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ORIGINAL ARTICLE

APPLICABILITY OF THE 2016 WHO DIAGNOSTIC CRITERIA FOR POLYCYTHEMIA RUBRA VERA IN SULAIMANIYAH CITY, KURDISTAN REGION, IRAQ

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ABSTRACT

Background: Polycythemia Rubra Vera (PRV) is a myeloproliferative neoplasm characterized by uncontrolled red blood cell production. This study aimed to evaluate the applicability of the 2016 WHO diagnostic criteria for PRV in Sulaimaniyah City, Kurdistan Region, Iraq, focusing on the incidence of JAK2 mutations and their association with patient demographics and clinical features.

Materials & Methods: A cross-sectional retrospective study was conducted on 100 PRV patients between February 2023 and February 2024 at Hiwa Hematology/Oncology Hospital and Shorsh Teaching Hospital. Patients were selected using a convenience sampling method, which involved including all eligible patients with available JAK2 mutation data and bone marrow examination results during the study period. Blood parameters, bone marrow examination, and JAK2 mutation status were analyzed. Statistical associations between JAK2 mutation status and age, gender, and clinical features (neutrophilia, thrombocytosis, splenomegaly) were assessed.

Results: The mean age of the patients was 52.91 ± 16.76 years, with 51% older than 50 years. Males comprised 68% of the cohort. Among the patients, 47% were JAK2 mutation-positive, and 53% were JAK2-negative. JAK2-positive patients were significantly older (mean age 57.51 ±17.84 years) compared to JAK2-negative patients (mean age 48.83 ± 14.73 years; $p=0.009$). A significant association was found between JAK2 mutation status and gender ($p=0.01$), with a higher proportion of males among JAK2-negative patients. JAK2-positive patients showed higher incidences of neutrophilia (25.5% vs. 3.8%; $p=0.002$), thrombocytosis (46.8% vs. 13.2%; $p<0.001$), and splenomegaly (53.2% vs. 9.4%; $p<0.001$) compared to JAK2-negative patients.

Conclusions: The study revealed that while the 2016 WHO criteria are generally effective for diagnosing PRV, the higher-than-expected proportion of JAK2-negative patients suggests the need for additional diagnostic considerations in this population. The significant associations between JAK2 mutation status and specific clinical features emphasize the heterogeneity of PRV presentations and the potential need for regional adaptations of the WHO criteria.

KEY WORDS: Janus kinase 2 mutation; Neutrophilia; Polycythemia Rubra Vera; Splenomegaly.

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INTRODUCTION

Polycythemia rubra vera (PRV), essential thrombocythemia (ET), primary myelofibrosis (PMF), unclassifiable myeloproliferative neoplasms (MPN-U) are four Janus kinase 2 (JAK2) mutation-prevalent MPN.[1] The mutation of JAK2 gain-of-function (JAK2V617F) was first detected in 2005 in PRV, ET and PMF when a guanine (G) to tyrosine (T) somatic mutation at nucleotide 1849/exon 14 resulted in the replacement of valine to phenylalanine at codon 617.[2]

In 2007, the World Health Organization (WHO)

published the criteria to diagnose Philadelphia-negative MPN, including PRV, ET and PMF, in which its criteria for PRV were 2 major and 3 minor criteria. Major criteria are haemoglobin (Hb) >18.5 g/dL in males, 16.5 g/dL in females, or other evidence of increased red cell mass (RCM), and the incidence of JAK2V617F or other JAK2 exon 12 mutations. The minor criteria consist of the following: (i) BM biopsy showing increased cellularity with growth in all three blood cell lineages (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation; (ii) serum erythropoietin (EPO) levels below the normal range; and (iii) the development of erythroid colonies in vitro. The diagnosis of PRV requires the presence of at least one major criterion together with either one minor criterion or the first major criterion along with two minor criteria.[3]

The JAK2 mutation test is now widely/mainly used in practice to confirm the PRV diagnosis despite its high cost. Approximately 96% of patients with PRV have a mutation in the JAK2 gene, which is elaborated in intracellular signaling in progenitor cells, a process that occurs after exposure of these cells to cytokines and makes them hypersensitive.[4]

Presently, there are two classification systems for MPN, including International Consensus Classification (ICC)[5] and WHO-5th edition (published in 2016 and revised in 2022).[6] The WHO-5th edition diagnostic criteria for PRV are almost alike of the ICC but no longer need RCM evaluation.[7] Both include 3 major criteria and one minor criterion.[7] First major criteria is Hb >16.5 g/dL in males and >16 g/dL in females, hematocrit (HCT) >49% in males and >48% in females, and RCM >25%.[7,8] On the other hand, the second main criterion involves a BM biopsy that demonstrates an increase in cell density adjusted for age, with the presence of three types of cells: RBC, WBC, and mature megakaryocytes which show no abnormal features. The third main criterion is the presence of either JAK2V617F or JAK2 exon 12 mutation. Simultaneously, the minor criterion is subnormal EPO value. A JAK2V617F allele burden (negative case) needs confirmation through repeated tests and/or highly sensitive assays (<1%) in which its interpretation should be considered the clinical scenario and the EPO value. The WHO-5th criteria for diagnosis of PRV needs meeting all three or the first 2 major criteria and the minor criterion.[1] Thus, based on the last edition of WHO diagnostic criteria, this study was designed to find the incidence of JAK2 mutation among patients with PRV and its correlation to splenomegaly, neutrophilia, and thrombocytosis.

MATERIAL AND METHODS

This cross-sectional, retrospective study looked at patients diagnosed with Polycythemia Vera (PRV) between February 2023 and February 2024 at Hiwa Hematology/Oncology Hospital and Shorsh Teaching Hospital in Sulaimaniyah, Iraq. Convenience sampling technique was used to pick patients based on the availability of medical information. This non-probability sampling technique was deemed appropriate due to the study's retrospective character and dependence on existing data.

The study included all eligible patients who met the inclusion criteria, which were the availability of JAK2 mutation data and bone marrow (BM) examination results. Patients were chosen based on their medical records, and the sample size was calculated using the number of available instances that met these criteria. We wanted to include a large number of examples to assure statistical power. Despite not doing a formal sample size calculation, a total of 100 patients because this number was sufficient to fulfill the study objectives with precision and dependability. Patients with PRV who had available data on JAK2 mutation status and BM examination were eligible for inclusion. Patients with hemoglobin levels over the WHO diagnostic standards for PRV were excluded, as this could imply a different diagnosis.

Each patient provided blood samples ranging from 5.0 to 7.0 milliliters. A Coulter automated hematology analyzer was used to measure hematological parameters such as hemoglobin (Hb), hematocrit (HCT%), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), and differential count. Wright staining was used to examine bone marrow for hypercellularity and trilineage growth, which included erythroid, granulocytic, and megakaryocytic proliferation.

JAK2V617F mutation analysis was carried out utilizing Real-Time PCR. The study followed scientific and ethical guidelines established by the Kurdistan Higher Council of Medical Specialties (KHCMS), Sulaimaniyah, Iraq. All participants provided written informed consent.

Data was analyzed using SPSS (version 26). Categorical data were studied using the chi-square test, while quantitative variables were evaluated using the Man-Whitney U test after normality testing with Kolmogorov-Smirnov. A p-value of <0.05 indicated statistical significance.

RESULTS

The mean age of the patients was 52.91 ±16.76 years, with an age range of 20 - 91 years. Most patients (51%) were aged >50 years, followed by 30-50 years (40%), then <30 years (9.0%). Also, most patients (68%) were males and 32% were females, as seen in Table (1).

Table 1: Sociodemographic data of the studied patients with polycythemia.

Variable		Number	%
Age (Years)	<30	9.0	9.0
	30-50	40	40.0
	>50	51	51.0
Gender	Female	32	32.0
	Male	68	68.0
Total		100	100

Additionally, genetic analysis indicated that most patients (53%) were JAK2 mutation negative, while 47% were JAK2 mutation positive. JAK2 mutation was significantly associated with age of the patients (p=0.009, using Mann whiney u test) as the age of JAK2 negative patients was 48.83 ± 14.73 years which was lower than JAK 2 positive patients (57.51 ± 17.84 years). Interestingly, the JAK2 mutation was significantly related to the patient's gender (p=0.01), as 79.2% (n=42) of the JAK2-negative patients were males, and 20.8% (n=11) were females. On the other hand, 55.3% (n=26) of the JAK2-positive patients were males, and 44.7% (n=21) were females. Notably, neutrophilia was significantly associated with JAK2 mutation (p=0.002), as only 3.8% (n=2.0) of the JAK2 negative patients had neutrophilia, and 25.5% (n=12) of the JAK2 positive had neutrophilia. Additionally, only 13.2% (n=7.0) of the JAK2-negative patients had thrombocytosis, and 46.8% (n=22) of the JAK2-positive had thrombocytosis (p<0.001). Lastly, splenomegaly was highly associated with JAK2 mutation (p<0.001), as 90.6% (n=48) of the JAK2-negative patients had no splenomegaly, and 9.4% (n=5) had splenomegaly, in which 7.5% (n=4.0) were mild, and 1.9% (n=1.0) were moderate. On the contrary, 46.8% (n=22) of JAK2-positive patients had no splenomegaly, and 53.2% (n=25) had splenomegaly, in which 34% (n=16) were mild, 14.9% (n=7.0) were moderate and 4.3% (n=2.0) were massive. Collectively, most JAK2 positive/negative patients were males, had no neutrophilia and thrombocytosis, but had mild splenomegaly, as shown in Table (2).

Moreover, the mean values of Hb, HCT and RCM were 16.79±0.50 g/dL, 52.01±2.86%, and 6.29±0.90. These values are higher than those established by WHO criteria-2016. There was no significant difference in Hb and HCT concentrations between JAK2 negative and JAK2 positive patients. On the other hand, the concentrations of RBC, total WBC, neutrophil and platelet were significantly lower in JAK2 negative (6.04 ± 0.53, 8.10 ± 3.42, 58.73 ± 11.86 and 265.75 ± 125.56, respectively) than JAK2 positive (6.58 ± 1.12, 10.86 ± 4.42, 68.77 ± 7.12 and 412.60 ± 155.64, respectively). On contrary, the level

Table 2: Correlation of JAK2 mutation to the patient's gender and clinical data

Variable		JAK2 Negative (n=53)	JAK2 Positive (n=47)	p-value
	Number (%)			
Gender	Female	11 (20.8)	21 (44.7)	0.01*
	Male	42 (79.2)	26 (55.3)	
Neutrophilia	No	51 (96.2)	35 (74.5)	0.002*
	Yes	2.0 (3.8)	12 (25.5)	
Thrombocytosis	No	46 (88.8)	25 (53.2)	<0.001**
	Yes	7.0 (13.2)	22 (46.8)	
Splenomegaly	No	48 (90.6)	22 (46.8)	<0.001**
	Yes	5.0 (9.4)	25 (43.2)	
	Mild	4.0 (7.5)	16 (34.0)	
	Moderate	1.0 (1.9)	7.0 (14.9)	
	Massive	0.0 (0.0)	2.0 (4.3)	

*: Significant difference, **: Highly significant difference using Chi-square test

Table 3: Correlation between JAK2 mutation and blood parameters among patients with polycythemia rubra vera

Hematological parameter	Total value	JAK2 Negative (n=53)	JAK2 Positive (n=47)	p-value
Total RBC (10^6/mm^3)	6.29±0.90	6.04 ± 0.53	6.58 ± 1.12	0.021*
Hb (g/dL)	16.79±0.50	16.88 ± 0.45	16.69 ± 0.54	0.063
HCT%	52.01±2.86	51.45 ± 2.14	52.64 ± 3.42	0.153
MCV (fl)	83.01±8.83	85.04 ± 5.69	80.66 ± 11.04	0.03*
MCH (pg)	26.74±3.65	28.06 ± 2.32	25.26 ± 4.29	<0.001*
MCHC (g/dL)	31.82±4.57	32.30 ± 1.59	31.26 ± 6.48	<0.001*
Total WBC (10^9/L)	9.39±4.14	8.10 ± 3.42	10.86 ± 4.42	<0.001*
Neutrophil%	63.70 ± 11.98	58.73 ± 11.86	68.77 ± 7.12	<0.001*
Lymphocyte%	29.94±10.84	34.05 ± 11.62	25.30 ± 7.66	<0.001*
Monocyte%	5.36±2.40	5.94 ± 2.31	4.70 ± 2.35	0.002*
Platelet (n/μL)	334.77±157.99	265.75 ± 125.56	412.60 ± 155.64	<0.001*

Values are presented as Mean±SD, *: Significant difference using Mann Whitney- U test.

Table 4: The correlation between BM status (hypercellularity for age with trilineage growth) and JAK2 mutation among patients with polycythemia rubra vera

Bone Marrow	JAK2 Negative (n=53)	JAK2 Positive (n=47)	p-value (Chi-square test)
Hypercellularity	42 (79.2%)	42 (89.4%)	0.168
No hypercellularity	11 (20.8%)	5.0 (10.6%)	

of MCV, MCH, MCHC, lymphocyte and monocyte were significantly higher in JAK2 negative (85.04 ± 5.69, 28.06 ± 2.32, 32.30 ± 1.59, 34.05 ± 11.62 and 5.94 ± 2.31, respectively) than JAK2 positive (80.66 ± 11.04, 25.26 ± 4.29, 31.26 ± 6.48, 25.30 ± 7.66 and 4.70 ± 2.35, respectively), as shown in Table (3).

Furthermore, regarding the BM examination, there was no significant correlation (p=0.168) between JAK2 mutation and hypercellularity for age with trilineage growth, including prominent erythroid, granulocytic, and mature-pleomorphic megakaryocytic proliferation. However, most patients in both groups (n=82) showed BM hypercellularity, of which 89.4% were JAK2 positive and 79.2% were JAK2 negative, as shown in Table (4).

DISCUSSION

This study aimed to evaluate the applicability of the 2016 WHO Diagnostic Criteria for Polycythemia Rubra Vera (PRV) in Sulaimaniyah City, Kurdistan Region, Iraq, where the local population presents distinct characteristics compared to global datasets.

Our findings suggest that while the WHO criteria are generally effective, there are notable differences that indicate a need for local adaptations. Our study revealed that the mean age of PRV patients was 52.91 ±16.76 years, spanning from 20 to 91 years. This finding is inconsistent with Jakovic et al., who reported an older age range of 58 to 72 years,[9] but aligns with Barbui et al., who observed a broader range of 28 to 84 years[10]. These discrepancies may be attributed to geographical, environmental, and ethnic variations. Furthermore, the predominance of males in our cohort (68%) is consistent with other studies,[9,10] reinforcing the gender bias observed in PRV.

The high proportion of JAK2-negative patients (53%) in our study contrasts sharply with global findings where JAK2-positive cases are more common.[10,11,12] This highlights a potential regional variance in genetic factors influencing JAK2 mutation prevalence. The WHO-2016 criteria's ability to accurately diagnose JAK2-negative PRV cases is crucial, as such cases may otherwise be missed or misdiagnosed under the previous WHO-2007 criteria.[13] Significant

associations were found between JAK2 mutation status and clinical features such as age and gender, with higher rates observed in older males.[14] This is consistent with Godfrey et al., who found a higher frequency of homozygous JAK2 mutations in older patients and males.[13] The findings suggest that age-related factors and gender-specific genetic predispositions may play a role in the acquisition of JAK2 mutations.

In terms of clinical correlations, our study identified significant associations between JAK2 mutation status and neutrophilia, thrombocytosis, and splenomegaly.[15] Specifically, JAK2-positive patients were more likely to exhibit neutrophilia, thrombocytosis, and splenomegaly, while JAK2-negative patients showed less frequent but milder splenomegaly. These findings are consistent with those of Jakovic et al.[9] and Barbui et al.[10], indicating that JAK2 mutation status can influence clinical manifestations of PRV.

The mean values for hemoglobin (16.79 ± 0.50 g/dL), hematocrit (52.01 ± 2.86%), and red cell mass (6.29 ± 0.90) in our study align with WHO-2016 criteria for PRV diagnosis.[7] However, no significant difference was observed between JAK2-negative and JAK2-positive patients in terms of hemoglobin and hematocrit levels. Notably, JAK2-negative patients had lower red blood cell, total white blood cell, neutrophil, and platelet counts compared to JAK2-positive patients. These variations in hematological parameters underscore the heterogeneity of PRV and the limitations of relying solely on JAK2 mutation status for diagnosis.[11] Regarding bone marrow morphology, no statistically significant relationship was found between JAK2 mutation status and bone marrow hypercellularity.[15] Despite this, a high prevalence of bone marrow hypercellularity was observed in both JAK2-positive and JAK2-negative patients, with a notable proportion showing signs of panmyelosis and megakaryocyte dysplasia, consistent with Nathany et al.[11] The shift from WHO-2007 to WHO-2016 criteria was driven by the need to improve diagnostic accuracy and address the limitations of the previous criteria, which often resulted in false negatives due to overly stringent hemoglobin thresholds.[16] The 2016 revision incorporates additional diagnostic criteria and acknowledges the importance of bone marrow morphology, making it a more comprehensive tool for identifying PRV.

CONCLUSIONS

This study assessed the applicability of the 2016 WHO Diagnostic Criteria for Polycythemia Rubra Vera (PRV) in patients from Sulaimaniyah City, Kurdistan Region, Iraq. We discovered that, whereas the 2016 WHO criteria were typically efficient in detecting PRV, the presence of JAK2 mutations varied significantly among individuals. Notably, 53% of the patients tested negative for JAK2 mutations, which was higher than expected given global data. The study also found that JAK2-positive patients were more likely to have neutrophilia, thrombocytopenia, and splenomegaly than JAK2-negative patients. There were no significant changes seen between JAK2 mutation status and important hematological indicators such as hemoglobin levels, hematocrit, or bone marrow hypercellularity. The data imply that, while the 2016 WHO criteria are valid, they may need to be amended or augmented with other diagnostic techniques in our local context to ensure correct PRV diagnosis, particularly in JAK2-negative individuals. More research is needed to determine whether other genetic or environmental factors influence PRV diagnosis in this population.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	SIN, HAG
Acquisition, Analysis or Interpretation of Data:	SIN, HAG, NSHK
Manuscript Writing & Approval:	SIN, HAG, NSHK

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.