Correlations of JAK2V617F Mutational status with Clinicohematologic Characteristics in Sudanese Patients with Primary Myelofibrosis

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Abstract: Primary myelofibrosis (PMF) is a *BCR*/*ABL*-negative myeloproliferative neoplasm (MPNs) characterized by dysregulated kinase signaling and release of abnormal cytokines. About 50-60% of PMF patients have been reported to have acquired somatic mutation in Janus kinase 2 gene (*JAK*-V617F mutation). The aim of this study was to fine out correlation between the *JAK2*-V617F mutational status and the clinicohematologic characteristics in Sudanese patients with PMF. 45 patients with PMF were involved in this study. Allele specific PCR was used to detected the *JAK2*-V617F, and quantitative real-time polymerase chain reaction (qRT-PCR) was used to determinate the percentage of mutated alleles in genomic DNA among *JAK2*-V617F positive MPNs. The *JAK2*-V617F mutation was detected in 51.1%. The mean allele burden of *JAK2*-V617F for positive patients was 69.3%. The prevalence of patients with high allele burden (i.e. *JAK2*-V617F allele burden exceeded 50%) was 31.1%. *JAK2*-V617F mutation associated with high Hb (P=.039), Hct (P=.04) in PMF patients. *JAK2* V617F allele burden was correlate with lower platelet count in (*P=.015)*.

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1. Introduction

Primary myelofibrosis (PMF) is a clonal stem cell disorder characterized by chronic myeloproliferation, atypical megakaryocytic hyperplasia, and bone marrow fibrosis. The disorder manifests clinically as anemia, splenomegaly due to extramedullary hematopoiesis (EMH), leukoerythroblastosis, and constitutional symptoms (Tefferi, 2014). Along with polycythemia vera (PV) and essential thrombocythemia (ET), PMF considered as classic Philadelphia (Ph) negative MPNs (Amy and Nicholas, 2013) (Dan and Cameron, 2010). approximately half of the patients with PMF have been reported to have an acquired somatic mutation, results from G to T mutation involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617 (Baxter et al., 2005) (Kralovics et al., 2005) (Shirane et al., 2015).

The JAK2 is a cytoplasmic, non receptor, tyrosine kinase that via its association with cytokine receptors serves as a signaling mediator for hematopoietic cytokines such as erythropoietin (Epo), and thrombopoietin (Tpo) to regulate cell proliferation and growth (Ross, 2012). *JAK2*-V617F mutation affects the noncatalytic 'pseudo-kinase' domain (JH2) of JAK2 and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2- mediated signaling pathways, leading to growth factor independent autonomous proliferation of hematopoietic precursors (Ross, 2012) (James et al 2005).

Correlation of *JAK2*-V617F mutation with clinical and hematological characteristic in MPNs haves demonstrated in several studies and reported variable results. In this current study we aimed to determine the relationship between JAK2 V617F mutational statuses, JAK2 V617F allele burden with clinicohematologic characteristics in Sudanese patients with PMF.

2. Material and Methods

Study population (patients): This cross sectional study, extended from 2013 to 2015. Forty-five patients diagnosed to have PMF according to the World Health Organization (WHO) criteria (James et al, 2009) were enrolled. Hematologic data obtained from patient's records.

Blood sampling and DNA extraction: 5 ml of peripheral blood from each subject was collected in EDTA K3 tube. Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol of innuPREP kit (Analytik Jena/Germany).

Analysis of the *JAK2* V617F mutation: The presence of JAK2-V617F mutation was assessed in 45 patients with PMF by Allele specific PCR. Amplifications were done in a total volume of 25 µL PCR mix containing 12.5 µL of TaqMan Universal Master Mix, 5 µL of nuclease-free PCR grade water, 2.5 µL of primers and probes mix and 50 ng/µL of DNA template. PCR conditions used were denaturation at 95℃ for 1 min, annealing at 58℃ for 40 s, and extension at 72℃ for 45 s. Products were electrophoresed on agarose gels and visualized using ethidium bromide staining. 23 PMF patients positive for *JAK2*-V617F mutation were analyzed for *JAK2*-V617F mutational load by using *JAK2* Muta*Quant*™ (Ipsogen Inc., New Haven, CT). qPCR reactions were performed in a final volume of 25 µl mix which containing 12.5 µL of TaqMan Universal PCR master mix, 1 µL of IPSOGEN PPM- VF or wild type primers and probe mix, and 6.5 µL of nuclease-free water, and 5 µL genomic DNA was used per well. The RQ-PCR conditions used were as follows: 50℃ for 2 min, heating at 95℃ for 10 min, and 50 cycles of 95℃ for 15 s and 63℃ for 90 s. The RQ-PCR was performed using a Rotor-Gene Q 5plex HRM instruments. Standard curves for both V617F and wild type were constructed using either a V617F or a wild-type plasmid of known value, provided by the manufacturer. The equation was calculated for each curve, and these equations were used to calculate the copy number of V617F and wild-type alleles in unknown samples. The allele burden of *JAK2* V617F is expressed as the percentage of V617F copies compared with the sum of V617F and wild-type copies.

Statistical analysis: Statistical analyses were performed using the SPSS version 11.2

3. Results

Forty-five PMF patients were enrolled in this study. At diagnosis the mean age was 57.00 years, mean hemoglobin 9.89 g/dL, RBC 4.01×10^9/L, WBC12.05×10^9/L and mean platelets count was 242.20×10^9/L.

Out of all patients, 23(51.1%) patient were JAK2V617F-positive, while 22(48.9%) were wild-type. The median allele burden of *JAK2*-V617F for positive patients was 69.3%. Patients divided into homozygous (had allele burden exceeded 50%) and heterozygous in which mutational load equal or less than 50%. Of 23 patients who were positive for *JAK2*-V617F mutation, 14 patients were homozygous 60.9% correspond 31.1% of whole patients.

The presence of *JAK2*-V617F mutation significantly associated with high Hb (P=.039) and Hct (P=.04). There were no significant differences in median age at presentation, total leukocyte count or erythrocyte count between JAK2V617F-positive and JAK2V617F-negative PMF patients (Table 1).

Homozygous patients had lowest platelets count compare with heterozygous *PMF patients* (*P=.015)*. There were no significant differences in mean age, WBC or Hb between Homozygous and heterozygous *PMF patients (Table 2*).

Table 1. Hematologic characteristics associated with the JAK2 V617F mutation status in PMF patients. P*, significant

Characteristics	Total	JAK2 Wild-type	JAK2- V617F	P
No of patients	45	22(48.9%)	23(51.1%)	
Age at diagnosis (yr, mean±SD)	57.00±16.64	54.31±16.55	59.56±16.68	.296
WBC ($\times10^9$/L, mean±SD)	12.05±10.78	11.20±10.30	12.95±11.43	.592
RBC ($\times10^9$/L, mean±SD)	4.01±1.41	3.64±1.19	4.40±1.55	.075
Hb (g/dL, mean±SD)	9.89±2.59	9.11±2.02	10.70±2.90	.039*
Hct (%, mean±SD)	29.28±7.59	27.02±5.88	31.65±8.55	.040*
Platelet ($\times10^9$/L, mean±SD)	242.20±206.27	272.04±222.64	213.65±189.83	.348

Table 2. Associations of hematologic parameters with the JAK2 V617F allele burden in PMF patients

Characteristics	JAK2V617F- heterozygous	JAK2V617F- homozygous	P
No of patients	9(39.1%)	14(60.9%)	
Age at diagnosis(yr,mean±SD)	58.44±14.49	60.28±18.44	.803
WBC ($\times10^9$/L, mean±SD)	9.87±9.91	13.25±11.16	.456
RBC ($\times10^9$/L, mean±SD)	3.30±0.84	4.19±1.50	.082
Hb (g/dL, mean±SD)	8.75±2.24	9.68±1.56	.288
Hct (%, mean±SD)	26.06±6.64	28.52±4.40	.340
Platelet ($\times10^9$/L, mean±SD)	329.55±184.36	139.14±157.44	.015*

4. Discussions

The *JAK2* V617F acquired somatic mutation is the most commonly described mutation associated with MPN. In PMF patients it have been detected in range (50-60%) (Levine et al, 2205) (Sultan and Irfan, 2015)]. Prevalence of JAK2V617F mutation in PMF patients in our study was 51.1%, similar to the reported data worldwide. The median allele burden of *JAK2*-V617F for positive patients was 69.3%, similar to findings by *Larsen TS* et al (Larsen et al., 2007) and Sang P et al (Sang et al., 2013). In our study, the homozygosity among JAK2V617F positive PMF patients was 60.9% correspond 31.1% of whole patients. Similarly these result were reported in numerous published studies (Singh et al., 2015) (Ipek et al., 2015) (Kralovics et al., 2005) (Yonal et al., 2015).

In numerous studies, the JAK2V617F mutation has been variably associated with higher indices of erythropoiesis, unchanged or decreased platelet count, increased bone marrow fibrosis, older age, and longer duration of disease (Kralovics et al.,2005) (Rudzki et al., 2007) (Speletas et al.,2007). The studies by Yonal I et al (Yonal et al., 2015), Benmoussa A et al (Benmoussa et al., 2011) and Ayad W et al (Ayad and Nafea, 2010) showed association between JAK2-V617F mutation and higher levels of haemoglobin and hematocrit in PMF patients. Similarly we have observed significant differences in Hb and Hct between JAK2V617F-positive and JAK2V617F-negative PMF patients. Also we observed no significant differences in mean age, WBCs, RBCs or platelets between mutated and un mutated patients, these results were consistent with the observations reported by Ha S et al (Ha et al., 2012), Sultan S et al (Sultan and Irfan, 2015) and Campbell J et al (Campbell et al., 2005).

Correlation analysis in our study showed association between JAK2V617F homozygous genotype and low platelets count (*P=.015)*, this observation was comparable with results in several studies [Yonal et al., 2015) (Rudzki et al., 2007). In contrast several studies [(Sultan and Irfan, 2015) (Ha et al., 2012), showed no correlations between JAK2V617F allele burden and mean age, WBCs, Hb, or Hct in PMF mutated patients. Similarly we observed on significant differences in Hb, WBCs, or mean age between homozygous genotype and heterozygous genotype groups. Over all the findings in our study were comparable with to the reported data worldwide.

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