Janus Kinase2 V617F Mutation in Sudanese Patients with Essential Thrombocythemia

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Abstract
Background: Janus Kinase2 (JAK2) is a cytoplasm tyrosine kinase involved in transduction of signal from growth factor receptor on auto phosphorylation following activation via ligand binding, JAK2 recruit STAT molecules which are then phosphorylated and translocate to the nucleus to act as transcription factor. Mutations of JAK2 gene have reported to be associated with all myeloproliferative disorders with variable frequency.

Objective: This study aimed to determine the frequency of JAK2 V617F mutation in Sudanese patients with Essential Thrombocythemia (ET) and investigate its correlation with platelet count, age of incidence, and patients' demographic data.

Material and Methods: A total of 50 patients with ET were enrolled in this study. Three milliliter (ml) of venous blood was collected from each subject and DNA was extracted from peripheral leukocytes by salting out method. JAK2 V617F mutation was detected by allele -specific competitive blocker polymerase chain reaction. Platelet was counted using automated hematology analyzer.

Results: A total of 50 Sudanese patients with essential thrombocythemia were enrolled in this study; 23(46%) of them were males and 27(54%) were females; their age range from 18 to 82 years. The results showed that 31(62%) of patients were positive for JAK2 V617F mutation. The platelet count was found higher in patients with the mutation than those without the mutation but the different was not statistically significant ((Mean±SD: 1071.6±543.5X10^3/µl and 956.3±508.9 X10^3/µl respectively, P.value: 0.85)). Also no statistically significant difference was found in mean age of incidence in patients with the mutation compared to those without the mutation (Mean±SD: 53.8±15.7 & 48.8±13.8 years respectively, P. value: 0.67).

Conclusion: About two third of the Sudanese patients with ET were found to have JAK2 V617F mutation. Presence of the mutation has no significant effect on platelet count or age of incidence.

Keywords: Essential thrombocythemia; Janus Kinase mutation; platelet count.
Introduction
The myeloproliferative disorders are a group of hematological conditions where there is primary disorder at the level of multipotent hematopoietic stem cell leading to increase production in one or more blood cell type. The main three disorders in group are polycythemia Vera (PV), essential thrombocythemia (ET), and idiopathic Myelofibrosis (IM) [1].
Essential thrombocythemia is an acquired myeloproliferative disorder characterized by sustained elevation of platelet count which is related to an expansion of megakaryocytic lineage and usually considered to be clonal disease arising in a multipotent stem cell. The disease can induce severe neurological complain, cardiac or peripheral arteries disabilities, and deep vein thrombosis [2].
Janus Kinase2 (JAK2) is a member of a group of cytoplasmic tyrosine kinases that are involved in transduction of signal from growth factor receptor on auto phosphorylation following activation via ligand binding. JAK2 recruit STAT molecules which are then phosphorylated and translocate to the nucleus to act as transcription factor [3]. JAK2 contributes to synthesis of erythropoietin, thrombopoietin, and granulocyte stimulating factor (GSF) receptors and serves as tyrosine kinase for them. A point mutation of JAK2 has been reported in 2005, resulting in Valine to Phenylalanine substitution at 617 amino acids; as a result JAK2 escape from auto regulatory system and become active without physiological signal [4].
Different groups reported variable frequency of JAK2 V617F mutation in ET ranging from 23%-57% [5-10]. It has been associated with an increase risk of arterial thrombosis but do not affect survival or leukemic transformation [11, 12].
This study aimed to determine the frequency of JAK2 V617F mutation in Sudanese patients with essential thrombocythemia and investigate its correlation with platelet count, age of incidence, and patients' demographic data.

Materials and methods
Study subjects and sample collection
This study is a descriptive cross-sectional study, conducted in Khartoum state, Sudan. Three milliliter (ml) venous blood samples were collected from a total of 50 Sudanese patients with ET in ethylene diamine tetra acetic acid (EDTA) blood tube for molecular and haematological investigations.

Molecular analysis
Genomic DNA was extracted from peripheral blood leukocytes using salting out protocol. JAK2 V617F mutation was detected by allele– specific competitive blocker polymerase chain reaction (ACB-PCR). Target DNA fragment was amplified by using Forward primer (5-GCATTT GG T TTTAATTATGGAGT-ATGTG-3) and Reverse primer (5-ACTG-
ACA CCT AGC TGT G ATCCTG-3). The thermocycling conditions included initial denaturation at 95°C for 5 minutes, then 45 cycles (each consist of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds), and final extension at 72°C for 10 minutes. PCR product was electrophoreses on 3% a agarose gel containing ethedium bromide and demonstrated by gel documentation system.

**Platelet count**
Platelets count was performed using automated hematology analyzer (Sysmex KX 21N)

**Data collection and analysis**
Data of this study was collected by structured interview questionnaire and from patients' medical files and analyzed by statistical package for social sciences (SPSS). Frequency of categorical variables was calculated and correlation of JAK2 mutation with categorical variables was tested by Chi-square test. Quantitative variables were represented as mean±SD. Means of platelet count and age of incidence were compared in patients with the mutation and those without the mutation using independent 2-sample t-test.

**Ethical considerations**
The study was approved by scientific research committee of the faculty of medical laboratory sciences, Al Neelein University and informed consent was obtained from each participant before sample collection.

**Results**
A total of 50 Sudanese patients with ET were enrolled in this study, 23(46%) of them were males and 27(54%) were females; their age range from 18 to 82 years (Mean±SD: 51.9±15.0). JAK2 V617F mutation was detected by allele specific polymerase chain reaction (AS-PCR); the size of the amplified mutant fragment was 139 bp. The results showed that 31(62%) of patients were positive for JAK2 V617F mutation. The platelet count was found higher in patients with JAK2 V617F mutation than those without the mutation but the difference was not statistically significant (Mean±SD: 1071.6±543.5X10^3/µl and 956.3±508.9 X10^3/µl respectively, P.value: 0.85). No statistically significant difference was found in mean age of incidence in patients with the mutation and those without the mutation (Mean±SD: 53.8±15.7 and 48.8±13.8 years respectively, P.value: 0.67). The mutation was more frequent in females (65%) than males (35%) but no statistically significant correlation was found between the mutation and gender (P.value: 0.06)

**Discussion**
It has been recently shown that the majority of patients with ET carry a single nucleotide mutation in JAK2 gene as a...
result of substitution of valine by phenylalanine in codon 617 exon 12 [5-10].
This study aimed to determine the frequency of JAK2 V617F mutation and study its correlation with patients' demographic and hematological data.

The result showed that 31 out of 50 patients (62%) with ET were positive for JAK2 mutation, 20(65%) of them were females and 11(35%) were males. The frequency of the mutation in different populations was variable, while some findings were similar to our results, others were different; In United Kingdom the frequency was 57% , France 43%, USA 32%,, and Turkey 62.1% [5,6,7,10,13]. These differences may be due to ethnic variations.

Platelet count was found higher in patients with JAK2 V617F mutation than those without the mutation but the difference was not statistically significant (P.value:0.85); this finding agrees with that of Poopak et al, who reported that, there is no significant difference in platelet count between patients with JAK2 mutation and those without it [14].

The mean age of incidence was slightly increased in the patients without the mutation but the difference was not statistically significant.

In the current study, no statistically significant correlation was found between gender and the mutation in spite of the occurrence of the mutation more frequently in females than males. This was in agreement with the finding reported by Ilhan et al who reported that, there is no difference in gender between mutated and unmutated patients [13].

In conclusion, about two third of the Sudanese patients with ET were found to have JAK2 V617F mutation. Presence of the mutation has no significant effect on platelet count or age of incidence.

References


