.1664	556
Male	Mean	38.94	8.239	5.782	16.325	363
(No: 48)	SD	17.699	2.9808	1.0141	2.3185	282

Table 3. Comparison of these parameters without gender discrimination based on the occurrence of the JAK2 mutation

	JAK2.Mutation		Mean	Std. Deviation	P-Value
Age		Negative	33.46	18.035	**0.024**
		Positive	49.18	12.766	
WBC		Negative	8.085	3.1692	0.339
		Positive	9.492	3.8905	
RBC		Negative	5.378	.8668	0.28
		Positive	5.965	1.6690	
Hb		Negative	15.223	2.7980	0.545
		Positive	16.027	3.6078	
PLT		Negative	368615.38	247101.713	0.319
		Positive	539000.00	541274.607	

Investigations based on the occurrence of JAK2 mutation in the female population also showed that the age parameter in positive and negative groups for the mutation had been significant (p=0.029), so that the age of the negative group was 28.13 ± 13.7 and in positive group it was 53.8 ± 11.8, and in other parameters in female group was not significant (Table 4). However, in the male population, the age of JAK2 positive patients was higher than Negative patients, but there was no significant difference in age and other factors (Table 4). Also, the comparison of all parameters in JAK2 positive mutation patients showed that there was no significant difference in the studied parameters in men and women (Table 5).

Table 4. Comparison of parameters of patients by gender based on the presence or absence of mutation

Gender	JAK2.Mutation		Age	WBC	RBC	Hb	PLT
Female	Negative	Mean	28	9.217	4.927	13.067	441000
		Std. Deviation	13.748	2.4744	0.8593	3.166	273040.29
	Positive	Mean	53.8	10.008	5.648	14.76	716000
		Std. Deviation	11.584	5.5996	2.2462	4.9013	710397.776
	P-Value		**0.029**	0.829	0.622	0.617	0.554
Male	Negative	Mean	35.1	7.745	5.513	15.87	346900
		Std. Deviation	19.462	3.3876	0.8658	2.4891	250149.933
	Positive	Mean	45.33	9.062	6.23	17.083	391500
		Std. Deviation	13.397	2.1678	1.162	1.9671	353253.309
	P-Value		0.277	0.411	0.179	0.328	0.319

Table 5. Comparison of blood parameters of JAK2 positive patients according to gender

	Gender	N	Mean	Std. Deviation	P-Value
Age	Female	15	53.80	11.584	0.297
	Male	18	45.33	13.397	
WBC	Female	15	10.008	5.5996	0.710
	Male	18	9.062	2.1678	
RBC	Female	15	5.648	2.2462	0.529
	Male	18	6.230	1.1620	
Hb	Female	15	14.760	4.9013	0.312
	Male	18	17.083	1.9671	
PLT	Female	15	716000.00	710397.776	0.349
	Male	18	391500.00	353253.309	

Discussion

The effect of gender on the occurrence of PV in many articles has been discussed. Many contradictory results have been published in this field. Our study showed that the prevalence of PV in women is higher than in men (62.5% vs. 37.5%), that was consistence with the results of Godferi et al in 2013 (9) and Payzin in 2014 (10). While a study conducted by Hamid et al. showed that the mutation rate was higher in men than in women, the correlation between mutation and gender was statistically significant (11). In a study conducted by Deamond et al, reported that in all age groups, PV risk was lower among women (12). In some publications, it is also reported that the risk of myeloid diseases is higher in young females, while in advanced ages it is overexpressed in males (13; 14). For this reason, in the difference of genotype and phenotype among JAK2-positive patients, gender should be considered when evaluating patients in terms of diagnosis, prognosis, and disease complications. As gender may influence the genotype or clonal expansion that affects variation in JAK2 allele burden, it will be important to investigate factors that determine susceptibility to mitotic recombination events.

The mean age of Jak2 positive cases in our study in men and women was 45 and 53%, respectively. In some similar studies, the effective roles of age were reported (11; 15). Our study also showed that the occurrence of this mutation in women is age-dependent, and the patients older with higher Hb levels were more prone to manifest PV than others. The mean ages of affected and not affected patients in women were 53.8 vs. 28, but this issue was not similar in men and among them, present in wider ages.

Cellular parameters were the other variables in our study. Like many other studies, our results showed that WBC, RBC, Hb, and Plt were higher in Jak2-positive patients and showed a positive relationship with JAK2 mutation. Gulbay et al (16) also reported that the difference in hematological parameters [white blood cell, hemoglobin (Hb), hematocrit (HCT), red blood cell distribution widths (RDW), and platelet count (PLT)] between JAK2 V617F-positive and JAK2 V617F-negative patients.

Conclusion

Our study showed that women with higher Hb levels than normal ranges may be more sensitive to manifest PV. So, evaluation of such parameters can be helpful in the early detection of PV in females. According to the results of this study, men with lower ages may also be expected to manifest PV with higher-than-normal Hb levels.

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Ethical statement

This study was conducted following the approval of the ethics code (RI.IAU.CHALUS.REC.1401.009) by the Ethics Committee of Islamic Azad University, Chalus branch

Conflicts of interest

The Authors had no Conflicts of interest

Author contributions

Zeinab Siahmargoie Performing the test; Mohammad taher Hojjati Supervision,
final validation of the article; Hadi Bazzazi Writing draft of the article and Data analysis; Khodaberdi Kalavi Data interpretation and technical consultant; Mana Zakeri Writing draft of the article and Statistical analysis; Hadi Joshaghani Clinical consultant and Writing draft of the article.

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