

JAK2 V617F Mutation in Patients with Thrombosis

ABSTRACT

Background: Thrombosis is a major disorder with serious complications. The JAK2^{V617F} mutation results in constitutive phosphorylation of JAK2 and activation of the cellular proliferation cascade. **The present study aimed to** clarify the prevalence of JAK2^{V617F} mutation in patients with thrombosis. **Subjects and Methods:** This case-control study was conducted on 60 subjects subdivided into 2 groups: 30 healthy subjects as a control group (Group I) and 30 patients diagnosed with thrombosis (Group II). All subjects were subjected to full history taking, and clinical examination and investigated for Molecular detection of JAK2^{V617F} mutation, FV (Factor V Leiden) – PTH (Prothrombin), and MTHFR (Methylene tetrahydro-folate reductase). **Results:** There was a statistically significant difference among the studied groups as regards the JAK2V617F mutation (P value < 0.05, odds ratio = 41.216, 95% C.I. 95% = 2.302 - 738.034, relative risk = 25, 95% C.I. 95% = 1.547– 404.012). This mutation was more significantly associated with venous thrombosis than arterial thrombosis (P = < 0.05). **Conclusion:** This study showed a significant association between JAK2^{V617F} mutation and thrombosis with more significant evidence in the venous than the arterial thrombosis.

Keywords: JAK2^{V617F}; Mutation; Thrombosis.

INTRODUCTION

Thrombosis is a disease with multifactorial causes. Genetic and environmental risk factors increase the risk of developing blood clots. It is caused by the Virchow triad interaction (*venous stasis, hypercoagulability, and endothelial injury*) [1].

JAK2 is a cytoplasmic tyrosine kinase that has a role in the signal transduction of multiple hematopoietic growth factor receptors. In patients with splanchnic venous thrombosis JAK2^{V617F} mutation has been reported as a marker of occult myeloproliferative neoplasms (MPNs). Cytokine-independent myeloproliferative disorders, mobilization of blood cell progenitors, and spontaneous formation of endogenous erythroid colonies were linked to this mutation [2].

JAK2^{V617F} is an acquired mutation located on the short arm of chromosome 9. It causes the substitution of valine by phenylalanine at position 617 of the pseudo kinase domain in the JAK2 protein. This mutation is present in polycythemia vera (95%), primary

myelofibrosis, and essential thrombocythemia (50-60%) [3].

This study aimed to clarify the prevalence of JAK2^{V617F} mutation in patients with thrombosis and investigate how far it is added to the thrombophilia screen or not.

PATIENTS AND METHODS

This is a case-control study done on 30 patients with newly diagnosed venous and arterial thrombosis which were selected from the Internal Medicine Department at Tanta University Hospitals. Written informed consent was taken from all participants of this study and the ethical committee at Tanta University approved this study to be conducted at the Clinical Pathology Department

Sixty subjects were enrolled in this study and classified as; Group I; 30 healthy subjects as a control group (18 males and 12 females). Their ages range from (40 - 62) with a mean value of (49.33 ± 5.92). Group II; 30 patients diagnosed with thrombosis (20 males and 10 females).

Their ages range from (42 - 66) with a mean value of (50.37 ± 5.82). Thrombotic patients include; Venous thrombosis (8 cases with deep venous thrombosis (DVT), 8 cases with portal vein thrombosis (PVT), 4 cases with cerebral vein thrombosis (CVT), and 2 cases with mesenteric vein thrombosis (MVT) and Arterial thrombosis (8 cases with myocardial infarction (MI). Subjects with hereditary thrombophilia and those with acquired risk factors for thrombosis, e.g., pregnancy, surgery, and immobilization were excluded.

Specimen collection: 7 ml of venous blood was withdrawn under complete aseptic conditions from all participants and divided into two parts; each in a separate tube as follows:

1. 1.8 ml of blood was taken into plastic tubes containing tri-sodium citrate 1/9 by volume (0.1 ml citrate with 0.9 ml blood) for PT, PTT, protein C, protein S, and antithrombin III assays by automated coagulation analyzer [STAGO-CC3304A319].
2. 5 ml of blood was taken in EDTA (*ethylenediaminetetraacetic acid*) tube for the following:
 - 2 ml for complete blood picture by an automated blood cell counter (*ERMA PCE-210 N cell counter*), then for peripheral blood film examination.
 - 3 ml was used for DNA extraction for:
 - FV-PTH-MTHFR Strip Assay by amplification of factor V, prothrombin, and MTHFR gene sequences in vitro.
 - Molecular detection of JAK2^{V617F} mutation by real-time polymerase chain reaction (QuantStudio 5 Real-Time PCR System).

For genotyping, genomic DNA was extracted using G-spinTM total DNA extraction kit according to the manufacturer's protocol [4]. DNA concentrations were determined by a Nanodrop spectrophotometer for measuring the

absorbance at 260 nm (A260) using a quartz cuvette [5].

Molecular detection of JAK2^{V617F} mutation by real-time PCR for the extracted DNA after exclusion of hereditary thrombophilia was done using:(1) TaqMan Probes for JAK2^{V617F} mutation detection (*Willowfort*): Wild type; VIC-5'-TCTC-CACAGACACATAC-3'BHQ, Mutant type; FAM-5'-TCCACAGAAACATAC3'-BHQ. (2) Primers flanking the mutant region: forward; 5'-AAGCTTTCTCACAAGCATTGGTTT-3', reverse; 5'-AGAAAGGCATTAGAAAGCCTGTAGTT-3', (3) HERA qPCR Kit (*Willowfort, WF1030200X*) and deionized nuclease-free water.

QuantStudio 5 Real-Time PCR System was used with a reaction mixture of 20 µl: [10 µl of HERA qPCR Master Mix, 1 µl of Primer (forward), 1 µl of Primer (reverse), TaqMan Probes: 0.5 µl of wild type and 0.5 µl of mutant type, 3 µL of DNA template (sample) and 4 µL of Nuclease free water). It was programmed for Pre-cycling heat HERA enzyme activation of DNA polymerase at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 10 seconds then annealing at 60°C for 30 seconds, and finally extension at 72 °C for 30 seconds.

Thrombophilia screening: FV-PTH-MTHFR Strip Assay was done according to the manufacturer's protocol by amplification of factor V, prothrombin, and MTHFR gene sequences in vitro from the pre-extracted genomic DNA. Plasma protein C and protein S and antithrombin III levels were assayed by the automated coagulation analyzer [STAGO-CC3304A319] by the colorimetric method for protein c [6] and antithrombin III [7] and by the immuno-turbimetric method for Protein S [6].

STATISTICAL ANALYSIS

In addition to the descriptive data, statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS) STATISTIC

VERSION 21 PROGRAM. Data were expressed as Range, mean \pm standard deviation (SD), and analyzed using the Chi-square (χ^2) test, the student's t-test, and Fisher's exact test to assess the significance of the difference between different parameters. $P < 0.05$ was accepted as significant.

RESULTS

Laboratory assessments of the measured parameters in the different submitted groups are presented in the following tables and figures:

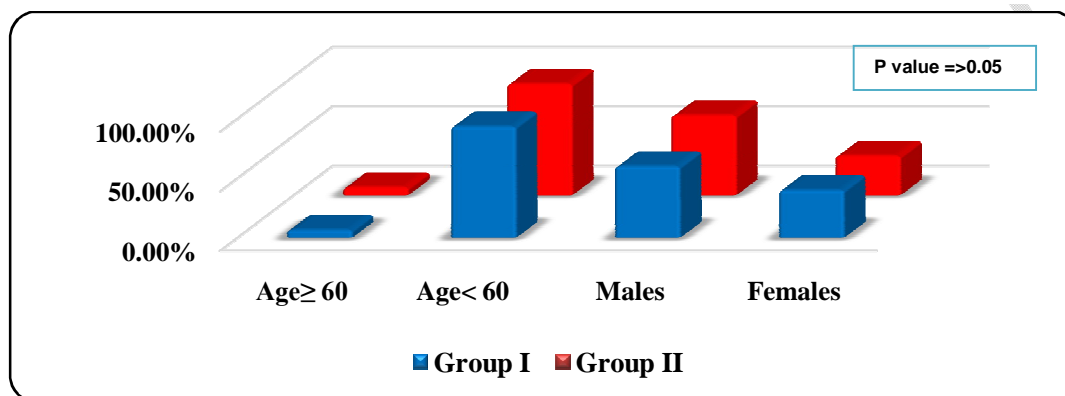


Figure [1]: Age and sex distribution among the studied groups.

- There was a statistically non-significant difference among the studied groups as regards age and sex.

TABLE (1): - Demographic and clinical characteristics of patients with thrombosis

	Group II (No=30)		Chi-square (χ^2) test	P Value
	Wild type (No=18)	Mutant JAK2 ^{V617F} (No=12)		
			Fisher exact test	
Age				
\geq 60	1 (5.6%)	1 (8.3%)	0.09	0.765
< 60	17 (94.4%)	11 (91.7%)		
Sex			2.5	0.114
Male	14 (77.8%)	6 (50%)		
Female	4 (22.2%)	6 (50%)		
Hb (g/dl)			15.63	0.00008
\geq 15	1 (5.6%)	9 (75%)		
< 15	17 (94.4%)	3 (25%)		
TLC ($10^3/\text{mm}^3$)			6.91	0.0086
\geq 10	8 (44.4%)	11 (91.7%)		
< 10	10 (55.6%)	1 (8.3%)		
Platelets ($10^3/\text{mm}^3$)			0.0001	< 0.05
\geq 300	5 (27.8%)	12 (100%)		
< 300	13 (72.2%)	0 (0%)		
Splenomegaly			10.21	0.0014
+ve	3\18 (16.7%)	9\12 (75%)		
-ve	15\18 (83.3%)	3\12 (25%)		
Thrombosis			0.01	< 0.05
Venous	10\18 (55.6%)	12\12 (100%)		
Arterial	8\18 (44.4%)	0\12 (0%)		

- There was a significant difference among the wild and mutant JAK2^{V617F} patients in the thrombotic group as regard hemoglobin (g/dl), total leukocyte count ($10^3/\text{mm}^3$), platelets ($10^3/\text{mm}^3$) count

splenomegaly (P value = 0.00008, 0.0086, < 0.05 and 0.0014) respectively, on the other hand, there was no effect of age and gender (P value = 0.765 and 0.114) respectively. It also showed that JAK2^{V617F} mutation was more significantly associated with venous thrombosis than arterial thrombosis (P value = < 0.05).

TABLE (2): - Clinical data for patients with JAK2^{V617F} mutation

	Age	Sex	Site	Venous\arterial	Splenomegaly	Hb gm/dl	WBCs (X 10 ³)	Platelets (X 10 ³)
Mutant Homozygous	52	♂	PVT	Venous	+ve	20.5	19	590
Mutant Homozygous	50	♀	PVT	Venous	+ve	18	11.5	666
Mutant Homozygous	55	♂	PVT	Venous	+ve	17.5	13.5	609
Mutant Heterozygous	48	♂	PVT	Venous	+ve	17	18	400
Mutant Heterozygous	50	♀	PVT	Venous	+ve	15	15	520
Mutant Heterozygous	66	♂	PVT	Venous	+ve	15	13	490
Mutant Heterozygous	49	♀	PVT	Venous	+ve	14.5	14.5	710
Mutant Heterozygous	48	♀	MVT	Venous	+ve	14.8	19	660
Mutant Heterozygous	43	♀	MVT	Venous	+ve	15	21	620
Mutant Heterozygous	48	♂	CVT	Venous	-ve	17	11.1	510
Mutant Heterozygous	55	♀	CVT	Venous	-ve	14.2	20	430
Mutant Heterozygous	44	♂	CVT	Venous	-ve	15	8.5	457

- The clinical data for patients with JAK2^{V617F} mutation, [30% heterozygous (9 cases) and 10% homozygous (3 cases)], there were higher levels of hemoglobin and platelets count in mutant homozygous patients if compared with mutant heterozygous patients, also there was a high prevalence of venous thrombosis (PVT) and splenomegaly in patients with JAK2^{V617F} mutation (both heterozygous and homozygous).

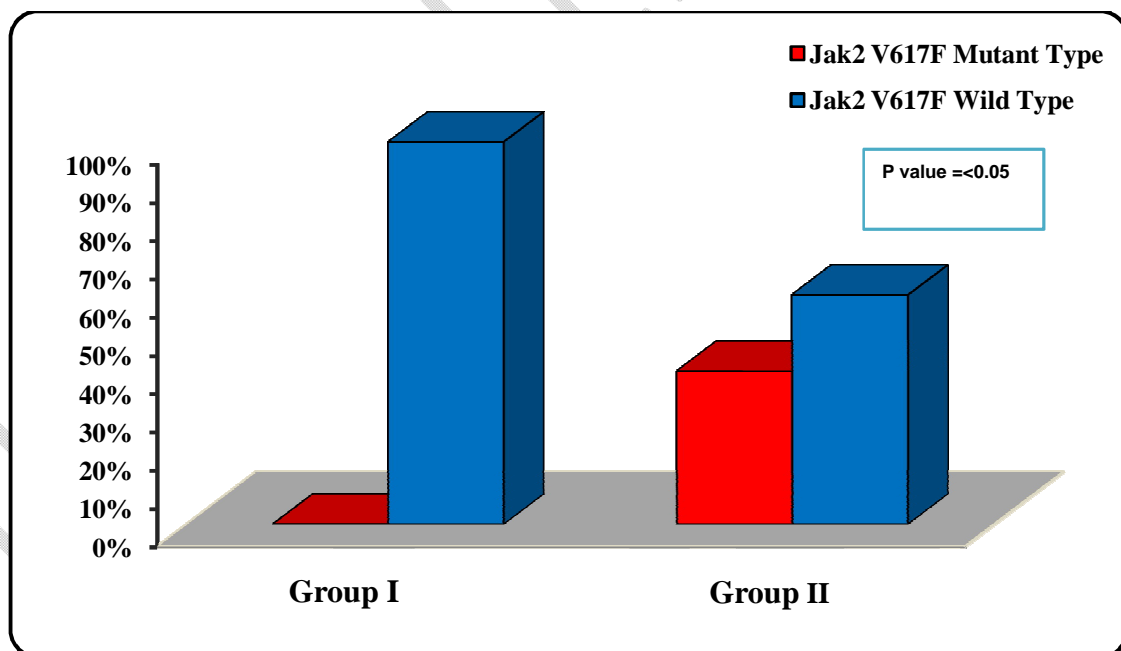


Figure [2]: JAK2^{V617F} mutation frequency distribution among the studied groups.

- There was a statistically significant difference among the studied groups as regards the JAK2^{V617F} mutation (P value < 0.05).

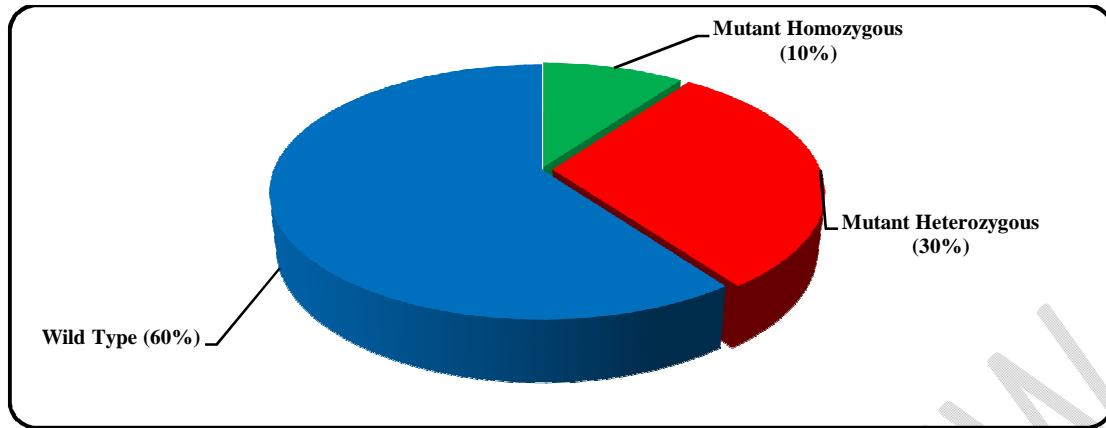


Figure [3]: JAK2^{V617F} mutation frequency distribution among the thrombotic group (Group II).

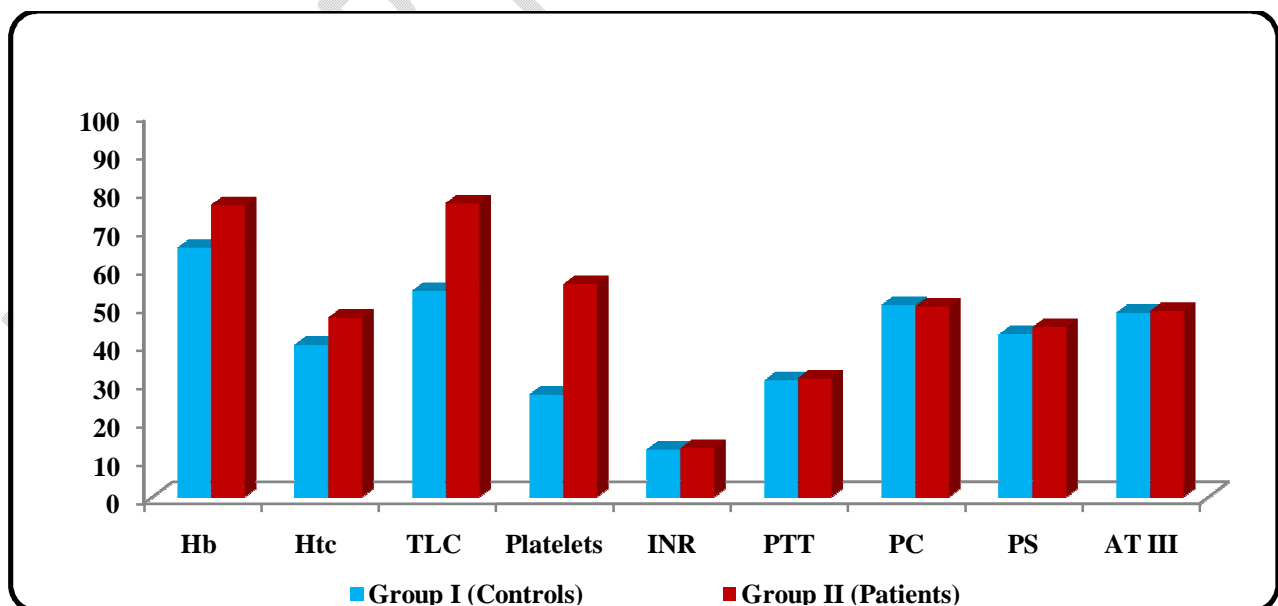
- The frequency distribution of JAK2^{V617F} mutation among the thrombotic group (Group II) was 12 (40%) thrombotic patients with Mutant JAK2^{V617F} [30% heterozygous and 10% homozygous] while 18 (60%) thrombotic patients were wild type.

TABLE (3): - Odds ratio (OR) and relative risk (RR) calculation

	Observation	95% Confidence Intervals		P value
		Low limit	Upper limit	
Odds ratio (OR)	41.216	2.302	738.034	0.012
Relative risk (RR)	25	1.547	404.012	0.024

• P value < 0.05 = significant.

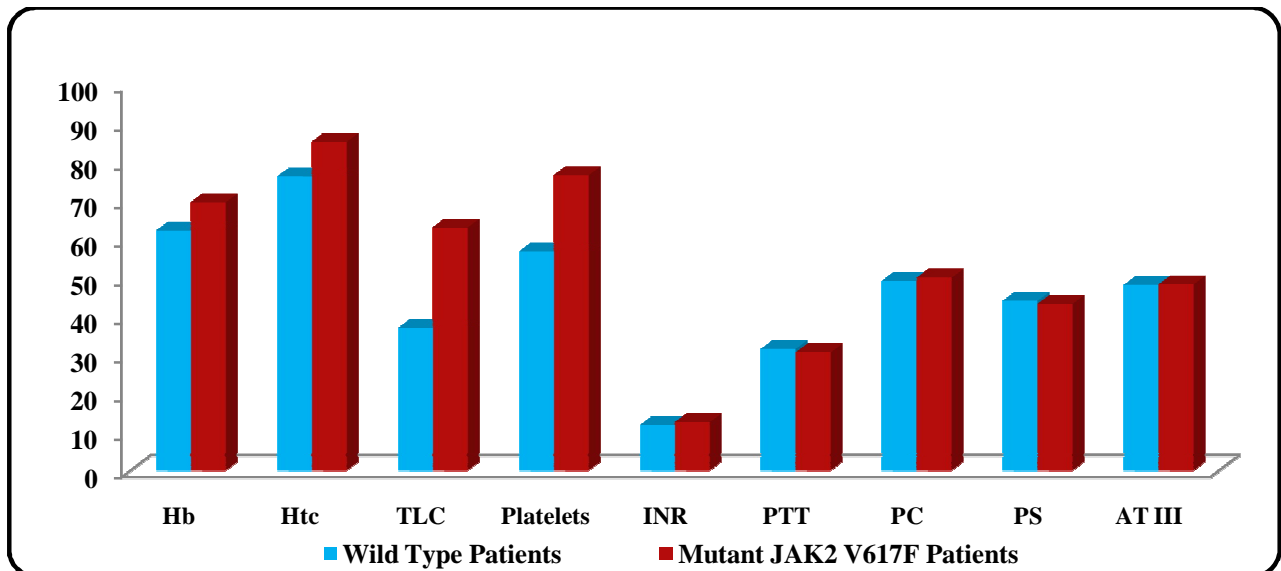
- There was a 41.216 times higher likelihood of having the JAK2^{V617F} mutation in patients with thrombosis compared to the control group (odds ratio = 41.216, 95% C.I. 95% = 2.302 – 738.034) with a significant level (p= 0.0115). Also, there was a relative risk of 25 meaning that people with JAK2^{V617F} mutation would be twenty-fifth as likely to contract the disease (thrombosis) (relative risk = 25, 95% C.I. 95% = 1.547– 404.012) with a significant level (p= 0.024).



Abbreviations: Hb = Hemoglobin, TLC = Total leukocyte count, INR = International Normalized Ratio, PTT = Partial Thromboplastin Time, PC = Protein C, PS = Protein S and AT III = Antithrombin III.

Figure [4]: Comparison between the studied groups as regard the different laboratory parameters

- As regards the mean value for Hemoglobin level (g/dl), Hematocrit percent (%), Total Leukocyte Count ($10^3/\text{mm}^3$), and Platelets count ($10^3/\text{mm}^3$), the present study revealed a significant increase in the thrombotic patient group (Group II) if compared to the control group (Group I), P values = (0.0031, 0.0038, < 0.0001 and 0.0067) respectively.



Abbreviations: Hb = Hemoglobin, TLC = Total leukocyte count, INR = International Normalized Ratio, PTT = Partial Thromboplastin Time, PC = Protein C, PS = Protein S and AT III = Antithrombin III.

Figure [5]: Comparison between the wild and mutant JAK2^{V617F} patients in the thrombotic group (Group II) with regard to the different laboratory parameters.

- As regards the mean value for hemoglobin level (g/dl), hematocrit percent (%), total leukocyte count ($10^3/\text{mm}^3$), and the platelets count ($10^3/\text{mm}^3$), the present study revealed a significant increase in the mutant JAK2^{V617F} thrombotic patients if compared to the wild type thrombotic patients, P values = (0.0030, 0.0035, 0.0037 and < 0.0001) respectively.

DISCUSSION

The development of thrombosis occurs through the interaction of a combination of factors, such as platelet activation, changes in viscosity and shear stress, increased level of red blood cells, changes in inflammatory cytokines, and activation of white blood cells. The integrity of the endothelium and coagulation factors play a cornerstone in thrombus formation [8].

Janus kinase is a family that consists of 4 members: JAK1, JAK2, JAK3, and TYK2. The role of JAK2 in signal transduction may be disrupted by JAK2 alterations [9].

The mutation in the JAK2 gene (V617F) has been detected in patients with

unexplained thrombosis or thrombosis in unusual sites [10]. The identification of the JAK2^{V617F} mutation appears to be useful in diagnosing patients with idiopathic PVT and may overcome the difficulties of diagnosing MPN in this setting [11].

The present study showed that there was a statistically non-significant difference between the studied groups as regards sex and age. These results agreed with *Ma* [12] who stated that there were no significant differences between the age or sex distribution between the two groups included in his study. Also, *Li et al.* [13] study agreed with the present study and stated that there was no significant difference found in the age

between patients with and without thrombosis.

The current study showed that there was a statistically significant difference between the studied groups as regards the JAK2^{V617F} mutation (P value < 0.05), with significantly higher rates of the JAK2^{V617F} mutation among the thrombotic patients, suggesting that JAK2^{V617F} mutation may contribute to thrombosis.

In the present study clinical data for patients with JAK2^{V617F} mutation, [30% heterozygous (9 cases) and 10% homozygous (3 cases)], showed higher levels of hemoglobin and platelets count in mutant homozygous patients if compared with mutant heterozygous patients, also there were significantly high prevalence of venous thrombosis (PVT) more than with arterial thrombosis (P value = < 0.05) and splenomegaly in patients with JAK2^{V617F} mutation (both heterozygous and homozygous).

These results agreed with the study of *González-Montero et al.* [9] who mentioned that half of patients (60 cases) with mesenteric and/or portal venous thrombosis diagnosis over a 7-year period showed no secondary cause and had JAK2^{V617F} mutation.

Dentali et al. [14] agreed with the present work and stated that there was a high prevalence of the JAK2^{V617F} mutation among patients with splanchnic vein thrombosis. Patients with splanchnic vein thrombosis that show no additional signs of hematologic disease other than the JAK2^{V617F} mutation at the time of thrombosis have an overt MPN development rate that is high during the follow-up.

Also, the current study results agreed with *Regina et al.* [15] who concluded that JAK2^{V617F} mutation has been found in up to

40% of patients with splanchnic venous thrombosis. In contrast, the prevalence of the mutation has been reported to be low and did not exceed 1.5% in patients with non-splanchnic venous thrombosis and without overt MPNs.

Li et al. [13] investigated the correlation between the JAK2^{V617F} mutation and thrombosis, their results agreed with current work and concluded that JAK2^{V617F} is an important diagnostic standard in patients with MPN, and has an important clinical significance. We showed that the mutation was found in 44 of 68 patients with MPN (64.7%), including 24 ET, 17 PV, and 3 PMF patients. These results indicate the high incidence rate of the JAK2^{V617F} mutation in patients with MPN. JAK2^{V617F} participates in the development of MPN and is correlated with thrombotic disease in MPN patients.

Finazzi et al. [16] reported that the incidence of thrombus in patients with the JAK2^{V617F} mutation is higher than that in patients without this mutation. *Kralovics et al.* [17] also reported that patients expressing the JAK2^{V617F} mutation are more susceptible to complications such as thrombosis.

Thrombotic events could occur mainly through an increase of direct activation or endothelium adhesion of blood cells or an over-expression of the JAK2 mutation in endothelial cells [18].

There are many studies showing an association of high JAK2^{V617F} allele burden in MPNs with thrombosis [19, 20, 21 & 22].

The prevalence of the JAK2^{V617F} mutation in patients with venous thrombosis had focused on by several studies. Individuals lacking clinical evidence of MPN showed JAK2^{V617F} positivity ranging from 12% to

74% in splanchnic vein thrombosis [11, 15, 23, 24, 25, 26, 27, 28, 29 & 30].

Furthermore, JAK2^{V617F} mutation was evaluated by other studies in patients with arterial thrombosis (acute myocardial infarction, ischemic stroke, and peripheral arterial thrombosis) and concluded absence or very low prevalence (0.7 – 1%) of JAK2^{V617F} [31, 32, 33, 34 & 35].

535 patients with arterial thrombosis were evaluated in six studies, and a very low prevalence of JAK2^{V617F} was observed (1.1%; 95% CI, 0.40– 2.29%) [36]. So, the JAK2^{V617F} mutation was not a risk factor for arterial thrombosis, and routine evaluation should not be done for these patients.

In the calculation of odds ratio and relative risk, the current study showed that there was a 41.216 times higher likelihood of having the JAK2^{V617F} mutation in patients with thrombosis compared to the control group (odds ratio = 41.216, 95% C.I. 95% = 2.302 – 738.034) with a significant level (p= 0.0115). The current study also showed that there was a relative risk of 25 meaning that people with JAK2^{V617F} mutation would be twenty-fifth as likely to contract the disease (thrombosis) (relative risk = 25, 95% C.I. 95% = 1.547– 404.012) with a significant level (p= 0.024).

Dentali et al. [14] systematic review evaluated the prevalence of the JAK2^{V617F} mutation (heterozygote and homozygote) in patients with venous thrombosis in usual and unusual sites. Twenty-four studies were included in the systematic review: six case-control studies and 18 retrospective cohort studies, with a total of 3508 patients. lower limb deep venous thrombosis, splanchnic vein thrombosis, pulmonary embolism, retinal vein occlusion, and CVT were the thromboembolic

events observed. The results of this meta-analysis were confined to 4 case-control studies and showed a strong association between JAK2^{V617F} and splanchnic vein thrombosis (OR 53.98; 95% CI, 13.1-222.45). So, JAK2^{V617F} is suggested as a strong predictor of the diagnosis of latent MPN in this situation. Considering that MPNs are among the most common acquired factors associated with splanchnic vein thrombosis [37], it seems reasonable to include JAK2^{V617F} testing in the routine evaluation of patients with splanchnic vein thrombosis.

Splanchnic vein thrombosis (SVT) cases are thought to have what is frequently termed ‘latent’ or ‘masked’ MPD [15].

Speletas et al. [38] concluded that Homozygous JAK2^{V617F} mutated patients showed significantly more complication and thrombotic risk than heterozygous JAK2^{V617F} mutated patients.

In contrast to the current study results, *Kumar et al.* [3] conducted a prospective case-control study screening of JAK2^{V617F} mutation and other thrombophilic risk factors on 52 cases of SVT, and 40 controls. From the total of 52 patients; 2 had MVT, 10 had BCS, and 40 were PVT/EHPVO. The JAK2^{V617F} mutation was seen in two cases. JAK2^{V617F} mutation was observed in 3.8% of SVT patients (lower frequency of mutation).

Singh et al. [39] study, showed a lower prevalence of JAK2^{V617F} mutation (0.67%). This was in concordance with a few other studies worldwide which found that this mutation was either absent or was present at a very low frequency (1%-2%) in patients with thrombosis [32, 33, 40, 41 & 42].

Also, Studies that evaluated the JAK2^{V617F} mutation in patients with venous thrombosis have found a very low prevalence

(0.1-3%) of JAK2^{V617F} [15, 25, 32, 33, 41, 42, 43 & 44].

Dentali et al. [14] also found only 8 presented the mutation of 248 patients with CVT, with a mean prevalence of 2.57% (95% CI, 0.97 – 4.91%). Although these results did not reach statistical significance, they do suggest an association.

Some authors speculate that, given the rarity of this condition, the small number of patients included in the studies [45]. The most important reason for this variety could be the difference in sensitivity of the techniques used for the detection of JAK2^{V617F} mutation [38].

All these studies further added to the dilemma of whether to include the JAK2^{V617F} mutation screening test in routine thrombophilia screening or not [46 & 47].

The present study revealed a significant increase in hemoglobin level (g/dl), hematocrit percent (%), total leukocyte Count ($10^3/\text{mm}^3$), and the Platelets count ($10^3/\text{mm}^3$) in the mutant JAK2^{V617F} thrombotic patients if compared to the wild type of thrombotic patients, with P values = (0.0015, 0.0018, 0.0019 and < 0.00001) respectively.

This agreed with *Ma* [12] who showed that the mean platelet count was significantly higher in patients with the JAK2^{V617F} mutation than in patients without the mutation with p-value = 0.013. At the same time, there were no significant differences in white blood cell count or hemoglobin levels between the two groups which were against the present work results at these points. JAK2^{V617F} mutation occurs frequently in CVST patients with thrombocytosis, approximately half (47.8%) of the CVST patients with thrombocytosis had the JAK2^{V617F} mutation,

suggesting that this mutation is prevalent among these patients.

Edelmann et al. [48] also agreed with the present study and concluded that the JAK2^{V617F} mutation can lead to increased platelet count.

The present study results were also in agreement with *Falanga et al.* [49] who observed higher platelet and leukocyte activation in JAK2^{V617F}-positive patients. Also, *Arellano-Rodrigo et al. and Passamonti et al.* [55 & 51] suggest the association of JAK2^{V617F} with increased platelet and leukocyte activation. Hence, in clinical practice, physicians should be alert to patients with higher platelet counts. Clinical management of these patients may be facilitated by early detection of the JAK2^{V617F} mutation.

In contrast to the current work, *Goodyer et al.* [52] found a normal peripheral blood count in seven (44%) of 17 cases with JAK2^{V617F}-positive patients. Also, *Regina et al.* [15] stated that the two groups (patients and controls) were similar in regard to the peripheral blood count parameters.

Thrombosis in JAK2^{V617F} mutation with an absence of erythrocytosis or thrombocytosis could potentially be caused by a possible JAK2-mediated effect with increased platelet and leukocyte activation [11].

In conclusion, this study showed that JAK2^{V617F} mutation was prevalent in 40% of patients with thrombosis and this is more evident in venous than arterial thrombosis so, the diagnostic value of JAK2^{V617F} mutation in the routine investigation for thrombophilia is beneficial in patients with splanchnic vein thrombosis.

Further studies including a large number of patients are needed for comprehensive statistical analysis.

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