

Coexistence of JAK2V617F Mutation and Philadelphia Chromosome in Sudanese Patients with Chronic Myeloid Leukemia

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Abstract

Background: Chronic myeloid leukemia (CML) is one of the myeloproliferative disorders results from an acquired genetic defect characterized by expansion of myeloid cell mass replacing normal haemopoiesis, it was the first malignant disorder reported in association with a chromosomal aberration, the Philadelphia chromosome (Ph). CML can also change into a fast-growing acute Leukemia that invades almost any organ in the body.

JAK2V617F mutation have been reported in patients with myeloproliferative disorders including polycythaemia vera , essential thrombocythaemia and myelofibrosis with different frequency; recently, some authors have reported the coexistence of JAK2V617F and BCR/ABL+ in CML patients expressing the p210 BCR–ABL oncoprotein.

The aim of this study was to determine the frequency of JAK2V617F mutation among Sudanese patients with Philadelphia positive CML.

Materials and methods: A total of 49 Sudanese CML patients were enrolled in this study, three milliliter (ml) of venous blood were collected from each patient in ethylene diamine tetraacetic

acid (EDTA). For molecular analysis DNA was extracted from EDTA anticoagulated blood sample by GF-1BLOOD DNA EXTRACTION KIT. JAK2 V617F mutation was detected using Allele-specific competitive blocker (ACB)-PCR assay.

Results: 19 out 49 patients were positive for the mutation, of them 11(22.4%) were males and 8(16.3%) were females; the correlation was not statistically significant.

No statistically significant correlation was found between the mutation and each of ethnic origin (*p. value* 0.33), hepatomegaly (*P. value* 0.12), and splenomegaly (*p. value* 0.30).

Conclusion: In summary JAK2V617F mutation is detected in 38.8% of Sudanese patients with Philadelphia positive CML.

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Introduction

Chronic myeloid leukemia (CML)- also known as chronic myelogenous leukemia and chronic granulocytic leukemia- is a clonal disease that results from an acquired genetic change in a pluripotential haemopoietic stem cell. This altered stem cell proliferates and generates a population of differentiated cells that gradually displaces normal haemopoiesis and leads to a greatly expanded total myeloid mass. One important landmark in the study of CML was the

discovery of the Philadelphia (Ph) chromosome in 1960, the next was the characterization in 1973 of the (9;22)(q34;q11) translocation, a third was the identification in the 1980s of the BCR – ABL (now renamed BCR– ABL1) chimeric gene and associated oncoprotein, and a fourth was the demonstration that introducing the BCR–ABL gene into murine stem cells in experimental animals caused a disease that simulated human CML (1).

The Janus kinases are a family of large cytoplasmic tyrosine kinases with molecular weights in the range of 120–140 kDa (1130–1142 aa). In mammals, there are four members of the Jak family: Jak1, Jak2, Jak3, and Tyk2. From C-terminal to N-terminal, Jaks consist of seven conserved domains, termed Jak homology (JH) domains 1-7. JH1 and JH2 domains exert the most important functions of Jaks. JH1 acts as a kinase domain, containing the ATP-binding region and the activation loop. The JH2 domain is the pseudokinase domain, which is highly homologous to tyrosine kinase domain, but lacks the catalytic activity due to the absence of necessary residues. The pseudokinase domain is believed to have autoinhibitory function and regulate both basal activity of the Jak kinases and cytokine-induced activation of the catalytic function (2,3).

The role of JAK2 in haematopoiesis is expression of hematopoietic growth factors receptors on the cell surface. These receptors transmit erythropoietin (EPO), thrombopoietin (TPO), cytokines, growth factors e.g. 1L-3, IL-5 and Granulocyte-Monocyte colony stimulating factor (GM-CSF) (4). It is believed that mutation disrupt the auto-inhibitory effect of JH2 on JH1 domain which lead to both constitutive activation and hypersensitivity to the effect of cytokines(5). There is constant activation of signal transducer and activation of transcription3 (STAT3), up regulation of anti-apoptotic protein Bcl-xL³ and enhanced AKT activity (6). This

deregulated signaling induces clonal expansion of hematopoietic progenitors that are independent of normal growth factor control (6).

Normally, the JAK/STAT pathway is tightly controlled to ensure normal blood cell production and function, but disruptions in the pathway can cause disease states. The acquired JAK2V617F mutation occurs in a spectrum of the Philadelphia chromosome (Ph)- negative chronic myeloproliferative disorders (CMPDs), which include polycythemia Vera, essential thrombocythemia, and myelofibrosis. However, an increasing number of cases of Ph positive CML with concomitant JAK2V617F mutation have recently been reported. There are few previous reports of the transformation of myeloproliferative disorders [PV (7,8), CIMF (9,10) or ET (11) into chronic myeloid leukaemia. The mutation is a G-T substitution at nucleotide 1849 in codon 617 of the JAK2 gene which results in substitution amino acid valine-to-phenylalanine that confers constitutive tyrosine kinase activity to the mutant gene.

In the present study, we aimed to determine the frequency of *JAK2V617F* mutations in Sudanese patients with Ph positive CML.

Materials and methods

This is a cross sectional study conducted at the radiation and isotopes center of Khartoum (RICK), Khartoum state, in the period from June to August 2014.

A total of 49 Sudanese patients with Ph positive CML were enrolled in this study; their age range between 23-80 years. Patients with negative Ph chromosome were excluded from the study.

Three milliliter (ml) of venous blood were collected from each patient in ethylene diamine tetraacetic acid (EDTA) container for molecular analysis.

Data was collected by using structured interview questionnaire.

DNA Extraction

Genomic DNA was extracted from EDTA blood sample using DNA extraction kit (GFI-1 BLOOD DNA EXTRACTION KIT, MALYSIA) according to the manufacturer's instructions.

JAK2V617F Mutation Analysis

JAK2 V617F mutation was detected using Allele-specific competitive blocker polymerase chain reaction (ACB-PCR). One micro liter (μ l) of DNA was amplified in a total volume of 20 μ L containing 0.5 μ l of normal forward primer: 5'-GCATTTGGTTTTAAATTATGGAGTATGTG-phosphate (a phosphate group was added to the 3' end to prevent extension from the normal primer, blocking amplification of the normal allele), 1 μ l of each mutant forward primer: 5'-GCATTTGGTTTTAAATTATGGAGTATGAT-3'), and reverse primer: 5'-ACTGACACCTAGCTGTGATCCTG-3'), 4 μ l Matser mix (GoTaq® Green Master Mix, Promega, USA) and 13.5 μ l sterile distilled water. The cycling conditions include initial denaturation at 95°C for 5 minutes; 45 cycles 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.

Five μ l of the PCR product (ready to load) was electrophoresed on 3% Agarose gel, stained with ethidium bromide and then demonstrated by gel documentation system (SYNGENE, JAPAN).

The presence of the 139-base-pair (bp) band indicated that the sample was positive for the JAK2 V617F mutation (mutated type) whereas absence of the band indicates that sample was negative for the mutation (wild type) as seen in figure (1).



Figure (1) Agarose gel electrophoresis image for JAK2V617F mutation analysis after PCR (100 bp ladder) show four positive samples (1,3,4 and 7) lanes from the right and three negative samples.

Statistical analysis

Data of this study was analyzed by statistical package for social sciences (SPSS), version 21. Frequency of JAK2V617F mutation and other qualitative variables were determined; age of the patients with and without the mutation was compared by independent 2-sample test; correlation between the mutation and gender, hepatomegaly, splenomegaly, and ethnic groups was tested by Chi-square test.

Ethical considerations

This study was approved by faculty of medical laboratory sciences, Al Neelain University and by RICK. Furthermore, informed consent was taken from each patient before sample collection.

Results

A total of 49 Sudanese patients diagnosed with ph'-positive chronic myeloid leukemia at RICK were enrolled in this study. Their ages ranged between 8-75 years (Mean $\pm$ SD: 41.4 $\pm$ 1.5); 33(67.3%) of them were males and 16(32.7%) were females. 38 patients (76.7%) were suffering from massive splenomegally and 10 patients (20.4%) were suffering from hepatomegally. 23 (68%) of the patients were belonged to the Afro-Asiatic ethnic group, 3(6%) were belonged to the Nilo-Saharan group and 12(24%) were belonged to the Niger-Congo group.

The investigated JAK2V617F mutation was positive in 19 (38.8%) out of 49 of the study subjects; of them 11(22.4%) were males and 8(16.3%) were females; the correlation was not statistically significant.

Comparison of age in the patients with the mutation and those without the mutation showed no statistically significant difference (Mean $\pm$ SD: 42 $\pm$ 17.2 and 41 $\pm$ 15.9 respectively; *P.value*: 0.814).

The distribution of the JAK2V617F mutation among ethnic groups showed that, 50% of the patient from the Niger-Congo group, 32.3% of those from the Afro-Asiatic group, and 66.7% of those from the Nilo-Sahara were positive for the mutation.

Sixteen out of 38 patients (42.1%) presented with splenomegaly and six out of 10 patients (60%) presented with hepatomegally also positive for the mutation.

No statistically significant correlation was found between the mutation and each of ethnic origin, hepatomegaly, and splenomegaly (Table 1).

Table 1 Correlation between JAK2V617F mutation and patients' demographic and clinical data

Variable	JAK2 (negative)		JAK2 (positive)	P.value
Gender	Male	22 (66.7%)	11 (33.3%)	0.20
	Female	8 (50%)	8 (50%)	
Splenomegaly	Yes	22 (57.9%)	16 (42.1%)	0.30
	No	8 (72.7%)	3 (27.3%)	
Hepatomegally	Yes	4 (40%)	6 (60%)	0.12
	No	26 (53.0%)	13 (26.5%)	
Ethnic origin	Afro-asiatic	23 (67.7%)	11 (32.3%)	0.33
	Nilo-Saharan	1 (33.3%)	2 (66.7%)	
	Niger-Congo	6 (50%)	6 (50%)	

Discussion

The presence of JAK2V617F mutation in patient with Philadelphia positive CML lead to advanced disease or early disease progression therefore its important to give the patient JAK2V617F inhibitor beside imatinib mesylate drugs (13).

The present study investigated the coexistence of JAK2V617F mutation and Philadelphia chromosome in Sudanese patients with CML.

The results showed that the frequency of JAK2V617F mutation in Sudanese patients with Philadelphia positive CML was 38.8%. This results agree with study done by Tabasumm *et al*

who reported the presence of JAK2V617F mutation in 44% of Philadelphia positive CML patients in Pakistan (12).

Another study also in Pakistan reported a frequency of 26.7 %, most of them showed early disease progression (13).

The result of the present study disagrees with many studies showed that no JAK2V617F mutation was detected in Philadelphia positive CML (14, 15), Also our result disagreed with Al-Kaabi *et al* who reported a frequency of only 1.1% for JAK2V617F mutation in Iraqi patients with Philadelphia positive CML (16) and Chonchon M.R *et al* who reported the presence of one case in 165 studied patients in central America (17).

These variations may cause differences in the nature of the disease in these different populations and may be due ethnic variations.

The explanation of the coexistence of both BCR/ABL translocation and JAK2V617F mutation in some patients may be due to two hypotheses that have been proposed (18). The first hypothesis which has been favored in the several literatures suggested that a single clone possesses one aberration and the patient's phenotype (e.g. CML feature) is dependent on the dominant clone (e.g. BCR/ABL translocation positive) which is determined by the selective pressure exerted by the specific treatment (e.g. hydroxyurea) prescribed for the other clone (e.g. JAK2V617F mutation positive) (19,20). The second hypothesis proposes that a single clone concurrently possesses both the BCR/ABL translocation and JAK2V617F mutation (15,16). The second hypothesis was supported by a recent study, which reported that the BCR/ABL translocation occurred in a pre-existing JAK2V617F mutation positive clone (17).

In this study no statistically significant difference was found in mean age in patients with the mutation and those without the mutation. This agrees with the finding of Pahore *et al* who reported same result (13).

No statistically significant correlation was found between the mutation and each of gender and ethnic origin of the patients; and also with hepatomegally and splenomegally.

In conclusion, JAK2V617F mutation was reported in a large proportion of Sudanese patients with Philadelphia positive CML; this should be considered when investigations of patients with CML are carried out, and JAK2V617F inhibitors should be implemented for those with the mutation.

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References

1. Hoffbrand A, Catovsky D , Tuddenham E , Green A . postgraduate haematology. Sixth edition,Wiley-blackwell, 2011, 687.
2. Saharinen P, Takaluoma K, Silvennoinen O. Regulation of the Jak2 tyrosine kinase by its pseudokinase domain. Mol Cell Biol. 2000;20:3387-3395.

3. Saharinen P, Vihinen M, Silvennoinen O. Autoinhibition of Jak2 tyrosine kinase is dependent on specific regions in its pseudokinase domain. *MolBiol Cell*. 2003;14:1448-1459.
4. Steensma DP. JAK2 V617F in myeloid disorders: Molecular diagnostic techniques and their clinical utility. *J Molecular Diagnostics*. 2006;8:397-409.
5. Delhommeau F, pisani DF, Jamis C, Casdivall N, Constantinescu S, Vainchenker W, Oncogenic mechanisms in myeloproliferative disorders. *Cell Mol Life Sci* 2006;63:2939-53; P MID :17131059;http://dx.doi.org/10.1007/s00018-006-6272-7.
6. McLornan D, Percy M, McMullin MF. JAK2 V617F: A single mutation in the myeloproliferative group of disorders. *Ulster Med J*. 2006;75:112-119.
7. Bocchia M, Vannucchi AM, Gozzetti A, et al. Insights into JAK2-V617F Mutation in CML. *Lancet Oncol*, 2007; 8: 864–6.
8. Mirza I, Frantz C, Clarke G, et al. Transformation of Polycythemia Vera to Chronic Myelogenous Leukemia. *Arch Pathol Lab Med*, 2007; 131: 1719–24.
9. Hussein K, Bock O, Seegers A, et al. Myelofibrosis Evolving During Imatinib Treatment of a Chronic Myeloproliferative Disease with Coexisting BCR-ABL Translocation and JAK2V617F mutation. *Blood* 2007; 109: 4106–7.
10. Bornhauser M, Mohr B, Oelschlaegel U, et al. Concurrent JAK2 (V617F) Mutation and BCR-ABL Translocation within Committed Myeloid Progenitors in Myelofibrosis. *Leukemia* 2007; 21: 1824–6.
11. Curtin NJ, Campbell PJ, Green AR. The Philadelphia Translocation and Pre-existing Myeloproliferative Disorders. *Br J Haematol*, 2005; 128: 734–6.

12. Tabassum N, Saboor M, Ghani R, Moinuddin M. Frequency of JAK2 V617F mutation in patients with Philadelphia positive Chronic Myeloid Leukemia in Pakistan. Pak J Med Sci 2014;30(1):185-188.
13. Pahore Z , Shamsi T , Taj M , Farzana M , Ansari S , Nadeem M , et al. JAK2V617F Mutation in Chronic Myeloid Leukemia Predicts Early Disease Progression.JCPSP 2011, Vol. 21 (8): 472-475.
14. Jelinek, J.; Oki, Y.; Gharibyan, V.; Bueso-Ramos, C. ; Prchal, J.T.; Verstovsek, S.; Beran, M.; Estey, E.; Kantarjian, H.M. and Issa, J.P. (2005). JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood. 106(10):3370- 3373.
15. Xiao, X.; Zhang, Y.; Zhang, G.S.; Zheng, W.L.; Xiao, L. and Liu, S.F. (2012). Coexistence of JAK2 V617F mutation and BCR-ABL1 transcript in two Chinese patients with chronic myelogenous leukemia. Acta Haematol. 127 (1): 47 -49.
16. AL- Kaabi M, Al-Obaidy Y , Sabir S , AL- Kadmy I. INCIDENCE OF JAK2 V617F MUTATION IN IRAQI PATIENTS WITH PHILADELPHIA POSITIVE CML.MJPMS,VOL 2 Issue 4, Oct – Dec 2013
17. Conchon, M.R.; Costa, J.L.; Novaes, M.M.; Dorlhiac-Llacer, P.E.; de Alencar Fischer Chamone, D. and Bendit, I. (2008). Simultaneous detection of JAK2 V617F mutation and Bcr-Abl translocation in a patient with chronic myelogenous leukemia. Int J Hematol . 88(2):243-245.
18. Park, S.H.; Chi, H.S.; Cho, Y.U.; Jang, S.; Park, C.J.; Kim, D.Y.; Lee, J.H. and Lee, K.H. (2013). Two Cases of Myeloproliferative Neoplasm with a Concurrent JAK2V617F

Mutation and BCR/ABL Translocation without Chronic Myelogenous Leukemia Phenotype Acquisition during Hydroxyurea Treatment. *Ann Lab Med.* 33(3):229-32.

19. Véronèse, L.; Tchirkov, A.; Richard-Pebrel, C.; Ledoux-Pilon, A.; Fleury, J.; Chaletteix, C.; Goumy, C.; Gouas, L.; Berger, M.G.; Vago, P.; Bay, J.O. and Tournilhac, O. (2010). A thrombocytosis occurring in Philadelphia positive CML in molecular response to imatinib can reveal an underlying JAK2(V617F) myeloproliferative neoplasm. *Leuk Res.* 34(4):e94-96.
20. Kim, Y.K.; Shin, M.G.; Kim, H.R.; Yang, D.H.; Cho, S.H.; Lee, J.J.; Chung, I.J.; Ryang, D.W. and Kim, H.J. (2008). Simultaneous occurrence of the JAK2V617F mutation and BCR-ABL gene rearrangement in patients with chronic myeloproliferative disorders. *Leuk Res.* 32(6):993-995.