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ORIGINAL ARTICLE

Prevalence of JAK 2 v617 Mutations in Malignant and Non-Malignant Tumors in the Eastern Province of the Kingdom of Saudi Arabia

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SUMMARY

Background: **Janus kinase II (JAK 2) mutation plays a critical part in the pathophysiology of myeloid pathologies and has been presented to be tangled in thrombotic obstacles of these sicknesses. This study documents the prevalence of JAK 2 v617 mutations in malignant and non-malignant tumors in the Eastern province of the Kingdom of Saudi Arabia.**

Methods: **A total of 112 patients were included in the current study between June 2022 and May 2023 at the Molecular Biology Laboratory of the King Fahad Hospital of the University, AlKhobar, Saudi Arabia. Laboratory data involved the hematological parameters (hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, hematocrit, white blood cells, red blood cells, and platelets) and real-time PCR JAK2 V617F mutation qualitative assay.**

Results: **The prevalence of JAK 2 disease among 112 patients was found to be (n = 13) 12%. White blood cell count was relatively higher in the positive patients, but the difference was statistically nonsignificant (p = 0.846). Similarly, the hemoglobin level among the positive patients was higher, 14.62 g/dL, but still not significantly higher (p = 0.075). However, red blood cell count in the JAK2 patients was significantly higher compared to the negative patients (p = 0.002). Similarly, the percentage of red blood cells measured by HCT test was also significantly higher among the JAK2-positive patients (p = 0.036) compared to the negative patients.**

Conclusions: **We believe these observations warrant a comprehensive search for activated tyrosine kinases in myeloproliferative disorders and hematological malignancies, as there are likely additional unidentified genetic events with biological and therapeutic significance. Additional in vitro and in vivo studies are needed to determine the cause of the specificity of JAK2 V617F for myeloid and lymphoid diseases.**

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INTRODUCTION

Janus kinases (JAKs) transduce gestures from lots of extracellular chemical mediators and act as perilous controllers of cell development, distinction, gene expression, and immune answers. Loss of ruling of JAK/STAT signaling is a vital constituent in numerous humanoid sicknesses [1]. JAK 2 is a cytoplasmic tyrosine kinase with a vital role in signal transduction from numerous hematopoietic growing factor receptors. Genetical errors in JAKs are the reasons for myeloproliferative tumors (MPTs) in people, which are clonal proliferative diseases distressing diverse myeloid lines [2]. Genetical errors are common in the well-known myeloproliferative tumors, specifically polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Up-to-date, the mutated JAK2 has been established in myeloid cells such as bone marrow cells, myelocytes, thrombocytes, and erythroblasts derivative of CD34 positive cells, but not in T lymphocytes [3]. Janus kinase 2 (JAK 2) mutation has a critical part in the pathophysiology of the myeloid pathologies and has been presented to be tangled in thrombotic obstacles of these sicknesses. Innovative advances in molecular genetics have extraordinarily upgraded diagnosis, differential diagnosis, as well as our understanding of the pathophysiology of such sicknesses, principally after extensive usage of JAK 2 repeated mutational defects. In contrast, the mutation was not existent in non-hematopoietic cells. The JAK2 mutation is an acquired dominant course that clues to insistent production of hematopoietic stem cells with reduced apoptosis, leading to various clinical appearances related to a rise in the quantity of thrombocytes, erythrocytes, and granulocytes in inconstant groupings. There are inadequate data concerning the JAK 2 mutations in the Saudi inhabitants [4]. A retrospective cohort study was done in King Abdullah Specialized Children's Hospital (KASCH) and King Abdulaziz Medical City (KAMC), Riyadh. In the departments of pediatric and adult hematology and oncology, they found only 0.53% of mutation [5]. About 15% of mutations were found in myeloid leukemias in the Riyadh region in 2022 [4]. Additionally, 7.7% of JAK2 mutations were found in a study done in King Faisal Specialist Hospital and Research Center (KFSHRC), Saudi Arabia [6].

MATERIALS AND METHODS

This was a retrospective study. One hundred and twelve patients' data were collected from the molecular laboratory of the King Fahad Hospital of the University, Al Khobar, Saudi Arabia. The patients' data were collected from June 2022 through May 2023. Data, including gender, age, complete blood counts, and real-time PCR JAK2 V617F mutation qualitative assay, were taken from pre-established data collection sheets.

JAK2 V617F mutation patients' samples were assessed by Rotor-Gene Q instrument (Qiagen) using Ipsogen JAK2 MutaScreen EZ kit to detect JAK2 V617F/G1849T mutation. The principle of multiplexed assay based on Two TaqMan® probes was used. One probe was a perfect match to the allele 1 sequence (e.g. the wild-type allele) and the other probe was a perfect match to the allele 2 (e.g. the allele with a mutation). Each probe was labelled with a distinctive fluorescent dye at its 5' end (reporter), such as FAM (green) for the mutated allele or VIC (yellow) for the wild type allele, and contained a non-fluorescent quencher at the 3' end. The probes also contained a minor grove binder (MGB), permitting the use of shorter probes with greater stability, and thereby a more accurate allelic distinction.

During the extension phase of the PCR, the perfectly matched probe was cleaved by the 5' → 3' exonuclease activity of Taq polymerase, separating the reporter dye from the quencher, and thus releasing detectable fluorescence. The not perfectly matched probe will be displaced rather than cleaved by the Taq polymerase, and no reporter dye will be released. The fluorescence signal (VIC or FAM) generated was collected at the end of the PCR (endpoint) and immediately indicated the presence of the targeted sequence(s) in the specimen (wild-type allele, mutated allele, or both).

Full blood counts of patients' samples were processed by Alinity HQ analyzer. All laboratory tests followed quality control for normal and abnormal results for quality purposes. Hematology and molecular genetic labs of King Fahad Hospital of the University, Al Khobar, Saudi Arabia, are CAP accredited.

Data were recorded as excel sheets in the patients' files. Then, they were transferred to SPSS (Statistical Package for Social Sciences), version 22 (IBM Corp., Armonk, NY, USA), for analysis purposes. Frequency and percentages were calculated for prevalence of JAK 2 disease and gender, whereas lab test counts are presented in mean and standard deviations. Comparisons of lab results with Jak 2 status and gender were done using non-parametric Mann-Whitney test. p-values less than or equal to 0.05 were considered statistically significant.

RESULTS

Prevalence of the JAK 2 mutation

In order to investigate the first objective of this study, the prevalence of JAK 2 disease among 112 patients was found to be (n = 13) 12%.

Table 1. Gender distribution between JAK 2 patients.

	JAK 2		Overall	p-value
Gender	Positive	Negative		
Male	8 (10.4)	69 (89.6)	77 (68.75)	0.398
Female	5 (14.3)	30 (85.7)	35 (31.25)	

Table 2. Age distribution among JAK 2 patients.

JAK 2	Age				
	n	Range	Mean	Standard deviation	p-value
Positive	13	30 - 73	50.08	13.67	0.141
Negative	99	1.50 - 90	42.31	18.22	
Overall	112	1.50 - 90	43.21	17.87	

Table 3. Comparison of lab test counts of JAK2-positive and -negative patients.

Lab test	JAK2 status	Mean	Standard deviation	Range	p-value
WBC ($\times 10^3$ cells/µL)	positive	11.35	5.25	(3 - 18.1)	0.846
	negative	10.57	14.26	(0.9 - 137)	
RBC (10^6 cells/µL)	positive	5.80	1.73	(2.51 - 8.55)	0.002*
	negative	4.67	1.10	(0.57 - 7.27)	
Hgb (g/dL)	positive	14.62	3.90	(8 - 19.1)	0.075
	negative	12.78	3.41	(6.3 - 20.6)	
HCT (%)	positive	45.82	12.31	(26.2 - 63.3)	0.036*
	negative	38.89	10.94	(4.1 - 66.2)	
MCV (fL)	positive	81.85	18.90	(54.7 - 120)	0.658
	negative	83.50	11.59	(43.6 - 107)	
MCH (pg)	positive	25.99	5.29	(18.7 - 36.8)	0.346
	negative	27.12	3.83	(16.2 - 34.4)	
MCHC (g/dL)	positive	31.98	1.28	(30.2 - 34.3)	0.194
	negative	32.52	1.44	(29.3 - 39)	
Plt ($\times 10^3$ cells/µL)	positive	556.77	288.09	(177 - 999)	0.085
	negative	404.74	297.78	(24 - 1,443)	

*** - Significant at 0.05 level of significance.**

Prevalence of JAK 2 mutation in gender and age groups

To investigate the second objective of this study, the statistical analysis showed that out of 77 male patients, JAK 2 was found in (n = 8) 10%, and out of 35 females, the prevalence of JAK2 was (n = 5) 14%, which show that the female's ratio was higher than males', but the association was not found to be statistically significant (p = 0.398) (Table 1). Average age of JAK2-positive patients was 50.08 ± 13 years, with a range of 30 to 73 years, whereas in negative patients the average was 42.31 ± 18 (range 1.50 to 90) years, which was less than

Table 4. Comparison of blood counts in male and female JAK2-positive patients.

Lab test	Gender	n	Mean	Standard deviation	p-value
WBC ($\times 10^3$ cells/µL)	male	8	12.36	5.40	0.4
	female	5	9.72	5.11	
RBC (10^6 cells/µL)	male	8	6.43	0.96	0.098
	female	5	4.79	2.31	
Hgb (g/dL)	male	8	15.96	3.30	0.121
	female	5	12.48	4.15	
HCT (%)	male	8	49.85	11.38	0.142
	female	5	39.38	11.98	
MCV (fL)	male	8	77.74	16.27	0.342
	female	5	88.44	22.81	
MCH (pg)	male	8	24.90	4.61	0.369
	female	5	27.74	6.37	
MCHC (g/dL)	male	8	32.26	1.30	0.331
	female	5	31.52	1.25	
Plt ($\times 10^3$ cells/µL)	male	8	637.00	311.34	0.218
	female	5	428.40	214.18	

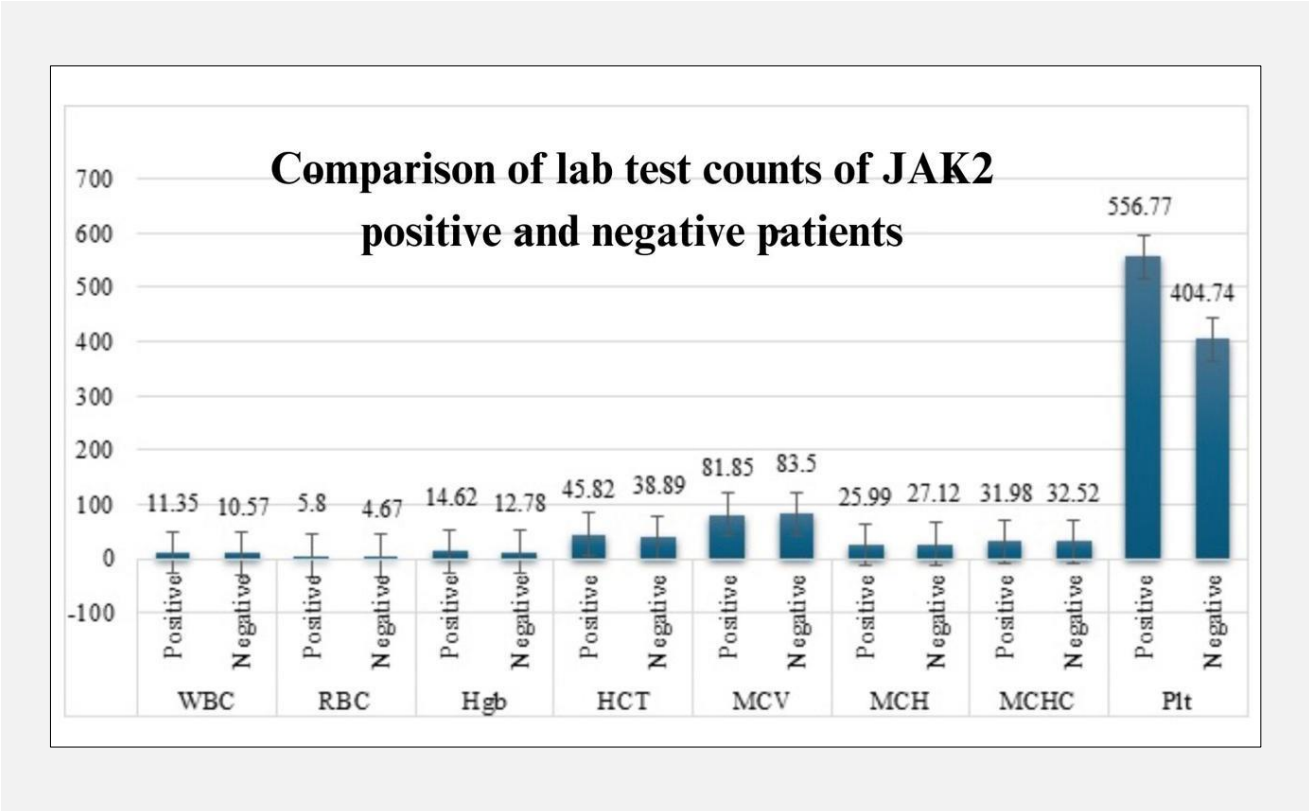

Figure 1. Comparison of lab test counts of JAK2-positive and -negative patients.

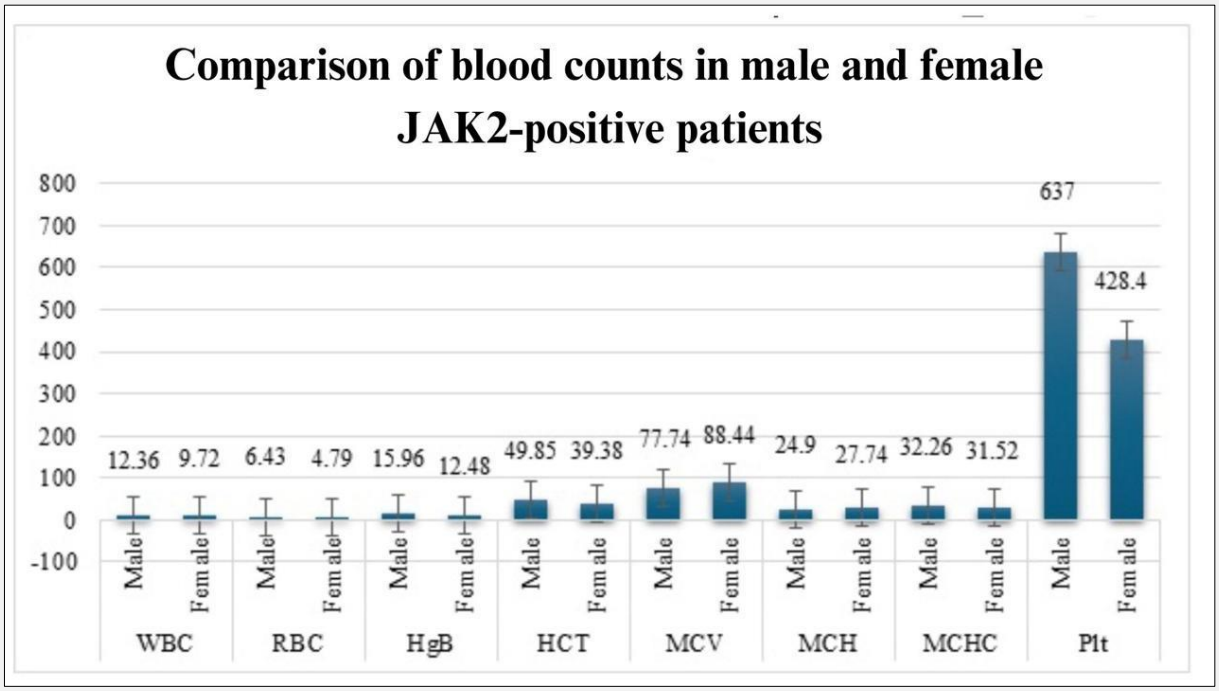

Figure 2. Comparison of blood counts in male and female JAK2-positive patients.

for the positive patients, but the difference was statistically nonsignificant (p = 0.141), as shown in Table 2.

Comparison of patients' complete blood count tests between the positive and negative JAK2 cases

Table 3 and Figure 1 show the comparison of average blood counts from the lab test between JAK2 patients. White blood cell count was relatively higher in the positive patients, but the difference was statistically nonsignificant (11.35 ± 5.25 vs. 10.57, p = 0.846). Similarly, the hemoglobin level among the positive patients was higher, 14.62 g/dL, but still not significantly higher (p = 0.075). However, red blood cell count in the JAK2 patients was significantly higher (5.80 ± 1.73) compared to the negative patients (4.67 ± 1.10) (p = 0.002). Similarly, the percentage of red blood cells measured by HCT test was also significantly higher among the JAK2-positive patients (45.82 ± 12.31 vs. 38.89 ± 10.9, p = 0.036) compared to the negative patients. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) counts were found higher in the JAK2-negative patients (MCV = 83.50 ± 11.5, MCH = 27.12 ± 3.83, and MCHC = 32.52 ± 1.44), but the difference was statistically nonsignificant (p = 0.658, 0.346, and 0.194, respectively).

The difference between male and female JAK2-positive patients' blood count was compared and is presented in Table 4 and Figure 2. WBC and hemoglobin level of male patients were found to be higher (12.36 ± 5.40 and 15.96 ± 3.30) than for the female patients, but the differences were statistically nonsignificant (p = 0.40 and 0.121, respectively). RBC, HCT, and platelets count were also found relatively higher among the male patients (RBC = 6.43 ± 0.96, HCT = 49.85 ± 11.38, and Plt = 637 ± 311.34) compared to positive females, yet again the differences were statistically nonsignificant (p < 0.05). None of the blood counts showed a significant difference between male and female positive patients (Table 4).

DISCUSSION

Janus kinases drive hematopoiesis and immunity, and aberrant JAK activation plays a crucial role in the pathogenesis of myeloid diseases. Several JAK mutations have been discovered, which cause JAK activation via a variety of mechanisms.

In the present study, the prevalence of JAK 2 V617 mutations in malignant and non-malignant tumors in the Eastern province of the Kingdom of Saudi Arabia was

observed using different hematological and molecular investigating tools.

The prevalence of JAK 2 mutation among 112 patients was found to be (n = 13) 12%. The lower prevalence was also found in the study done by Xu et al. (1%) in 2007 in China [7]. Similarly, Nielsen et al. showed lower JAK 2 mutation prevalence (1%) in 2011 [8]. Cordua et al. conducted a research study on a Danish general population and revealed that the JAK 2 mutation prevalence was 3.1% in 2019 [9]. In Egypt, Ebid et al. found the prevalence of the JAK 2 mutation was highest in patients with PV, where 56 out of 70 cases (80%) carried the mutation, followed by ET, with 6 out of 24 (25%), and IMF, with 2 out of 16 (12.5%) [10]. In 2012, Gari et al. revealed a higher prevalence of the JAK 2 mutation with 91% in PV, 40% in ET, and 25% in MF in Saudi Arabia, due to the long duration of their study (2001 - 2010) [11]. A retrospective cohort study was done in King Abdullah Specialized Children's Hospital (KASCH) and King Abdulaziz Medical City (KAMC), Riyadh, in the departments of pediatric and adult hematology and oncology; they found the prevalence of the JAK 2 mutation to be 0.53% [5]. Our result is consistent with a study done in Riyadh region in 2022, which showed the prevalence of the JAK 2 mutation to be 15% [4]. Additionally, 7.7% prevalence of the JAK 2 mutation was found in a study done in King Faisal Specialist Hospital and Research Center (KFSHRC), Saudi Arabia, which is in line with our result [6].

Gender-wise, 77 (69%) patients were male and 35 (31%) were female. Out of the 77 male patients, the JAK 2 was found in 10%, and out of the 35 females, the prevalence of JAK2 was 14%. These findings are consistent with a study done by AlGhasham et al. in 2014 [12].

The hemoglobin level (Hb), red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) among the JAK2-positive patients were higher compared to JAK2-negative patients, and these results are similar to the studies done by Tefferi and Pardanani in 2015, Syeed in 2019, and Malhan in 2014 [13-15]. Similarly, white blood cell count and platelet count were relatively higher in the positive patients, but the difference was statistically nonsignificant, and these results are similar to the results of Singdong et al., 2016, Rumi et al., 2014, Tefferi et al., 2014; Kim et al., 2015a; Kim et al., 2015b, and Malhan, 2014 [13,15-19].

CONCLUSION

JAK2 mutation testing will rapidly become a frontline test for individuals with a suspected diagnosis of an MPD, and the same genetic event can play a role in the pathogenesis of a wide spectrum of myeloid and lymphoid malignancies. These observations warrant a comprehensive search for activated tyrosine kinases in MPD'S and hematological malignancies, as there are likely additional unidentified genetic events with biological and therapeutic significance. Additionally, *in vitro* and *in vivo* studies are needed to determine the cause of the specificity of JAK2 V617F for myeloid and lymphoid diseases, as second mutations, host modifiers, differential cytokine receptor expression, and other factors may influence the ultimate phenotype of hematopoietic progenitors that acquire the JAK2 V617F mutation. Our data also suggest that different genetic events may lead to JAK-STAT pathway activation in different malignancies. Additional data are needed to have a better understanding of the JAK 2 mutation.

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Ethical Approval:

This study was approved by the Institutional Review Board of Imam Abdulrahman bin Faisal University Dammam IRB (log number: IRB-PGS-2023-11-355).

Declaration of Interest:

There exists no conflict of interest for any of the researchers.

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