JAK2-V617F Mutation and BCR-ABL Rearrangement in Chronic Myeloproliferative Neoplasms

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Abstract: Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell that leads to overproduction of mature, functional blood cells in the bone marrow. Chronic myeloid leukemia (CML) is a myeloproliferative disorder that is defined by its causative molecular lesion, the BCR-ABL fusion gene, while vast numbers of Philadelphia negative MPNs are characterized by the presence of JAK2 V617F mutation. Detection of JAK2 V617F mutation so convincingly establishes the presence of a clonal disorder. The present work aimed to study the expression of JAK2 V617F mutation by real-time PCR in chronic myeloproliferative disorder patients as well as the study the BCR/ABL gene rearrangement by FISH. **Subject and Methods:** The subjects of this study consist of 40 patients of newly diagnosed MPNs and 10 apparently healthy individuals serving as a control group. The patients were subjected to routine laboratory investigation, detection of BCR/ABL fusion gene by FISH technique and detection of JAK2 mutation expression in MPNs by real time PCR. **Results:** BCR/ABL fusion gene was detected in 100% of CML patients, while it was absent in other MPNs. JAK2 mutation was detected in (80%) of polycythemia vera (PV) cases, (60%) of essential thrombocythemia (ET) cases, (70%) of myelofibrosis (MF) cases and it was absent in CML. **Conclusion:** JAK2 V617F mutation is a risk factor for MPNs to develop approving the strong association between the JAK2 mutations and these disorders, which when present, can definitively confirm the diagnsosis. JAK2 mutation testing should be considered as a front-line screening test for suspected MPNs, and its use as a first-intention diagnostic test may spare some patients further investigations.
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1. Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell that leads to overproduction of mature functional blood cells (red blood cells, platelets and white blood cells) in the bone marrow. The cardinal features of the three main myeloproliferative disorders are an increased red-cell mass in Polycythaemia Vera, a high platelet count in Essential Thrombocythemia, and bone marrow fibrosis in Idiopathic Myelofibrosis. These three disorders share many characteristics, including marrow hypercellularity, a tendency to thrombosis and hemorrhage, and a risk of leukemic transformation on the long term. Chronic myeloid leukemia is a myeloproliferative disorder that is defined by its causative molecular lesion, the *BCR-ABL* fusion gene, which most commonly results from the Philadelphia translocation (Ph) [(1)].

JAK2 "Just another kinase" or "Janus kinase" is a tyrosine kinase involved in cytokine receptor signaling [(2)], it has a critical role in mediating the signaling pathways of thrombopoeitin (TPO), erythropoietin (EPO) and other cytokines involved in haemopoiesis. JAK2 is activated by the binding of these ligands to cytokine receptors [(3)]. The JAK family of tyrosine kinases includes JAKs 1-3 and tyrosine kinase 2 (TYK2). JAKs are expressed equally in all cells with the exception of JAK3, which is found only in hematopoietic cells [(4)]. The most important structural domain of the JAK molecule is the enzymatic kinase domain (JH1), which phosphorylates tyrosine on target proteins. The pseudokinase domain (JH2) has no enzymatic activity and is thought to inhibit the kinase domain, while the FERM domain is important in regulating binding of the JAK proteins to cytokine receptors[(3)].

The acquisition of a mutation in the Janus kinase 2 (JAK2) genes by hematopoietic cells has been described as a genetic, defect underlying myeloproliferative disorders. The mutation leads to constitutive activation of JAK2, a tyrosine kinase involved in cytokine receptor signaling [(2)]. So the impact of the JAK2 V617F mutation on the cytokine signaling pathways suggests that it plays an important role in the pathogenesis of MPNs and represents a

major breakthrough in molecular understanding of the myeloproliferative disorders (MPDs) that may have significant implications for diagnosis and treatment [4].

Janus kinase signaling is activated in hematological malignancies by a number of mechanisms including the down regulation of negative regulators of JAK-STAT pathways, amplification of the JAK2 locus and the involvement of JAK2 in chromosomal translocations

G-T mutation results in phenylalanine being substituted for valine at position 617 (V617F) in the pseudokinase/JH2 domain and results in a protein with increased kinase activity and hyper-responsiveness to cytokine signaling [5].

There is a much higher frequency of JAK2 mutation in PV while it is less frequent in IMF and ET [6]. The recently discovered JAK2 V617F point mutation, found in 50-60% of ET patients, has been reported to be associated with a higher risk of thrombotic events[7]. The mutation has been screened for in a number of other hematological malignancies and was found in some cases of atypical MPD [8], in a subset of patients with MDS perhaps in association with 5q- and rarely in AML unless it is secondary to a previous MPD [9]. No cases have been described in lymphoid malignancies[10] although a distinct mutation, JAK2 L611S, was discovered in one case of pre-B-ALL during a screen for JAK2 mutations using denaturing high-performance liquid chromatography [11]. The discovery and the study of JAK2 V617F mutation represent a major advance in the molecular understanding of MPNs that may have implications for diagnosis and treatment. JAK2 V617F, a somatic point mutation that leads to constitutive JAK2 phosphorylation and kinase activation, has been incorporated into the WHO classification and diagnostic criteria of myeloid neoplasms [12]. The discovery of the JAK2 V617F mutation in the classical myeloproliferative neoplasms (MPNs) essential thrombocytosis, polycythemia vera, and primary myelofibrosis has ushered in a new era of scientific discovery in these diseases, resulting in a molecular classification and an improved understanding of disease pathogenesis[13].

Aim of work:

The present work aimed to study the expression of JAK2 V617F mutation by real-time PCR and study the BCR/ABL gene rearrangement by FISH in different MPNs and report their prevalence in Egyptian patients.

2. Subjects and Methods

Subjects:

The subjects of this study were selected from Hematology/Oncology Unit of Internal Medicine Department, Tanta University and National Cancer Institute (NCI) Cairo University. They were classified into the following groups:

Group I: Consists of 10 apparently healthy individuals matched in age and sex with the patients group to serve as a control group.

Group II: Consists of 40 patients of newly diagnosed MPNs (10 patients were PV, 10 were ET, 10 were MF and 10 were CML).

Methods:

The patients were subjected to the following:

I-Detailed history and thorough clinical examination searching for important signs of prognostic significance mainly pallor, purpura, bleeding, hepatomegaly, splenomegaly, lymphadenopathy and fever.

II-Radiological study mainly abdominal sonar and CT for detection of clinically undetected organomegaly or lymphadenpathy.

III-Laboratory investigation including: Complete blood count, B.M. aspiration and/or biopsy, LDH estimation, cytochemical staining (LAP score), conventional cytogenetic, detection of BCR-ABL rearrangement by FISH and detection of JAK2 mutation by allele specific real Time PCR.

Informed written consent was taken from every patient and control before enrollment in the study. The research was approved by the ethical committee of research of Tanta University.

Sampling:

eight ml venous blood was collected from every patient and control under complete aseptic condition and divided: 1 ml put in EDTA vacutainer tube for CBC; 3 ml put in plain vacutainer tube for serum LDH; 2ml in EDTA vacutainer tuber for real time PCR to study JAK2 mutation and 2 ml put in vacutainer tube containing non-preservative heparin for study of BCR-ABL fusion gene by FISH.

Detection of JAK2 mutation by real time PCR:

1- DNA was extracted from the venous blood using QIA amp DNA blood minikit from Qiagen (Applied biosystem, step I version).

2- The putative short fragment spanning the mutation site of JAK2 V617F mutation was amplified using real time PCR with optimized primers and probes mix. The primers flanking the mutant region included:

F.primer 5'-AAGCTTTCTCACAAGCATTTGGTTT-3'.

R.primer 5'-AGAAAGGCATTAGAAAGCCTGTAGTT-3'

They were employed together with Taqman probes which were specific for either:

Wild type:

VIC-5'-TCTC-C ACAGACACATAC-3'MGB.

Or mutant JAK2 allele:

FAM-5'-TCCACAGAAACATAC-3'-MGB.

Cell culture and *in situ* hybridization:

PB and/or BM specimens were cultured for 48 and 72 hours at 37°C in RPMI medium supplemented with 10% fetal bovine serum without the addition of any mitrogen (unstimulated). Colcemid (0.02ug/ml) was added to the cultures 30 minutes before harvest. After 30 minutes of hypotonic treatment with 0.075M KCl, the cells were fixed with methanol and acetic acid (3:1) and cells were made into slide preparations.

FISH assay: was performed according to manufacture's instructions, hybridization mixture (10ul) was then applied to each slide, which was cover slipped and sealed. Hybridization solution contained (hybridization buffer, purified water, and the specific probe). Specific probe (LSI) BCR-ABL dual colour dual fusion translocation probes (Vysis, Inc, Downers Grove, TL 60515 USA) for detection of t(9;22)(q34,q11) was used. Hybridization was performed for 10 hours at 37°C in a humidified chamber. Post hybridization washes consisted of rinses in 0.4x SSC at 37°C and 2xSSC at room temperature. Finally, nuclei were counter stained with DAPI. Cells were analyzed under a fluorescence microscopes equipped with Quips spectra vision hardware and software.

Statistical methods:

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). A p-value < 0.05 was considered significant and of < 0.001 was considered highly significant.

3. Results

The subjects of this study were classified into the flowing groups: **Group I:** Consisted of 10 apparently healthy individual serving as a control group. **Group II:** Consisted of 40 patients of newly diagnosed MPNs which were classified into:

Group A: 10 are PV. Group B: 10 are ET. Group C: 10 are MF. Group D: 10 patients are CML. Table (1) shows insignificant difference between the control individuals and the MPNs patients as regards to age and sex (Table 1).

As regards the laboratory findings in the studied groups, Hb concentration: the comparison revealed statistical significant difference between the two groups (p=0.041). WBCs count: the comparison revealed statistical significant difference between the two groups regarding JAK2 mutation (p=0.012). Platelets count: the comparison revealed statistical significant difference between the two groups regarding JAK2 mutation (p=0.028). LAP Score: the comparison revealed highly statistical significant difference between the two groups regarding (p=0.01) (Talbe 2). Serum LDH level: the comparison revealed highly statistical significant difference between the two groups regarding with p-value 0.001 (Table 3).

Table (1): Distribution of the studied cases according to age and sex

		Control N =(10)	CMPNs patients N=(40)	*p*. value
Age	**Range**	37-45	33-48	0.639
	Mean ±SD	41.10±3.24	40.42±3.08	
Sex	**Male (N%)**	5(50%)	26(65%)	0.995
	Female (N%)	5(50%)	14(35%)	

Table (2): Important clinical data of the studied cases compared with the control

Clinical data		Control N =(10)	CMPNs patients N=(40)	*p*. value
Splenomeglay N (%)	**Present**	0(0%)	32(80%)	**0.001**
	Absent	10(100%)	8(20%)	
DVT N (%)	**Present**	0(0%)	2(5%)	**0.046**
	Absent	10(100%)	38(95%)	

Table (3): Laboratory findings of the studied groups

Laboratory Data		Control N =(10)	CMPNs patients N=(40)	*p*. value
HB(g/dl)	**Range**	10.50-18.5	6.48-20.55	**0.041**
	Mean ±SD	14.63±3.26	11.78±4.16	
TLC(x10^3/cmm)	**Range**	7.20-13.84	3.48-79.45	**0.012**
	Mean ±SD	11.32±4.32	23.3±24.2	
Platelets (x10^3/cmm)	**Range**	190-620	131.2-1124.9	**0.028**
	Mean ±SD	346.5±125.03	494.4±334.2	
LAP score N(%)	**Decreased**	-	8 (20%)	**0.01**
	Normal (12-120)	8(80%)	18(45%)	
	Hight(>120)	2(20%)	14(35%)	
Serum LDH	**Range**	200-480	236-1200	**0.001**
	Mean + SD	337.7±81.16	690.1±281.4	

Cytogenetic abnormalities (BCR-ABL) gene rearrangement of the studied cases as compared with the controls: BCR-ABL was absent in GI while as regards to GII it was present in 10 out of 40 patients (25%). The comparison revealed highly statistical significant difference between the two groups (p=0.007) (Table 4).

Table (4): BCR-ABL gene rearrangement of the studied cases as compared with the controls

Laboratory Data		Control N =(10)	CMPNs patients N=(40)	*p.* value
Cytogenetic abnormalities BCR-ABL	Present	0(0%)	10(25%)	0.007
	Absent	10(100%)	30(75)	

All the ten individuals of the control group (100%) had normal JAK2 expression (wild type) while none of the ten individuals (0%) had JAK2 mutation expression. 19 patients with CMPNs out of 40 (47.5%) had wild JAK2 expression, while 21 patients out of 40 **(**52.5%) had mutant JAK2 expression in whom 20 out of 21 patients (95.2%) were heterozygous and 1 out of 21 patients (4.8%) was homozygous. This revealed a statistical highly significant difference between the two groups regarding JAK2 mutation (p=0.001) (Table 5).

Table (5): Statistical comparison between CMPNs patients and the control group as regards JAK2 mutation

	Control N =(10)	CMPNs patients N=(40)	*p.* value
JAK2 wild type	10(100%)	19(47.5%)	0.001
JAK2 mutant	0(0%)	21(52.5%)	
heterozygous	0(0%)	20(95.2%)	
homozygous	0(0%)	1(4.8%)	

In PV patients:

There was no significant difference between the control individuals and the PV patients with mutant JAK2 as regards to age and sex. The main clinical presentations were splenomegaly in 6 patients out of 8 patients (75%) with significant difference in comparison to control group (p= 0.025), and there is none of the PV patients with mutant JAK2 showed DVT with no significant difference in comparison to control group (p = 0.059) (Table 6).

Hb concentration: there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals (p=0.009)**.** *WBCs count:* revealed a near significant difference between the PV patients having mutant JAK2 expression and the control individuals (p=0.057). *Platelets count*: there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals (p=0.032). *LAP Score*: there was statistical significant difference between the PV patients having mutant JAK2 expression and the control individuals (p=0.029). *Serum LDH levels*: there was insignificant difference between the PV patients having mutant JAK2 expression and the control individuals (p=0.075) (Table 6).

In ET patients: As regards to age and sex, there was insignificant difference between the control individuals and the ET patients with mutant JAK2. Splenomegaly was present in 5 out of 6 patients (83.3%) with significant difference in comparison to control group (p=0.035). DVT was present in 2 out of 6 patients with mutant JAK2 with significant difference in comparison to control group (p=0.050) (Table 6).

Hb concentration: revealed a near significant difference between ET patients with mutant JAK2 expression and the control individuals (p=0.051). *WBCs count*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and the control individuals (p=0.856). *Platelets count*: there was statically significant difference between ET patients having mutant JAK2 expression and the control individuals (p=0.001). *LAP Score*: there was statistical significant difference between the ET patients having mutant JAK2 expression and the control individuals (p=0.011). *Serum LDH levels*: there was statistical significant difference between ET patients having mutant JAK2 expression and the control individuals (p=0.001) (Table 6).

In MF patients: As regards to age and sex, there was insignificant difference between the control individuals and the MF patients with mutant JAK2. Splenomegaly was present in 6 out of 7 patients (85.7 %) with significant difference as comparison to control group (p=0.002). None of the patients with mutant JAK2 showed DVT with no significant difference in comparison to control group (p =0.073) (Table 6).

Hb: There was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals (p=0.003). *WBCs count*: there was significant difference between the MF patients having mutant JAK2 expression and the control individuals (p=0.003). *Platelets count*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals (p=0.001). *Serum LDH levels*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals (p=0.001). *LAP Score*: there was statistical significant difference between the MF patients having mutant JAK2 expression and the control individuals (p=0.039) (Table 6).

Table (6): Statistical comparison between PV, ET, MF patients with mutant JAK2 and the control group as regards clinical and laboratory data

		PV N =(8)	ET N =(6)	MF N =(7)	Control N =(10)
Age		37-48	34-47	36-43	37-45
	M± SD	42.3±5.6	39.5±6.3	37.9±7.1	41.6±6.2
	p	0.523	0.741	0.652	-
Sex	**Male**	5(62.5%)	3(50%)	5(71.4%)	5(50%)
	Female	3(37.5%)	3(50%)	2(28.6%)	5(50%)
	p	0.253	0.636	0.963	-
Splenomegaly	**Present**	6(75%)	5(83.3%)	6(85.7%)	0(0%)
	Absent	2(25%)	1(16.7)	1(14.3%)	10(100%)
	p	0.025	0.035	0.002	-
DVT No(%)	**Present**	0	2(33.3%)	0	0
	Absent	8(100%)	4(66.7%)	7(100%)	10(100%)
	p	0.059	0.050*	0.073	-
HB(g/dl)	**M± SD**	17.63 ±1.25	11.32 ±1.88	8.11 ±1.63	13.61 ±3.25
	p	0.009	0.051	0.003	-
TLC(x10^3/cmm)	**M± SD**	12.36 ±1.25	11.35 ±2.88	5.32 ±1.84	10.25 ±2.88
	***p* Value**	0.057	0.856	0.003	-
Platelets (x10^3/cmm)	**M± SD**	633.2 ±112.5	1147.1 ±369.1	197.1 ±65.8	513.5 ±71.5
	p	0.032	0.001	0.001	-
LAP score N (%)	**Low**	0	0	0	0(0%)
	Normal	3(37.5%)	4(66.7%)	3(42.7%)	8(80%)
	High	5(62.5%)	2(33.3%)	4(57.3%)	2(20%)
	P	0.029	0.011	0.039	
Serum LDH	**M± SD**	320.6 ±84.5	698.5 ±156.8	946.8 ±195.2	369.2 ±47.8
	P	0.075	0.001	0.001	-

In PV patients: There was insignificant difference between the PV patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In ET patients:** There was insignificant difference between the ET patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In MF patients:** There was insignificant difference between the MF patients having wild type JAK2 expression and those with mutant JAK2 as regards to age and sex. **In CML patients:** No one showed JAK2 mutation. 7 males out of 10 patients (70%) and 3 females out of 10 patients (30 %) showed wild type JAK2 expression (Table 7).

Table (7): Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards age and sex

		PV		ET		MF		CML	
		mutant JAK2 N=8	Wild type JAK2 N=2	mutant JAK2 N=6	Wild type JAK2 N=4	mutant JAK2 N=7	Wild type JAK2 N=3	mutant JAK2 N=0	Wild type JAK2 N=10
Age	**Range**	37-48	33-45	34-47	35-46	36-43	37-43	-	33-48
	Mean ±SD	40.39 ±3.21	37.5 ±6.25	38.9 ±4.2	38.6 ±3.58	40.2 ±1.52	39.22 ±2.96	-	40.42 ±3.08
p. value		**0.325**		**0.536**		**0.658**		**-**	
Sex	**Male (N%)**	5(62.5)	1(50)	3(50)	3(75)	5(71.4)	2(66.7)	-	7(70)
	Female (N%)	3(37.5)	1(50)	3(50)	1(25)	2(28.6)	1(33.3)	-	3(30)
p. value		**0.874**		**0.685**		**0.741**		**-**	

In PV patients: as regards splenomegaly, there was no significant difference between patients of PV having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.242); on the other hand there was no PV patients having DVT. **In ET patients:** there was splenomegaly in 5 patients out of 6 ET patients having mutant type JAK2 expression (83.3%), while 2 patients out of 4 ET patients (33.3%) having wild type JAK2 expression. As regards splenomegaly there was no significant difference between patients of ET having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.366). As regards to DVT no significant difference between patients of ET having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.196). **In MF patients:** there was no significant difference between patients of PV having mutant type JAK2 expression and those with wild type JAK2 expression (p=0.147) as regards to splenomegaly and there was no MF patients having DVT. **In CML patients:** There was splenomegaly in 10 patients out of 10 CML patients (100 %) having wild type JAK2 expression and there was no CML patients having DVT (Table 8).

Table (8): Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards to clinical data

Clinical data	PV		ET		MF		CML	
	mutant JAK2 N=8	Wild type JAK2 N=2	mutant JAK2 N=6	Wild type JAK2 N=4	mutant JAK2 N=7	Wild type JAK2 N=3	mutant JAK2 N=0	Wild type JAK2 N=10
Splenomeglay	6(75)	2(100)	5(83.3)	2(50)	6(85.7)	1(33.3)	-	10(100)
***p*. value**	**0.242 NS**		**0.366 NS**		**0.096 NS**		**-**	
DVT	0(0)	0(0)	2(33.3)	0(0)	0(0)	0(0)	-	0(0)
***p*. value**	**-**		**0.196**		**-**		**-**	

In PV patients: *Hb concentration*: there was no statistical significant difference between the PV

patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression (p=0.536). *WBCs count*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression (p=0.523). *Platelets count*: there was a near significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression (p=0.056). *LAP Score*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression (p=0.361). *Serum LDH levels*: there was no statistical significant difference between the PV patients having mutant JAK2 expression and those PV patients having wild type JAK2 expression (p=0.449) (Table 9).

In ET patients: *Hb*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression (p=0.741). *WBCs count*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression (p=0.321). *Platelets count*: there was highly statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression (p=0.002). *LAP Score*: there was no statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression (p=0.523). *Serum LDH levels*: there was statistical significant difference between the ET patients having mutant JAK2 expression and those ET patients having wild type JAK2 expression (p=0.029) (Table 9).

In MF patients: *Hb*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression (p=0.325). *WBCs count*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression (p=0.489). *Platelets count*: there was statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression (p=0.014). *LAP Score*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression (p=0.365). *Serum LDH levels*: there was no statistical significant difference between the MF patients having mutant JAK2 expression and those MF patients having wild type JAK2 expression (p=0.637). CML patients: All CML patients had wild type JAK2 expression (Table 9).

Table (9): Statistical comparison of mutant and wild type JAK2 expression in PV, ET, MF and CML patients as regards to laboratory Data

Laboratory Data		PV		ET		MF		CML	
		mutant JAK2 N=8	Wild type JAK2 N=2	mutant JAK2 N=6	Wild type JAK2 N=4	mutant JAK2 N=7	Wild type JAK2 N=3	mutant JAK2 N=0	Wild type JAK2 N=10
HB(g/dl)	Mean ±SD	17.63 ±1.25	18.23 ±2.32	11.32 ±1.88	10.52 ±2.99	8.11 ±1.63	7.99 ±2.11	-	12.16 ±2.51
p. value		0.536		0.741		0.325		-	
TLC ($x10^3$/cm m)	Mean ±SD	12.36 ±1.25	11.41 ±1.58	11.35 ±2.88	10.47 ±1.88	5.32 ±1.84	8.74 ±4.11	-	59.27 ±22.29
p. value		0.523		0.321		0.489		-	
Platelets ($x10^3$/cm m)	Mean ±SD	633.2 ±112.5	365.2 ±86.3	1147.1 ±369.1	796.1 ±59.6	197.1 ±65.8	219.8 ±144.1	-	209.1 ±154.1
p. value		0.056		0.002		0.014		-	
LAP score N(%)	low	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	8(80)
	Normal	3(37.5)	1(50)	4(66.7)	3(75)	3(42.8)	2(33.7)	0(0)	2(20)
	High	5(62.5)	1(50)	2(33.3)	1(25)	4(57.1)	1(33.3)	0(0)	0(0)
p. value		0.361		0.523		0.365			
Serum LDH	Mean ±SD	320.6 ±84.5	363.2 ±79.9	698.5 ±156.8	809.8 ±23.1	946.8 ±195.2	886.7 ±180.3	-	886.8 ±256.3
p. value		0.449		0.029		0.637		-	
BCR-ABL (%)	Pres.	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	10(100)
	Abse.	8(100)	2(100)	6(100)	4(100)	7(100)	3(100)	0(0)	0(0)

In PV patients: 2 patients out of 10 patients (20%) had wild type JAK2 expression while 8 patients out of 10 patients (80%) had JAK2 mutation expression in which 7 patients (87.5%) had heterozygous mutation while one patient (12.5%) had homozygous mutation. **In ET patients:** 4 patients out of 10 patients (40%) had wild type JAK2 expression while 6 patients out of 10 patients (60%) had JAK2 mutation expression in which 6 patients (100 %) had heterozygous mutation while no patient (0%) had homozygous mutation. **In MF patients:** 3 patients out of 10 patients (30%) had wild type JAK2 expression while 7 patients out of 10 patients (70%) had JAK2 mutation expression in which 7 patients (100%) had heterozygous mutation while no patient (0%) had homozygous mutation. **In CML patients:** 10 patients out of 10 patients (100%) had wild type JAK2 expression while no patient out of 10 patients (0%) had JAK2 mutation expression (Table 10).

Table (10): Statistical comparison between PV, ET, MF and CML patients as regards JAK2 mutation

	PV N=10	ET N=10	MF N=10	CML N=10	*P.* value
JAK2 wild type	2(20%)	4(40%)	3(30%)	10(100%)	0.002
JAK2 mutant	8(80%)	6(60%)	7(70%)	0(0%)	
Heterozygous	7(87.5%)	6(100%)	7(100%)	0(0%)	
Homozygous	1(12.5%)	0(0%)	0(0%)	0(0%)	

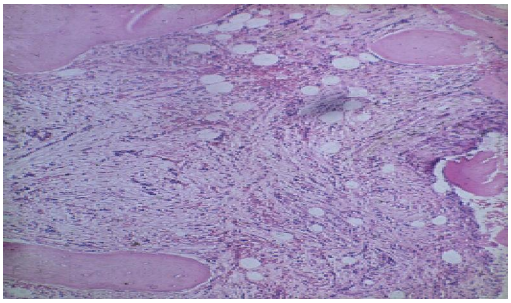


Fig. (1): This slide shows a trephine biopsy specimen of idiopathic myelofibrosis patient which has been cut into thin sections and stained with haematoxylin and eosin (H&E). The pink areas are bone. The area between the bone trabiculae should contain a mixture of hematopoietic cells and fat cells. In this specimen the normal bone marrow has been largely replaced by fibrous tissue.

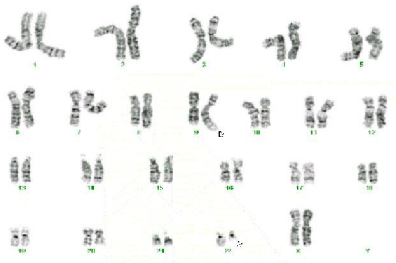


Fig.(2): Karyogram of CML patient showing BCR/ABL fusion gene.

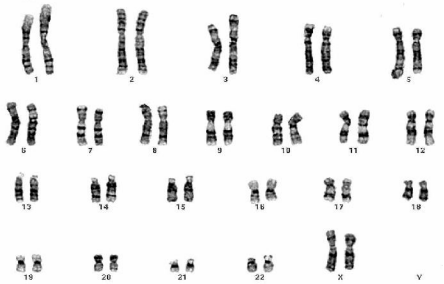


Fig. (3): Karyogram of PV female patient showing negative Philadelphia.

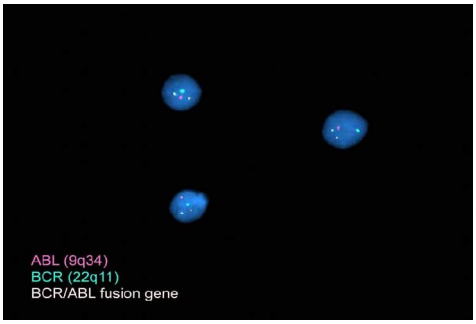


Fig. (4): A case of CML showing BCR/ABL fusion gene (Ph chromosome) one orange signal, one green signal and two fusion signals.

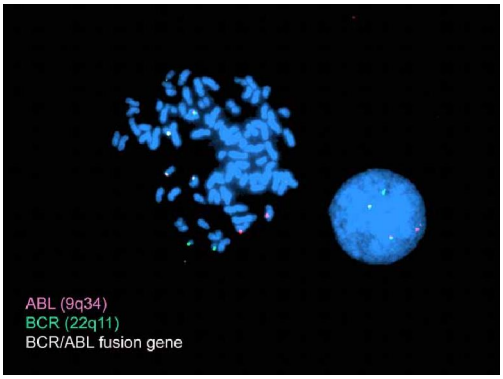


Fig.(5): A case of CML in blast crisis showing hyperdiploid metaphase, two normal 9, two normal 22 and two derivative 9, two derivative 22. One interphase BCR/ABL fusion gene (double fusion). A case of double Philadelphia.

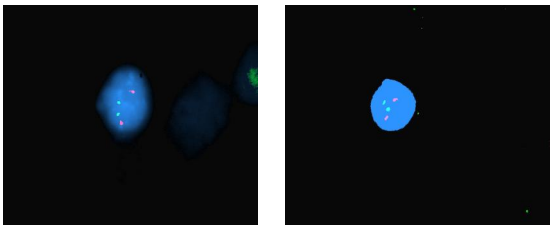
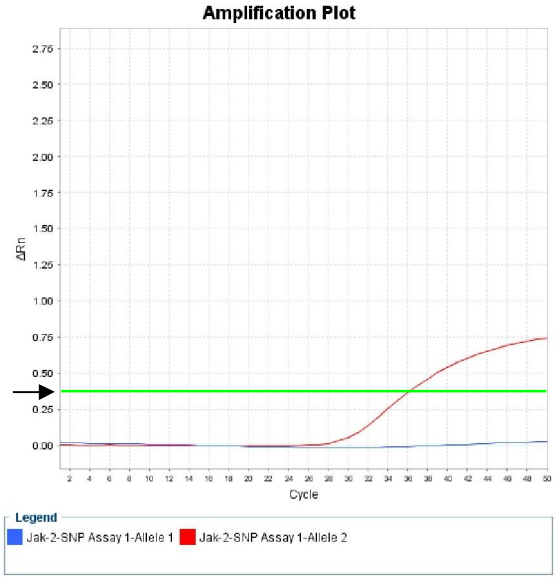
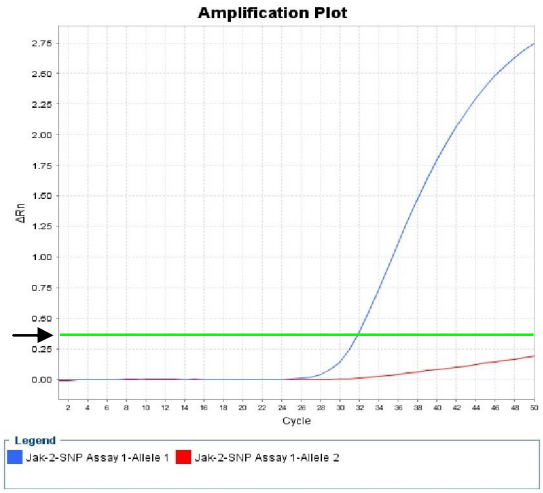


Fig.(6): A case of Philadelphia negative PV showing interphase cells with two orange signals and two green signals i.e. two normal chromosome 9 and two normal chromosome 22.



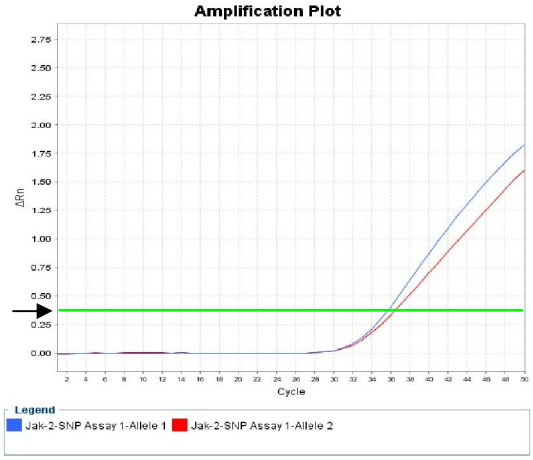
Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

Fig (7): Print out of step one software PCR analysis show normal gene (wild type) is expressed (above the green line), this patient is negative for JAK2 mutation expression.



Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

Fig (11): Print out of step one software PCR analysis show normal gene (wild type) is not expressed (below the green line), this patient is positive for JAK2 mutation expression of homozygous type (above the green line).



Allele 1 = Mutant JAK2 expression
Allele 2 = Wild type JAK2 expression

Fig (8): Print out of step one software PCR analysis show normal gene (wild type) is expressed (above the green line), this patient is positive for JAK2 mutation expression of heterozygous type (above the green line).

4. Discussion:

The JAK family of tyrosine kinases includes JAKs 1-3 and tyrosine kinase (TYK2). JAKs are expressed ubiquitously in all cells, with the exception of JAK3, which is found only in hematopoietic cells[4].

JAK2 V617F is a gain of function mutation that contributes to the expansion of the MPN clone by increasing the tyrosine phosphorylation activity that promotes cytokine activity and include erythrocytosis. The mutation is primarily associated with PV, ET and MF and is not found in CML[10].

Substitution of phenylalanine for valine, both are hydrophobic non polar amino acids, at position 617 of the JAK2 protein, within the JH2 pseudokinase domain[14].

Loss of JAK2 auto inhibition results in constitutive activation of the kinase, analogues to other mutations in MPDs and leukemia that; aberrantly activate tyrosine kinases [14].

Subsequently, 10 other mutations in exon 12 of the JAK2 gene were discovered[15]. These mutations were associated with PV and idiopathic erythrocytosis in patients negative for V617F mutation. Taken together, the prevalence of JAK2 mutations is ~ 99% in PV and ~50% in both ET and MF [16].

A positive JAK2 V617F mutation detection strongly favours MPNs as it is usually negative in normal individuals, secondary erythrocytosis, reactive tbrombocytosis, secondary myelofibrosis, leukaemoid reaction, lymphomas, sarcomas, CML, AML (unless those evolving from MPNs), and MDS (except for those associated with features of MPNs), and it is a sensitive marker detecting MPDs as the underlying etiology of splanchnic venous thrombosis affecting splenic, portal and hepatic veins [3].

Kiladjian et al., [17] had developed an algorithm in which screening of JAK2 can be the initial test in establishing a diagnosis of MPN. In addition, the sensitivity, standardization and convenience of JAK2 analysis and also many, authors have speculated that patients with the Jak2 mutation, but without' overt MPN may have 'latent' or 'early' MPNs and be at much higher risk of developing overt disease at a later date.

As JAK family members play a crucial role in the immune system; for example, inherited JAK3 deficiency causes severe combined immunodeficiency, and JAK2 plays an important role in cardiovascular signaling systems. Development of inhibitors that inhibit V617F without undesirable side effects may therefore be challenging[18].

The JAK2 mutation also affects response to treatment. Among patients with Essential Thrombocythemia, those with the V617F mutation are more sensitive to Hydroxyurea than are patients without the mutation. The response to therapy can now be directly monitored through quantification of JAK2-positive cells in peripheral blood [19].

The present work aimed to study the expression of JAK2 V617F mutation expression by real-time PCR in chronic myeloproliferative disorder patients and study the BCR/ABL rearrangement by FISH.

This study included 40 patients with newly diagnosed chronic myeloproliferative disorders; 10 cases were polycythaemia Vera (PV), 10 cases were essential thrombocythemia (ET), 10 cases were

myelofibrosis (MF), and 10 cases were chronic myeloid leukemia (CML).

In the present study, none of the ten individuals of the control group (0%) had shown JAK2 mutation expression while in CMPNs patients, 21 patients (52.5%) had JAK2 mutation expression, and this is consistent with ***Kralovics et al.,***[20] who reported in his study that prevalence of JAK2 mutation among examined CMPN patients was (52.5%) of cases, while none of the control group (0%) had shown JAK2 mutation expression.

Twenty MPNs patients out of 21 patients (95.2%) had heterozygous JAK2 mutation, while only one patient (4.8%) had homozygous mutation and this is supported by ***Nelson and Steensma,***[14] who implied that prevalence of heterozygosity may be due to mixed clonality or heterozygosity for the autosomal mutation (encoded on chromosome 9p24, and also this agrees with ***Kralovics et al.,***[20] who reported higher frequency (98%) of the JAK2 V617F heterozygous mutation in CMPDs cases.

In the current study, the frequency of JAK2V617F mutation among PV patients was (80%). This is consistent with ***Jones et al.,***[21] who reported JAK mutation in (81%) of PV patients, ***Zhao et al.,***[22] whose study reported that JAK2 mutation was expressed in (83%) of PV patients, ***James et al.,***[5] whose study reported that JAK2 mutation was expressed in (89%) of PV patients and ***Bock et al.,*** [23] who reported (90%) of JAK2 mutation expression among PV patients.

Baxter et al., [24] and ***Kreft et al.,*** [25] reported a higher frequency of JAK2 mutation expression among PV patients and this is due to the use of a more sensitive technique i.e. allele-specific PCR methodology.

In PV patients, only one patient (12.5%) expressed homozygous mutation, while 7 patients (87.5%) expressed heterozygous mutation. ***Scott et al.,*** [26] stated that homozygous JAK2 V617F favors the diagnosis of PV over ET. ***Baxter et al.,***[24] stated that (30%) of PV patients expressed homozygous mutation in their granulocytes while approximately (90%) of them expressed the homozygous mutation when the hematopoietic progenitor cells were examined and this difference suggests that V617F homozygosity promotes the development of PV. Our study had shown that only two patients (20 %) didn't express JAK2 mutation and this is consistent with ***Scott et al.,***[27] who explained in his study that the few patients with PV who didn't show the homozygous JAK2 V617F mutation often have a different mutation in the same gene, one of a number of different mutations on exon 12.

In ET patients, our study showed the prevalence of JAK2 mutation among 6 patients (60 %) and this agrees with ***Baxter et al.,***[24] whose study showed (57%) frequency of JAK2, mutation expression, while this disagreed with ***Horn et al.,***[28] who reported higher incidence of JAK2 mutation (74%) among ET patients and ***Levine et al.,***[10] who found JAK2 mutation expression in only 32% of ET patients. Six positive JAK2 mutation ET patients (60%), were heterozygous while none of the ET patients (0%) expressed homozygous mutation. Similarly, ***James et al.,*** [5] reported in their study that the large majority of patients with JAK2 V617F positive ET are heterozygous. Also ***Baxter et al.,***[24] stated that homozygosity is rarely detectable in peripheral blood cells from patients with ET.

In MF patients, 7 patients (70 %) had JAK2 V617F mutation. ***Kralovics & Skoda,***[29] reported that 57% of MF patients had the mutation, while ***Jelinek et al.,***[30] reported that 95% of MF patients had JAK2 mutation. ***Levine el al.,***[10] stated that only 32% of MF patients expressed JAK2 mutation. Our study showed that (100%) of patients with JAK2 V617F positive MF were heterozygous while none of the MF (0%) patients showed homozygous mutation and this disagrees with ***Baxter et al.,***[24] who reported the prevalence of homozygosity in his study and that (85%) of patients with JAK2 V617F positive MF were homozygous, and also ***Vannucchi et al.,***[31] stated that homozygosity seems to be related to an increased risk of transformation in myelofibrosis. However, definitive identification of V617F zygosity in individual cases is complicated by the fact that it is not possible to distinguish between a relatively small homozygous clone and a larger heterozygous clone when mixed populations of cells are analyzed [20].

On comparing positive JAK2 mutation in PV, ET and MF patients with the controls as regards clinical and laboratory data higher hemoglobin level were found in JAK2 positive PV patients with a highly statistical significant difference of a p-value (0.009*) and this is consistent with ***James et al.,***[5], ***Vannucchi et al.,***[31] and ***Villeval et al.,***[32] who stated that V617F positive PV patients had significant higher Hb levels. There was no significant difference between positive JAK2 mutation and the control individuals in TLC with p value (0.057). On the contrary ***James et al.,***[5] reported that JAK2 positive PV patients had lower total leukocytic counts. Also there was statistical significant difference in PV with mutant JAK2 as regards platelets with *p* value (0.032) and LAP score with p value (0.029) while, there was no statistical significant difference as regards serum LDH level with *p* value (0.075).

As for ET positive JAK2 mutation patients, they had higher platelet count compared to the other groups with a highly statistical significance and a p-value 0.001. This agreed with ***Khwaja***,[3] who reported that JAK2 positive ET patients tend to have higher platelet

count than the control individuals. Also there is highly statistical significance difference with a *p*-value 0.001as regards to serum LDH level and LAP score while there was no significance difference as regards Hb with a *p* value (0.051) and TLC with a *p* value (0.856).

As for MF positive JAK2 mutation patients, splenomegaly was prevalent among MF patients (85.7%), and this is consistent with ***Villeval et al.,***[32] who reported in his study that the incidence rate of splenomegaly showed significance as regards the mutation loads. Also the leukocytic count was higher in control individuals with significant difference in comparison to those with JAK2 positive and a *p*-value 0.003, platelet count with p-value 0.001, HB with *p*-value 0.003, serum LDH level with *p*-value 0.001 and LAP score with *p*-value (0.039).

As for CML none of the patients showed JAK2 mutation expression while all of them were Philadelphia positive and this comes in consistency with ***Haferlach et al.,***[33] and ***Jelinek et al.,***[30] who reported absence of JAK2 mutation in Philadelphia positive CML cases in their studies.

There was no statistical significant difference found between the PV, ET, MF and the CML regarding age and sex prevalence. This disagrees with ***Campbell et al.,***[34] who suggested in his study that sex prevalence might influence the phenotypic presentation of JAK2V617F positive disease, as JAK2 positive PV is more frequent in males while JAK2 positive ET is more frequent in females.

There was statistically non significant difference between positive & negative JAK2 mutation CMPNs patients regarding age, sex prevalence, splenomegaly, DVT, Hemoglobin level, Total leukocytic count, LAP score and serum LDH level. While there was significant difference between positive and negative JAK2 mutation CMPNs patients regarding platelet count in ET and BCR-ABL abnormality in CML patients.

On comparing PV, ET, and MF as regards JAK2 mutation expression, PV was the most prevalent in mutation expression (80%) among the three groups of CMPNs, and this approves with ***Baxter et al.,*** [24] who reported that JAK2 is present in hematopoietic cells in the majority of PV patients, and that the difference in JAK2 allele burden was highly significant between the MPNs diseases, and this is also explained by ***Wernig et al.,***[35] who stated that when mutant JAK2 is transfected into animal models, it becomes sufficient to develop a CMPNs mimicking PV which tends to terminate in myelofibrosis, while for the PV negative for the JAK2V617F it was referred to the newly diagnosed JAK2 gene mutation in exon 12.

Results of this study showed that JAK2V617F mutation expression was associated with increased risk of CMPNs including PV, ET and MF and that this mutation is a risk factor for these disorders to develop. In approval with this finding and owing to the strong association between the JAK2 mutations and MPNs, the World Health Organization (WHO) included the JAK2 mutations among their major diagnostic criteria for PV, ET, MF[31].

Also, this comes in consistency with ***Tefferi and Vardiman,***[37] who stated that the most important role of JAK2 mutation testing at present seems to be during the initial evaluation of patients with myeloproliferation. Given the high specificity of the mutation for clonal myeloid disease, JAK2 V617F, when present, can definitively confirm the diagnosis; so JAK2 mutation testing should be considered as a front-line screening test for suspected MPNs, and its use as a first-intention diagnostic test may spare some patients further investigation. However, the differences in the reported rates are likely because of differences in diagnostic precision and assay sensitivity. A direct sequencing technique was used for the detection of the JAK2 V617F mutation in some studies and this has a lower sensitivity than techniques using PCR amplification of the mutant allele, such as allele-specific PCR or an amplification refractory mutation system PCR[34]. Additionally, early studies indicate that the JAK2 allele burden decreases with successful therapy, disappears in some patients, and reappears during relapse. Thus, JAK2 testing appears useful for both diagnosis, management and follow up[38].

Conclusion

We concluded from this study that JAK2 V617F mutation plays a fundamental role in the pathogenesis and development of CMPNs, and that its detection is very useful to confirm diagnosis and to provide an early detection assay of the CMPNs, and definitely the presence of JAK2 mutation should be interpreted in conjunction with other laboratory and clinical findings as well as to rule out a false diagnosis of reactive thrombocytosis, myelofibrosis or secondary erythrocytosis. Also it may be of use in the management and the follow up of the patients and as a biological marker for monitoring the response to treatment.

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3/29/12