DETECTION OF JAK-2 MUTATION BY PCR TECHNIQUE IN CHRONIC MYELOPROLIFERATIVE DISORDERS

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ABSTRACT

At this study we aimed to detect the prevalence of JAK2 V617F mutation in various myeloproliferative neoplasms (MPNs) that represent a major advance in molecular understanding of CMPN disorders in order to identify its diagnostic value.

In this study we evaluated it's clinical and laboratory correlates in 40 patients with MPNs. The mutation was detected by allele-specific PCR.

The mutation was detected in 8 patients: 80% (8/10) of those with polycythemia vera, 63.6% (7/11) of those with essential thrombocythemia, 62.5% (5/8) of those with chronic idiopathic myelofibrosis and 0% (0/11) of those with chronic myeloid leukemia. The patients carrying the mutation were older ($p = 0.003$) and have splenomegaly in polycythemia vera group ($p = 0.05$) but no statistical difference was found between positive and negative JAK2 carriers as regarding HB, TLC ,PLTs count or gender type ($P > 0.05$).

This study implies that the JAK2–V617F mutation may be useful for the diagnosis, classification and the management of patients with MPDs.

Keywords: JAK2 V617F; Polycythemia vera; Essential thrombocythemia; chronic Idiopathic myelofibrosis; Myeloproliferative neoplasms.

1. INTRODUCTION

Chronic myeloproliferative Neoplas-ms (CMPNs) are clonal hematopoietic stem cell disorder characterized by proliferation of one or more myeloid cell lineage in the bone marrow and increased number of mature and immature cells in the peripheral blood.

CMPNs include polycythemia Vera (PV), esssential thrombocythemia (ET), chronic idiopathic myelofibrosis (CIMF) and chronic myeloid leukemia (CML), plus rare types such as chronic neutrophilic leukemia (CNL), hyperesinophilic syndrome (HES) and chronic esinophilic leukemia (CEL). There are other disorders overlap with both myeloproliferative and myelodysplastic disorders and classified as (MDS/MPN) such as atypical CML (aCML), chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia(JMML) in which proliferation is accompanied by dysplastic features of ineffective hematopoiesis in other lineages. [1]

Although there are strict diagnostic criteria for MPN subtypes, precise categorization remains a subject of debate and furthermore, it is difficult to differentiate some cases from reactive disorders. Only CML is characterized by a pathognomonic molecular marker, the breakpoint cluster region-Abelson (BCR-ABL) fusion gene which is a constitutively activate tyrosin kinase that is believed to be the primary and probably the only driving force behind chronic phase CML. The primary abnormalities driving excess proliferation in most other cases have been obscure. However, several lines of evidence have implicated aberrant tyrosin kinase have been identified in some cases of aCML, CMML, and HES/CEL such as platelets derived growth factor receptor alpha (PDGFRA), platelets derived growth factor receptor beta (PDGFRB), fibroblast growth factor receptor 1 (FGFR1), and the recently diagnosed Janus-Kinase 2 (JAK2) genes. [2]

The Jak2-V617F mutation causes substitution of Phenylalanine for Valine at position 617 of the Jak2 gene. This mutant Jak2 has enhanced kinase activity and when expressed with the presence of erythropoietin receptor in cells it causes hyperactivation of erythropoietin induced cell signaling. [3]

The molecular pathogenesis of BCR-ABL negative MPNs was poorly understood until the identification of JAK2 V617F mutation. The identification of JAK2 V617F in PV, ET, CIMF and other MPNs may represent an important advance in our understanding of the myeloproliferative neoplasms. [4]

2. MATERIAL AND METHODS

2.1. Patients

This study included 10 healthy subjects as control group and 40 patients with newly diagnosed MPNs; 10 with PV, 11 with ET, 8 with MF and 11 with CML. These patients were referred from hematology outpatient clinic to different laboratories of zagazig university hospitals between December 2011 and June 2013. Patients were subjected to complete history taking and general medical examination The following laboratory Investigations were done CBC BM. Smear and biopsy BCR-ABL fusion gene by PCR technique to diagnose CML cases and detection of JAK2 mutation by Allele specific PCR technique.

Formed consents were obtained from every healthy control and patient before sampling.

2.2. **Allele-specific PCR for the detection of JAK2–V617F mutation**

The JAK2–V617F mutation was detected according to the protocol of Baxter et al,. (2005) [5] with some modifications. the primers used for multiplex PCR were

J1 (reverse):

5_-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3_,

J2 (forward specific):

5_-AGCATTTGGTTTTAAATTATGGAGTATATT-3_,

and J3 (forward-internal control):

5_-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3_.

We added 1 ul of each diluted (1:3) primer and 3 ul extracted DNA and 21 ul D.W. to master-mix tube and put the mix tube in thermal cycler for 2 min at 94 ◦C followed by 35 cycles each cycle was performed at 94 ◦C for 45 s, 59 ◦C for 45 s, 72 ◦C for 60 s then after all cycles the tube was incubated for 5 min at 72 ◦C after the last cycle. The PCR products were analyzed on 2% agarose gels. The primers J1 and J3 amplify a 364 bp product (both mutant and wild-type alleles and serves as an internal control), while the primers J1 and J2 amplify a 203 bp product (when the patient carries the JAK2–V617F (Fig.1)

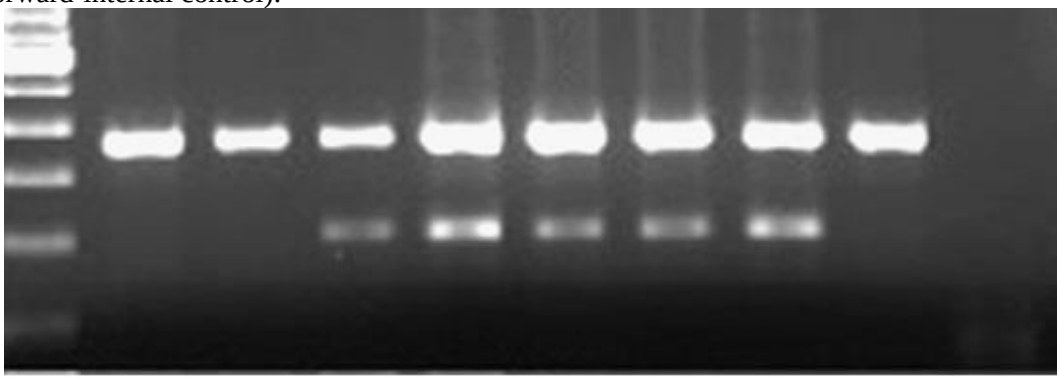

Fig.1: JAK2–V617T mutation established by allele-specific PCR. An allele-specific PCR protocol was performed amplifying a 364 bp product (both mutant and wild-type alleles and serves as an internal control) and a 203 bp product (when the patient carries the JAK2–V617F mutation).

2.3. **Statistical analysis**

The comparison between positive and negative carriers for JAK2 mutation as regarding quantitative parameters (Hb, TLC and PLTs count) was performed by independent sample T-Test, while the association of JAK2 mutation with non quantitative parameters as (gender and splenomegaly) was performed using chi- square test. Also ANOVA was performed to compare quantitative parameters among different groups.The associations was expressed with the corresponding 95% confidence interval (CI). A variable was considered significant when $p \leq 0.05$. The analysis was performed using IPM SPSS Version 20.

3. RESULTS

3.1. **Prevalence of JAK2–V617F mutation in patients with MPNs**

Twenty patients out of 40 patients (50%) were positive for the presence of the JAK2–V617F mutation. More specifically, the prevalence of mutation in the different subtypes of MPNs was 80% in PV (8 out of 10 patients), 63.6% in ET (7 out of 11 patients), 62.5% in IMF (5 out of 8 patients) and 0% in CML (0 out of 11 patients) . Characteristics of the patients are shown in Table 1, which also displays the information according to JAK2–V617F mutational status. In all cases, allele-specific PCR was performed.

3.2. **Correlations of JAK2–V617F mutation with clinical and laboratory findings in MPNs patients**

Statistical comparison between positive and negative Jak2 mutation in PV, ET and MF groups revealed non significant difference as regarding Plts count, TLC and Hb concentration, but high significant difference as regarding age whith older age in positive Jak2 mutation group. Also there was no significant relation between splenomegaly or gender and positive mutation CMPN group. Tables [1,2]

Table 1: comparison between positive and negative Jak2 mutation groups as regarding various data

		CMPNs				
	Jak2	N	Mean	St.d	T test	Sig.
Age	+ve	20	47.3	8.84	3.23	0.003 HS
	-ve	9	35.8	9.05		
HB	+ve	20	14.3	5.6	1.09	0.285 NS
	-ve	9	11.97	4.5		
TLC	+ve	20	10.7	4.4	-.408	0.687 NS
	-ve	9	11.7	8.8		
PLTs	+ve	20	610	409.3	0.162	0.872 NS
	-ve	9	584	363.3		

Table 2: relations between JAK2 presentation and splenomegaly and gender

	Jak2 +ve	Jak2 -ve	Chi square	P value	Sig.
CMPN +ve spleen	16	5	1.86	0.173	NS
CMPN -ve spleen	4	4			
CMPN Male	12	4	0.607	0.436	NS
CMPN Female	8	5			
CMPN no.	20	9			
PV +ve spleen	6	0	3.75	0.05	Sig.
PV -ve spleen	2	2			
PV male	5	1	0.104	0.747	NS
PV female	3	1			
PV no.	8	2			
ET +ve spleen	5	2	0.505	0.477	NS
ET -ve spleen	2	2			
ET male	3	2	0.052	0.819	NS
ET female	4	2			
ET no.	7	4			
MF +ve spleen	5	3	All cases have splenomegaly (non statistic)		
MF –ve spleen	0	0			
MF male	4	1	1.74	0.187	NS
MF female	1	2			
MF no.	5	3			

3.3. **Prevalence and correlations of JAK2–V617F mutation in patients with ET**

Statistical comparison between positive and negative Jak2 mutation in ET group revealed non Significant difference As regarding Plts count,TLC and Hb concentration and age. Also there was no relation between gender and splenomegaly in ET and JAK2 expression. Tables [2,3]

Table 3: comparison between positive and negative Jak2 mutation in ET group as regarding various data

Group Statistics							
	ET.jak2	N	Mean	St.d	T test	Sig.	
Age ET	+ve	7	44.14	6.04	1.76	0.111	NS
	-ve	4	37.50	5.912			
hb. ET	+ve	7	11.40	1.47	1.504	0.167	NS
	-ve	4	10.00	1.53			
tlc. ET	+ve	7	9.79	3.14	1.43	0.188	NS
	-ve	4	16.18	11.59			
plt. ET	+ve	7	1065.0	279	0.95	0.413	NS
	-ve	4	928.25	196			

3.4. Prevalence and correlations of JAK2–V617F mutation in patients with PV

Statistical comparison between positive and negative Jak2 mutation in PV group revealed highly significant difference between positive and negative PV patients as regarding age With higher age in positive jak2 patients but non Significant difference was found as regarding Plts count, TLC and Hb concentration. Also there was significant relation between splenomegaly and positive mutation in PV. and no relation between was found between gender and JAK2 expression in PV group. Tables [2,4]

3.5. Prevalence and correlations of JAK2–V617F mutation in patients with CIMF

Statistical comparison between positive and negative Jak2 mutation in CIMF group revealed non significant difference as regarding Plts count, TLC and Hb concentration and age. Also there was no relation between gender and splenomegaly in CMPNs and JAK2 expression. Tables [2,5]

Table 4: comparison between positive and negative Jak2 mutation in PV group as regarding various data

Group Statistics							
	pv.jak2	N	Mean	St.d	T test	Sig.	
HB. PV	+ve	8	20.46	2.56	0.834	0.663	NS
	-ve	2	19.60	0.707			
PV. age	+ve	8	54.50	7.05	4.99	0.001	HS
	-ve	2	25.50	9.19			
TLC. PV	+ve	8	14.5	2.585	1.49	1.75	NS
	-ve	2	11.45	2.616			
PLT. PV	+ve	8	503.12	92.2	1.76	0.116	NS
	-ve	2	374.50	94.04			

Table 5: comparison between positive and negative Jak2 mutation in CIMF group as regarding various data

Group Statistics							
	MF.jak2	N	Mean	St.d	T test	Sig.	
Age MF	+ve	5	8.48	1.795	0.24	0.98	NS
	-ve	3	9.5	0.62			
hb. MF	+ve	5	5.86	2.22	1.329	0.39	NS
	-ve	3	5.833	3.61			
tlc. MF	+ve	5	143.8	84.95	0.13	0.990	NS
	-ve	3	265	179.9			
plt. MF	+ve	5	40.2	6.72	9.2	0.32	NS
	-ve	3	40.33	9.5			

4.DISCUSSION

We observed that the JAK2–V617F mutation was present in a high proportion of our MPNs patients and it was correlated with old age and splenomegaly. This agrees with Matthaios et al., (2006) [6] who found also correlation with higher Hb and low erythropoietin levels.

None of control group individuals (0%) had Jak2 (V617F) mutation this agrees with (Amy et al., 2005) [2] who studied JAK2 mutation in 679 individuals from which 160 normal subjects as healthy controls and the other with MPNs, they did not found JAK2 mutation in any of their control cases. On the other hand; our results disagree with (Sidon et al., 2006) [7] who detect very low levels of JAK2 V617F mutation in 5 (10%) of 52 clinically healthy individuals with unclear significance for these findings until now, Is follow up is recommended in positive healthy subjects, does it increase, and may mean prediction of new cases these remains questions.

In our study we detect JAK2 mutation in 50 % of cases, in PV patients, 8 patients (80 %) had positive Jak2 mutation while 2 patients (20%) had negative Jak2 mutation. In ET patients,7 patients (63.6 %) had positive Jak2 mutation while 4 patients (36.4%) had negative Jak2 mutation. In MF patients,5 patients (62.5%) had positive Jak2 mutation while 3 patients (37.5%) had negative Jak2 mutation. The eleven cases of CML were negative for JAK2 mutation.

In this study, the frequency of Jak2 mutation among CMPNs was 50% this is less than results of Kralovics et al., (2005) [8] who reported the prevalence of the mutation to be 66% but their study did not include CML. So if we exclude CML In our study Jak2 mutation will be (68.7%) among PV, Et and MF and this agrees with them.

In the current study, the frequency of Jak2 mutation among PV patients was 80% this is consistent with Matthaios et al., (2007) [6] Who found Jak2 mutation among PV patients to be (81.4%), and also consistent with James et al., (2005) [9] who found the prevalence of Jak2 mutation in PV patients to be (89%), while Baxter et al., (2005) [5] found it to be (97%). So JAK2 V617F was considered as major criteria for diagnosis of PV according to WHO (2008). [10]

In our study, the frequency of Jak2 mutation among ET patients was 63.6% this agrees with Matthaios et al., (2007) [6] Who found Jak2 mutation among ET patients was (69.3%) this also come in consistence with Baxter et al., (2005) [5] who found the prevalence of Jak2 mutation in ET patients to be (57%). while Levine et al., (2006) [11] found it to be with lower frequency (32%). JAK2 V617F was considered as major criteria for diagnosis of ET according to WHO (2008). [10]

In this study, the frequency of Jak2 mutation among MF patients was 62.5% this agrees with Matthaios et al., (2007) [6] Who found Jak2 mutation among MF patients in (58.3%) this also come in consistence with Kralovics et al., (2005) [8] who found the prevalence of Jak2 mutation in MF patients to be (57%), but contrary to Levine et al., (2005) [12] who detected lower frequency (32%), Also JAK2 V617F was considered as Major criteria for diagnosis of CIMF according to WHO (2008). [10]

In our study there were 11 CML cases 9 of them were typical with positive BCR-ABL fusion gene detected by PCR technique while 2 cases were atypical with negative BCR-ABL gene, but none of CML cases carried the JAK2 mutation.

The 2 Cases were considered as atypical CML because they did not have the criteria of CMML or JMML (no monocytosis and not juvenile) and were negative for BCR-ABL by PCR. Atypical CML is beyond our scope of study as it is classified by WHO (2008) as MPN-MDS, so further investigation on atypical CML cases should be continued on larger scale to identify the percentage of atypical CML with positive JAK2 mutation or JAK2-PCM1 fusion as they were found in some cases of atypical CML. [13]

On comparing PV, ET and MF patients with control group, PV group revealed statistically significant difference as regarding age, Platelets count and Hb level with p-value (< 0.05). This agrees Villeval et al., (2006) [14] who reported higher Hb level in PV. The ET group revealed statistically significant difference as regarding age, Platelets count and Hb level with p-value (< 0.05). While MF group revealed statistically significant difference as regarding age and lower Hb level with p-value (< 0.05).

On comparison between positive Jak2 mutation PV, ET and MF we found significant difference as regarding age, Platelets count, TLC and Hb level with p-value (< 0.05). but non significant difference was found as regarding sex and splenomegaly. This agrees James et al., (2005) [9] who reported that positive Jak2 mutation PV has higher Hb levels than other positive mutation groups.

We found no significant difference between positive and negative JAK2 patients of CMPNs, PV, ET and MF as regarding Hb, Plts count, and TLC but there was significant difference between positive and negative JAK2 mutation groups as regarding age with higher age incidence in CMPNs patients and PV patients.

Statistical relation between JAK2 V617F and both splenomegaly and gender type revealed significant relation between positive JAK2 mutation and splenomegaly in PV group only, on the other hand no relation between JAK2 mutation and spleen enlargement in all other groups. Also no relation was found between JAK2 mutation and sex type in all studied groups.

On comparing PV, ET and MF as regarding Jak2 mutation we found significant difference among groups with p-value (<0.05), PV had the most prevalent mutation (80%) this agrees with Baxter et al., (2005) [5] who reported that Jak2 mutation is present in hematopoietic cells in the vast majority of PV patients and that the difference in jak2 allele burden was highly significant between the PMNs disease entities.

Our study showed that the prevalence of Jak2 V617F mutation expression among MPNs patients was 50 % and it was more prevalent in PV patients (80%) than in both ET (63.6%) and MF patients (62.5%).

Despite this high expression of Jak2 mutation in PV and its relative lower expression in ET and MF it was found That Jak2 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 these findings and owing to strong association between the Jak2 mutation and MPNS, the WHO now includes the Jak2 mutation among their major diagnostic criteria for PV, ET and CIMF. [15]

in another study it was found that detection of Jak2 mutation establishes the presence of clonal disorder and so Jak2 mutation should be performed as a front-line screening test for suspected MPNs and this may spare suspected patients further investigation. [9]

The difference in the reported rates may be due to difference in precision and sensitivity of methods used, e.g. direct sequencing used in some studies has lower sensitivity than techniques using PCR amplification. [16]

In addition earlier studies indicated that Jak2 mutation burden decreases with successful myelo suppressive therapy and may disappear in some patients and reappear on relapse, so quantitative Jak2 V617F may be useful not only in diagnosis but also in follow up and management. [17]

In cases of MPNs with negative JAK2-V617F other mutations should be studied e.g. Other JAK2 mutations or translocations and/or MPLW515L mutation.

Other mutations and translocations should be studied in JAK2 V617F negative MPNs: 1- JAK2 exon 14 other than JAK2 V617F such as 611, 616, 620 and 627 mutations.

2- JAK2 exon 12 mutations which are clustered in 537 to 543 B.

3- JAK2 fusion genes which are involved in myeloid and lymphoid leukemias and include PCM1-JAK2, BCR-JAK2, TEL/ETV6-JAK2.

4- MPLW515 Mutations in JAK2 V617F-negative ET and CIMF. [18]

JAK2 exon 12 mutations compared with JAK2 V617F is characterized by lower TLC and PLT count with higher Hb values, screening for exon 12 mutations in JAK2 V617F negative patients with clinical PV, unexplained erythrocytosis, venous thrombosis or Budd-Chiari syndrome may be helpful for establishing diagnosis. [3]

Compared with JAK2 mutations, JAK2 fusions are associated with more aggressive courses as AML, ALL, atypical CML and CIMF, e.g. pericentriolar material 1 (PCM1)-JAK2 fusion gene is found in atypical CML, CIMF CEL, AML and ALL. [19]

In November 2011, the JAK1/JAK2 inhibitor, ruxolitinib (Jakafi), became the first US Food and Drug Administration (FDA)–approved drug for patients with intermediate- or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis. Results showed patients (n=309) who received ruxolitinib had a significant reduction in spleen volume (at least 35%) at 24 weeks when assessed by MRI or CT compared with placebo. So more studies on Jak2 inhibitors should be continued for other CMPNs specially those positive for other JAK2 mutations or translocations. [20]

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