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The prevalence of the *JAK2* V617F Exon 14 mutation in Sudanese patients with Myelo Proliferative Neoplasms (MPNs)

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The understanding of the pathogenesis of myelo proliferative neoplasms has been aided significantly by the discovery of the *JAK2* V617F exon 14 mutation and provided with additional capabilities for analyzing and managing this type of disease. The aim of this study was to determine the frequency of *JAK2* V617F exon 14 mutation in Sudanese patients with myelo proliferative disorders referred to Fedail Hospital and Radioisotopes Centre Khartoum (RICK) in Khartoum State-Sudan., and to investigate the differences of laboratory parameters between patients with *JAK2* V617F exon 14 positive myelo proliferative neoplasms (MPNs) and *JAK2* V617F exon 14 wild type MPNs.A total of 166 patients with MPNs; 76 with polycythemia Vera (PV), 76 with essential thrombo cythemia (ET) and 14 with primary myelofibrosis (PMF), and 11 healthy individuals were included in a study conducted from 2014 to 2018. DNA was isolated from peripheral blood leukocytes by QIAamp mini kit, and *JAK2* V617F exon 14 mutation gene detected by quantitative real-time PCR (qRT-PCR) technology (QuantStudio 12K Flex) using TaqMan® Mutation Detection Assay and Sanger sequencing to confirm the results of TaqMan and to identify the type allele of mutations.The*JAK2* V617F exon 14 was detected in 61.3% in all MPNs patients. The prevalence of *JAK2* V617F exon 14 mutations was 68.6% in PV, 50% in ET and PMF patients. Mutation was not detected in 11 healthy adult people. The presence of *JAK2* V617F exon 14 mutations was not associated with total WBCs count and PLTs count for PV patients, however the mutation positively correlates with high total RBCs count (p = .005), Hb concentration (p =.018) and HCT (p =.016) in PV patients, and with high total WBC count (p =.000) in ET patients. A *JAK2* V617F exon 14 Sanger sequencing was done for 114 of the 166 patients to confirm the results of TaqMan and to identify the type allele of mutations; 64 PV, 38 ET and 12 PMF. The majority of *JAK2* V617F exon 14 positive ET and PMF patients were heterozygous, no *JAK2* V617F exon14 homozygous allele was detected in PV patients.The*JAK2* V617F exon 14 mutation could be frequently detected in the Sudanese patients with MPNs, the vast majority of polycythemia patients and around half of the essential thrombocythemia and primary myelofibrosis have the mutation.

Keywords: *JAK2* V617F exon 14 mutation; Chronic Myelo proliferative Neoplasms; Polycythemia Vera;

INTRODUCTION

Myelo proliferative disorders (MPDs) are a group of disorders characterized by clonal proliferation of one or more types of cells of

myeloid series (Levine and Gilliland 2008). In these disorders, bone marrow shows increased numbers of progenitor cells of myeloid lineages while peripheral blood shows increased number of immature and mature cells accompanied with chance for potential transformation into AML (Jones, Kreil et al., 2005, Jones and Cross 2013).

Traditionally, MPDs used to be classified into the "classic" and "atypical" categories. Classic category includes chronic myelogenous leukemia, polycythemia Vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIM). The WHO has introduced a classification for MPDs in 2008. According to this classification, MPDs include chronic myeloid leukemia (CML), polycythemia Vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). In addition to some uncommon types as chronic neutrophilic leukemia (CNL), hyper eosinophilic syndrome (HES) and chronic eosinophilic leukemia (CEL) (Rumi, Pietra et al., 2014).

This classification is based mainly on the predominant myeloid cell lineage expanded in the blood. PV is characterized by excessive production of erythrocytes, increased red cell mass as well as splenomegaly due to extramedullary hematopoiesis (Zaleskas, Krause et al., 2006). High platelet count is necessary in ET together with susceptibility for thrombosis and hemorrhage (Schwemmers, Will et al., 2007). Finally Idiopathic myelofibrosis (IMF) shows bone marrow fibrosis, variable count of cells of myeloid series and hepatosplenomegaly (Jones and Cross 2013).

According to WHO 2008 classification, the hallmark of chronic myelogenous leukemia (CML) is the presence of a t (9; 22) BCR-ABL1 fusion genes in cells of myeloid series which is present in about 90% of patients (Shet, Jahagirdar et al., 2002). This mutation is detected by standard cytogenetic analysis in 95% and can be detected by more sophisticated methods (as FISH and RT-PCR) in the remaining 5% of cases (Cortes, Talpaz et al., 1995). Lack of specific diagnostic molecular markers for other typical MPDS represented a major problem over years. So, diagnosis depended on clinical and pathological features together with exclusion of other specific genetic abnormalities such as BCR-ABL1 fusion (Murugesan, Aboudola et al., 2006).

The Janus kinase/signal transducers and activators of transcription (*JAK*)/(STAT) pathway perform an essential role in the initiation of signal transduction. The presence of *JAK2* is essential for normal hematopoiesis. This fact has been confirmed by defects in erythropoiesis which appeared in JAK2 deficient mice (Gery, Cao et al., 2009). *JAK2* is composed of two main domains, the first is an enzymatically active kinase domain (*JAK* homology 1 [JH1]) and a catalytically inactive pseudokinase domain (JH2). The JH2 domain plays an inhibitory role that generally inhibits the kinase activity of *JAK2* (Hsu 2007).

In 2005, Janus kinase *JAK2* mutation was discovered in some myelo proliferative disorders. The discovery of this mutation was represented a great progress in the understanding of BCR-ABL negative myelo proliferative disorders (Gonzalez, De Brasi et al. 2014). The most common mutation of *JAK2* is a substitution of valine with phenylalanine at position 617 in the JH2 domain (Levine, Loriaux et al. 2005), which affects the function of the inhibitory pseudokinase JH2 domain causing an increased activity of progenitor cells of myeloid origin and excessive production of mature cells of myeloid origin (Etheridge, Roh et al. 2014, Gonzalez, De Brasi et al., 2014). Less commonly are mutations in exon 12, beside *JAK2* mutation has been suspected to have a role in the development of AML in a single case report (Elnaggar, Agersborg et al., 2012).

The presence of *JAK2*V617F mutation in hematopoietic cells causes hypersensitivity to cytokines and factor independent growth (Elnaggar, Agersborg et al., 2012). The presence of the erythropoietin, thrombopoietin or granulocyte-colony stimulating factor receptor/s is essential for this mutation to work, leading to functional hyperactivity and increased sensitivity for hematopoietic growth factor such as interleukin 3 (IL-3), stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and insulin-like growth factor-1 (IGF-1) (Brière 2007, Hsu 2007, Kaushansky 2007, Bellido and teBoekhorst 2012).

The aim of work is to study the prevalence of *JAK2* V617F Exon 14 mutation in Sudanese patients with myeloproliferative disorders were referred Fedail Hospital and Radioisotopes Centre Khartoum (RICK) in Khartoum State-Sudan.

MATERIALS AND METHODS

Study Population (Patients)

Over a period of about 4 years (2014-2018), a total of 166 patients with chronic MPNs; 76 with PV, 76 with ET and 14 with PMF, and 11 healthy adult individuals were randomly enrolled. Hematologic data obtained from patient records

and hematological diagnoses and subtyping results were reconfirmed according to the 2008 WHO criteria.

Blood Sampling and DNA Extraction

5 ml of peripheral blood from each subject were collected in EDTA K3 containing vacutainer tube. Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol ofQIAamp DNA blood mini kit for DNA extraction which was obtained from QIAGEN Inc., Chatsworth, CA, USA, Cat. No. 51104.described by the manufacturer, then it was stored at –20℃ until the PCR was performed.

Analysis of the *JAK2* V617F Exon 14 Mutation

Mutation analysis of *JAK2* V617F exon 14 was performed using quantitative real-time PCR (qRT-PCR) technology (QuantStudio 12K Flex) using TaqMan® Mutation Detection Assay and Sanger sequencing to confirm the results of TaqMan and to identify the type allele of mutations.

Quantitative Determination of *JAK2* V617F exon 14 mutation by TaqMan

The TaqMan® Mutation Detection Assays detect and measure somatic mutations in genes that are associated with cancer. The TaqMan® Mutation Detection Assays are powered by castPCR™ technology, which refers to Competitive Allele-Specific TaqMan® PCR. The castPCR™ technology is highly specific and sensitive, and it can detect and quantitate rare amounts of mutated DNA in a sample that contains large amounts of normal, wild type gDNA. In a mutation detection experiment, a sample of unknown mutation status is run in individual real-time PCRs with one or more assays that target mutant alleles within a gene and the corresponding gene reference assay. After amplification, the C_T values are determined by the Applied Biosystems® real-time PCR instrument software. Data files containing the sample C_T values can be exported from the real-time PCR instrument software and imported into a data analysis tool. Life Technologies Mutation Detector™ Software is recommended for post-PCR data analysis of mutation detection experiments.

In mutation analysis calculations, the difference between the C_T value of each mutant allele assay and the C_T value of the gene reference assay is calculated. This ΔC_T value represents the quantity of the specific mutation allele detected within the sample; it is used to determine sample mutation status by comparison to a pre-determined detection ΔCT cutoff value.

Detection of *JAK2* V617F by Sequence Analysis

To confirm the results of TaqMan and to identify the type allele of mutations. Sequence Analysis was performed using allele-specific PCR. Amplifications were done in a total volume of 25 µL PCR mix containing Genomic DNA template 1 µl, 2.5 µl (10x) PCR buffer (50 µl PCR buffer, 1.5 µl MgCl2), 10Mm dNTPs 0.5 µl, Forward primer (200 ng/ml) 0.5 µl, Reverse primer (200 ng/ml) 0.5 µl, 0.1 µl of Taq DNA polymerase (plantiumtaq), and 19.9 µl of deionized distilled water. PCR were; Forward primer 5'-CTCCTCTTTGGAGCAATTCA-3' and Reverse primer 5'-GAGAACTTGGGAGTTGCGATA-3'. The PCR conditions used were denaturation at 95℃ for 1 min, annealing at 50℃ for 35 s, and extension at 72℃ for 45 s. Products were electrophoresed on agarose gels and visualized using ethidium bromide staining. Then DNA sequencing protocol were done for the samples.
Data were analyzed by a computer program IBM (SPSS) version 20.

RESULTS

The JAK2-V617F exon 14 mutation was detected in 61.3% of all patients with MPNs. The prevalence of the mutation in subtypes were 68.6% for PV, 50% for ET and PMF. JAK2-V617F exon 14 mutation was not detected in 11 healthy adult people. At the time of diagnosis, PMF had the highest mean WBC count (69.29 ×109/L). PV had the highest RBCs count, Hb concentration and HCT (6.74±1.24 ×109/L, 16.26±3.17 g/dL and 50.82±8.16 %). While ET had the highest Platelets count (1188.81±548.41 ×109/L). the presence of JAK2-V617F exon 14 was found to be significantly associated with high total RBCs count (P= .005), Hb concentration (P=.018) and HCT (P=.016) in PV patients. There was an association between JAK2 exon 14 (V617F) and erythrocytosis in PV patients (P=.005). No significant association detected between JAK2 exon 14 (V617F) and total WBCs and PLTs count in PV patients. However, JAK2 exon 14 (V617F) patients had a high WBC count and PLTs count overall. No significant difference in total RBCs, Hb concentration, Hct or PLTs count was observed between positive JAK2 exon 14 (V617F) and negative JAK2 exon 14 (V617F) ET patients,

Table 1. Hematologic parameters according to myeloproliferative neoplasm (MPN) subtypes

Characteristics	PV	ET	PMF	Total
Age at diagnosis (yr, mean±SD)	46.50±6.87	47.53±9.07	50.71±10.52	47.33±8.30
WBC (×109/L, mean±SD)	21.39±29.87	33.34±51.56	69.29±61.31	30.90±45.60
RBC (×109/L, mean±SD)	6.74±1.24	4.84±1.19	4.71±1.89	5.69±1.59
Hb (g/dL, mean±SD)	16.26±3.17	10.87±3.01	9.71±1.73	13.24±4.09
Hct (%, mean±SD)	50.82±8.16	36.39±10.41	32.43±6.15	42.66±11.84
Platelet (×109/L, mean±SD)	421.09±452.59	1188.81±548.41	308.71±283.86	757.90±624.41
NO of JAK2-Exon 14 (V617F) positive (%)	68.6%	51.4%	50.0%	61.3%

Table 2; Hematologic parameters associated with the JAK2 V617F exon 14 mutation statuses

	PV			ET			PMF		
Characteristics	JAK2 WT 22 (31.4%)	JAK2-V617F 48 (68.6%)	P.value	JAK2 WT 19 (50%)	JAK2-V617F 19 (50%)	P.value	JAK2 WT 6 (50%)	JAK2-V617F 6 (50%)	P.value
Age at diagnosis (yr, mean±SD)	48.27±8.80	46.25±5.99	.014	47.67±9.60	47.63±7.95	.990	40.00±4.09	57.50±4.72	.000
WBC (×109/L, mean±SD)	29.55±37.29	15.88±22.28	.186	12.78±9.25	69.68±50.96	.000	49.67±39.92	71.33±53.98	.448
RBC (×109/L, mean±SD)	6.73±.63	6.83±1.39	.005	4.67±1.46	4.58±.96	.829	6.00±2.37	3.83±.41	.052
Hb (g/dL, mean±SD)	17.09±3.07	16.15±2.89	.018	9.89±3.27	11.05±1.78	.184	9.33±1.86	10.50±1.64	.277
Hct (%, mean±SD)	53.27±6.24	50.67±7.95	.016	33.44±9.04	37.16±6.15	.151	31.00±6.45	35.00±5.62	.279
Platelet (×109/L, mean±SD)	289.18±162.06	480.48±547.79	.155	426.11±317.81	437.79±333.05	.914	448.33±397.65	212.67±75.31	.184

p-value significant at <0.05

Table 3; JAK2 V617F exon 14 Mutation status among myelo proliferative neoplasm (MPN)

Disease subtypes	Total No. of subjects	JAK2-V617F wild-type N (%)	JAK2-V617F mutant type N (%)	JAK2-V617F heterozygous N (%)	JAK2-V617F homozygous N (%)
PV	64	34 (53.1%)	30(46.9%)	30(46.9%)	0 (0%)
ET	38	23 (60.5%)	15 (39.5%)	12 (85.7%)	2 (14.3%)
PMF	12	6 (50%)	6 (50%)	5 (83.3%)	1 (16.7%)

Although ET patients who harbour the mutation have highest WBCs, RBCs, Hb, Hct, and platelets count. JAK2 exon 14 (V617F) mutation was found in a significant association with high total WBC count (P=.000) in ET patients. Although JAK2 exon14 (V617F) positive PV and PMF patients had a high mean of age, the difference was not significant statistically. A JAK2 V617F exon 14 Sanger sequencing was done for 114 of the 166 patients to confirm the results of TaqMan and to identify the type allele of mutations; 64 PV, 38 ET and 12 PMF. The majority of JAK2 V617F exon 14 positive ET and PMF patients were heterozygous, while there is no JAK2 V617F exon14 homozygous allele was detected in PV patients. These results are summarized in (Tables 1, 2 and 3).

DISCUSSION

The JAK-STAT is utilized by the Janus kinase (*JAK*), which is bread of intracellular, non-receptor tyrosine kinases, to change the energy of, or transduce, cytokine-mediated signals. These Janus kinase-signal transducers and activators of transcription may be the key constituents of cytokines receptor systems and growth regulators. Therefore, *JAK* activation results in cell differentiation, migration, propagation and apoptosis. These cellular mechanisms are a requisite part for some processes including hematopoiesis, immune development, mammary gland development and lactation, abiogenesis and sexually dimorphic growth (Igaz, Toth et al., 2001, O'Shea, Gadina et al., 2002). *JAK2* particularly provides a ciphering or encoding function for Tyrosine-protein kinase linked to the cytoplasmic domains of type I and II cytokine receptors (Kralovics, Passamonti et al., 2005).

The *JAK2* V617F exon 14 mutations is the most frequent mutation associated with Philadelphia-negative MPNs; it was detected in >70% of MPNs (Kralovics, Passamonti et al., 2005). The frequency of the *JAK2* V617F exon 14 mutations varied between the MPNs subtypes; it can be found in approximately 95% cases of PV and about 50-60% cases of ET or PMF (Baxter, Scott et al., 2005, Kubo, Toh et al., 2015). In numerous published studies, the *JAK2* V617F exon 14 mutations was detected in 80% - 97.0% in PV Zhu et al., 2011; Jones A, 2005; Ebid et al., 2015; Ayad and Nafea, 2011; Speletas et al., 2007).

In agreement with these reports, in our study, the *JAK2* V617F exon 14 mutations has been detected in 61.3% of patients with MPNs. The prevalence of the mutation was 68.6%for PV, 50% for ET and PMF. Also the findings of this study were similar to the results obtained by Baxter et al. (Baxter et al., 2005); they detected the *JAK2* V617F exon 14 mutations in 29 (57%) of 51 with ET, eight (50%) of 16 with idiopathic myelofibrosis, and 71 (97%) of 73 patients with PV. Similarly, Ayad et al. (Ayad and Nafea, 2011) detected the JAK2 V617F exon 14 mutations in 81.4% of PV, 50% of ET, 46.1% of PMF. The study by Karkucak et al. (Karkucak et al., 2012) was in line with this study. They found that 80% of the PV group and 42% of the ET group were positive for the *JAK2* V617F exon 14 mutations. Also, our study was consistent with the study by Zhu et al., who detected the mutation in 83.9%, 60% in PV and ET, respectively (Zhu et al., 2011).

In summary, in our study, the prevalence of *JAK2* V617F exon 14 mutation in Sudanese MPNs patients is consistent with previous studies of the prevalence of the *JAK2* V617F exon 14 mutation in MPN patients worldwide.

The finding of the *JAK2* V617F exon 14 mutations in several related disorders led to the hypothesis that its presence or absence or its quantity could modify the disease phenotype. In clinical studies, correlations of *JAK2* V617F exon 14 mutation with parameters such as age, white blood cell (WBC) count, Hb concentration, Hct, platelet count, spleen size, disease duration, pruritus, and fibrosis in subjects with MPN have been evaluated frequently(Baxter, Scott et al., 2005). In numerous studies, the *JAK2* V617F exon 14 mutations has been variably associated with higher Hb, WBC, older age and unchanged or decreased platelet count (Speletas, et al., 2006; Kittur, et al., 2007; Benmoussa, et al., 2009; Yonal, et al., 2015). Another study by Campbell et al. showed that patients with the mutation present with higher haemoglobin levels, higher white cell counts in PV (Campbell et al., 2005). In the study of Singh et al., it has been shown that *JAK2* V617F exon 14 negative patients with PMF were significantly associated with more severe anaemia, younger age and thrombocytopenia (Singh et al., 2015). Several studies (Rudzki et al., 2007; Yonal et al., 2015) revealed a positive correlation with the *JAK2* V617F exon 14 mutation and higher Hb, WBC, older age and lowest platelet count in patients with ET. In contrast, other studies (Ha, et al., 2012; Sultan and Irfan, 2015) showed no significant correlations between V617F mutation and patient age, white blood cell count, Hb, Hct, or the platelet count for ET or PMF patients.

In our study, a significant association between older age and *JAK2* V617F exon 14 positive PMF patients was observed (p= .000). The JAK2 V617F exon 14 positive PV patients had high Hb concentration level and high HCT in comparison with JAK2 V617F exon 14 negative patients, these difference was statically significant (p= .018) (p= .016). Also, a significant association was observed between JAK2 V617F exon 14 positive PV patients and total RBCs (p= .005).

In contrast, no significant differences in age, total RBCs, Hb concentration, HCT or platelets count were observed between *JAK2* V617F exon 14 positive and *JAK2* V617F exon 14 negative ET

patients, no significant difference in total WBC was found between *JAK2* V617F exon 14 positive and *JAK2* V617F exon 14 negative PMF patients, even though PMF patients who harbor the mutation have the highest total WBC count. No significant differences were detected between *JAK2* V617F exon 14 mutation and total WBC, or platelets count in PV patients, whoever *JAK2* V617F exon 14 positive patients had slightly high total WBC count and the most upper platelets count. Although, *JAK2* V617F exon 14 positive PV and ET patients high mean platelets count, but the difference was not statically significant. *JAK2* V617F exon 14 positive ET patients had high mean age, the difference was not significant statically.

CONCLUSION

The *JAK2* V617F exon 14 mutation could be frequently detected in the Sudanese patients with MPNs, the vast majority of polycythemia patients and around half of the essential thrombocythemia and primary myelofibrosis have the mutation.

The permission for the study was taken from the research committee at University of Khartoum and the Ministry of Health, Khartoum state. Informed consent was from each participant before the collection of the sample.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Elrashed Babiker Yasin: was responsible for designing the research project, data collection, specimen's analysis, laboratory work, and writing of the paper and communication with the authorities, he is the corresponding author.
Professor Anwar Alkordofani: was responsible for data analysis and writing the manuscript.
Dr. Bahaeldin K. Elamin PhD: was responsible for designing the research project, data collection and communication for the approval.
Dr. Ashraf Dallol PhD: was responsible for the logistic support of the research project.

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