Mintage Journal of Pharmaceutical & Medical Sciences
RESEARCH ARTICLE

INCIDENCE OF JAK2 V617F MUTATION IN IRAQI PATIENTS WITH PHILADELPHIA POSITIVE CML

[1]Marwah Hasan Abd Ali AL- Kaabi[1], Yusra Ghiath Yaseen Al-Obaidy[1] , Shahla´a Fadhil Sabir[1] and Israa Mohamed Safi AL- Kadmy[2]

[1]National Center Of Hematology/Al –Mustansiriya University [2]Department of Biology /College of science /Al –Mustansiriyah university
Email: marwah523@gmail.com

Received -16-08-13; Reviewed and accepted -25-08-13

ABSTRACT

Objective: The aim of this study was to detect the occurrence of JAK2 V617F mutation in CML Iraqi patients. Methods: In this study 89 blood samples were collected from Iraqi CML patients who receiving imatinib mesylate or nilotinib as a treatment at National Center of Hematology during the period January 2013-June 2013, then DNA were extracted and ARMS-PCR technique was performed to detect the JAK2V617f mutation. **Results:** Out of 89 patients, JAK2V617F mutation was detected in only one patient (1.1%), a 52 year-old woman who was on Imatinib mesylate therapy for five years. Conclusion: Such rare patients with co-existingBCR-ABL translocation and JAK2V617F mutation must be identified in view of the possibility of targeted therapies.

Key words: Chronic myeloid leukemia, JAK2 V617F mutation, ARMS-PCR.

INTRODUCTION

Chronic myelogenous leukemia (CML), also referred to as chronic myeloid, chronic myelocytic, or chronic granulocytic leukemia, was the first malignant disorder reported in association with a chromosomal aberration, the Philadelphia chromosome (Ph^1) [1]. CML is characterized by clonal expansion of bone marrow stem cells leading to selective granulocytic hyperplasia with or without thrombocytosis. Ph chromosome is a microscopically visible short 22q resulting from an asymmetrical reciprocal t (9; 22) (q34; q11.2) chromosomal translocation which creates a BCR-ABL1fusion gene with three different principal protein products [1].

JAK2 is a member of the Janus kinase (JAK) family of non-receptor tyrosine kinases comprising of four family members (JAK1, JAK2, JAK3, and TYK2) [2]. These are cytoplasmic tyrosine kinases that mediate intracellular signalingfor most cytokines, including many interleukins (ILs), the interferons (IFNs) and several hormones [3]. Mutations and translocations in the JAKgenes leading to constitutively active JAK proteinsare associated with a variety of hematopoietic malignancies, including the myeloproliferative disorders(JAK2), acute lymphoblastic leukemia (JAK2), acute myeloid leukemia (JAK2, JAK1), acute megakaryoblastic leukemia (JAK2, JAK3) and T-cell precursor acute lymphoblastic leukemia (JAK1) [4].

JAK2 plays a major role in myloid disorders [5]. JAK2 V617F mutation is characterized by a G to T transverse at nucleotide 1849 in exon 12 of the JAK2 gene, located on the chromosome 9 p, leading to a substitution of valine to phenylalanine at amino acid position 617 in the JAK2 protein [5]. This mutation plays a fundamental role in the pathogenesis and development of Chronic myeloproliferative neoplasms (CMPNs), and that its detection is very useful to confirm diagnosis and to provide anearly detection assay of the CMPNs [6].

Indeed, the JAK2V617F mutation is present in the great majority of patients with PV and approximately half of patients with ET and chronic idiopathic myelofibrosis (CIMF) [7]. After Jelinek*et al., [8]* reported the absence of the JAK2 mutation in chronic phase Ph positive CML in approximately 100 patients; it was thought that the JAK2 mutation and the BCR-ABL translocation were mutually exclusive. However, an increasing number of cases of Ph-positive CML with concomitant JAK2V617F mutationhave been recently reported.The recent recognition that JAK2 may bean important therapeutic target emphasizes the importance of identifying such rare patients with co-existing BCR-ABL translocation and JAK2V617F mutation [7].

MATERIALS AND METHODS

Patients and sampling

This study conducted between January 2013-June 2013 including 89 Iraqi cases of chronic myeloid leukemia who receiving imatinib mesylate or nilotinib as a treatment at National Center of Hematology. The study was approved by the Review Ethical Committee. They were diagnosed clinically, hematologically and cytoginically as CML. Hematological parameters including complete blood picture and differential count were taken from patient's record at time of sampling. The patients were randomly selected concerning to age, gender, disease duration, disease phase and treatment. Screening for JAK2 V617F mutation was performed to all patients.

Sample collection

Two ml of vein puncture blood sample was taken from 89 CML patients by using disposable syringe, and then dispensed in tube containing EDTA as anticoagulant and kept immediately at 4 ºC for DNA extraction.

DNA extraction and ARMS PCR amplification:

DNA was extracted from peripheral blood using Wizard Genomic DNA Purification kit from Promega USA. The JAK2V617F mutation was detected by ARMS-PCR technique. This technique uses 4 primers as follows; a forward outer primer (FO), a reverse outer primer (RO), a forward inner wild type specific primer (Fwt) and a reverse inner mutant specific primer (Rmt).

The oligonucleotide PCR primers specific for the JAK2 V617F mutation, melting temperature, pmol /µg was listed in table (1). The forward primer from one set and the reverse from the other generate a control 463-bp band in all cases. The reverse inner mutant specific primer and the forward outer primer generate a 279-bp mutant fragment. In the presence of the wild-type JAK2, the reverse outer primer and the forward inner wild type specific primer produce a fragment of 229-bp.

PCR reaction was performed according to the procedure that suggested by the manufacture company (Promega, UAS) within a total volume of 25 µL containing 3.5 µl (25-1250 ng) DNA, 12.5 µL of Go Taq® Green Master Mix, 2x (Promega, UAS), 0.5 µl of each FO, RO and Fwt, and 1µl of Rmt primer with adding 6.5 µl of nuclease free water to get final volume of 25 µl.

Table 1: The primers that were used in PCR amplification [9]

Primer name	Nucleotide sequences (5 ' ⟶ 3')	Tm	pmol/µg
Forward outer (FO)	TCCTCA GAACGTTGATGGCAG	54.2	230
Reverse outer (RO)	ATTGCTTTCCTTTTTCACAAGAT	48	228
Forward inner wild type specific (Fwt)	GCATTTGGTTTTAAATTATGGAGTATATG	52.9	197
Reverse inner mutant specific primer (Rmt))	GTTTTACTTACTCTC	55.1	338

The conditions of PCR amplification steps was carried on the thermal cycler (C 1000 thermal cycler, BIO-RAD, UAS) as follows: an initial denaturation step at 94°C for 6 min, followed by 40 cycles of denaturation at 94°C for 40 sec., anneling at 56°C for 45 sec., extension at 72°C for 45 sec., and a final extension step of 10 min at 72 °C [9].
Finally, the amplified PCR products were submitted to electrophoresis using 1% agarose gel with ethidium bromide (0.5 µg/mL) for 30 min (10 Volts/ cm²) and DNA ladder (100bp) was used to assess PCR product size, then PCR products were visualized by UV light at 336 nm, and photographs were taken by using digital camera.

RESULTS AND DISCUSSION

This study included 89 CML patients, all of the CML patients were previously diagnosed according to clinical presentations and morphological criteria of bone marrow examination and was confirmed by cytogenetic assay for t (9:22). Out of the 89 CML patients, 48 (53.93%) were males and 41(46.06%) were females (M:F ratio 1.1:1) and mean age was ranging from (18- 75) years. Disease duration of the patients ranging from 6 months to 12 years and all the patients were undergoing treatment, 78 (87.6%) of the patient were on imatinib were on Nilotinib (Tasigna; Novartis Pharma).

Screening analysis for the JAK2V617F mutation in this study was detected in only one patient (1.1%), a woman (52 years) who was on imatinib for five years. Figure (1) shows the positive result mesylate (Gleevec; Novartis Pharma) and 11 (12.3%) Although many studies had proposed that there was no coexistence of JAK2 V617F mutation and BCR-ABL translocation [5,8,10], the present study gives evidence for coexistence of these two diseases specific mutation is possible. The result of this study is consistence with a number of studies that described the coexistence of JAL2 V617F mutation in patients with Philadelphia positive CML [11,13,16,17,18,19].

These cases with coexistence of JAK2V617F mutation and BCR/ ABL translocation have been recently reported up to 28 cases [11,16,20]. Among these reports, the majority of the patients either had pre-existing BCR/ ABL-positive CML and developed JAK2V617F mutation while undergoing tyrosine kinase inhibitor treatment [12,13] or developed BCR/ABL-positive CML with a pre-existing JAK2V617F mutation-positive MPN [14,15]. In contrast, a small number of patientsshowed simultaneous occurrence of both JAK2V617F mutation and BCR/ABL translocation, with a CML phenotype in the bone marrow (BM) with development of symptoms or morphology associated with JAK2V617F mutation and with MPN only after imatinib treatment [11,16,17,18]

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 [11]. 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. hydoxyurea) prescribed for the other clone (e.g. JAK2V617F mutation positive) [12,13]. The second hypothesis proposes that a single clone concurrently possesses both the BCR/ABL translocation and JAK2V617F mutation [17,18]. 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 [11]. Such cases should take into account

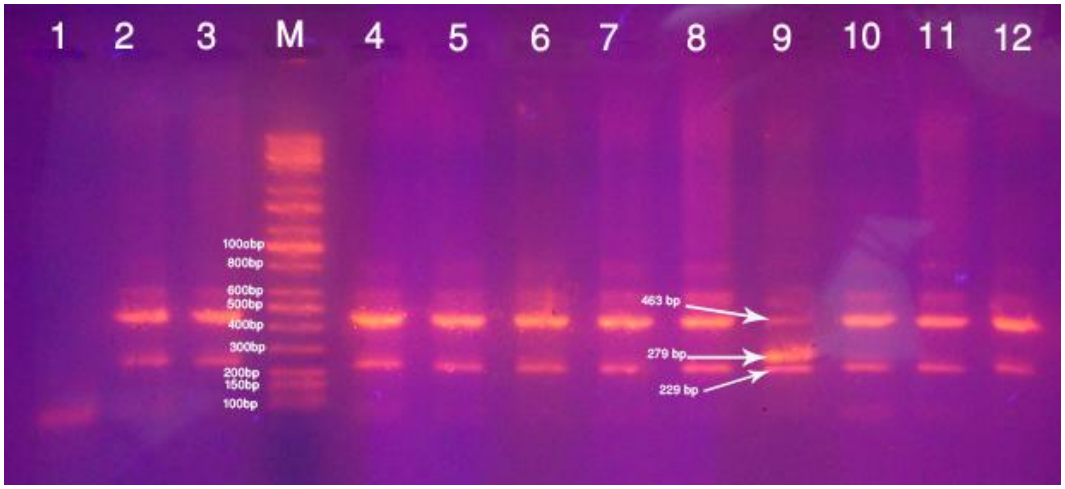

Figure 1: Agarose gel electrophoresis (1% agarose, 10 v/cm) for JAK2 V617F mutation (Amplified size 279bp) LaneM: 100bp DNA ladder, 1: negative control (water),negative result in lanes: 2,3,4,5,6,7,8,10,11, and 12.Positive result in lane: 9.

Since the WHO does not currently address the classification of mutation myeloproliferative neoplasms that have more than one genetic abnormality [16].

Together with previous reports, we suggest that screening for JAK2 V617F mutation should be considered in any Ph+ CML patients with chronic MPD since the Identification of a JAK2-V617F mutation in the presence of significant amounts of BCR-ABL transcripts will certainly and significantly alter the therapeutic strategy in such cases.

ACKNOWLADGEMENTS

We would like to thank all the working staff of the National Center of Hematology for their help and support, special thanks for Dr. Ala´a F. Alwan, Mrs. Ahlam Hafid, Mrs. Ansam Ghalib and Mr. Ali Bayan.

REFERENCES

1. Naeim, F.; Rao, P. N.; Song, S. X. and Grody, W. W. (2013). Chapter11: Chronic Myelogenous Leukemia. Atlas of Hematopathology. Elsevier Science. Pages 155-165.
2. Purandare, A. V.; Lorenzi, M. V. and Lombardo, L. J. (2010). Chapter 13: Janus Kinase (JAK2) Inhibitors for the Treatment of Myeloproliferative Neoplasm (MPN). Annual Reports in Medicinal Chemistry. v. 45:210-227.
3. Menet, C. J.; Rompaey, L. V. and Geney, R. (2013).Chapter (4). Advances in the Discovery of Selective JAK Inhibitors. Progress in Medicinal Chemistry. Elsevier B.V.52: 153-223.
4. Vainchenker, W.; Dusa, A. and Constantinescu, S.N (2008).JAKs in pathology: Role of Janus kinases in hematopoietic malignancies and immunodeficiencies *Semin Cell Dev Biol.* 19(4):385-93.
5. *Karimzadeh, P.; Ghaffari,S. H.; Ferdowsi, S.; Chahardouli,B.; Saltanatpouri,Z.; Einollahi, N.; Mousavi, S. A.; Bahar, B.;Dargahi, H.;Togheh, G.; Alimoghaddam, K. ; Ghavamzadeh, A. and Nadali, F.(2010).Comparisions* of ARMS-PCR and AS-PCR for the Evaluation of JAK2V617F Mutation in Patients with non-CML Myeloproliferative Neoplasms. *IJHOSCR.* 4(2):10-13.
6. Zahra, M.K.; El-Fadaly, N.H,; Aboul-Enein, K.M.; Elgamal, B.M.; Amira, Y. Abd El-Naby and Eman, A. Amer.(2012). JAK2-V617F Mutation and BCR-ABL Rearrangement in Chronic Myeloproliferative Neoplasms. *Life Sci J.* 9(2):403-413.
7. Jallades, L.; Hayette, S.; Tigaud, I.; Johnston, A.; Coiffier, B.; Magaud, J.P. and Ffrench, M. (2008). Emergence of therapy-unrelated CML on a background of *BCR-ABL*-negative *JAK2*V617F-positive chronic idiopathic myelofibrosis. *Leuk Res.* 32(10):1608-161
8. 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 foundin CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. *Blood*. 106(10):3370- 3373.
9. Nadali, F.; Ferdowsi, Sh.; Karimzadeh,P.; Chahardouli, B.; Einollahi, N.; Mousavi, S.A.; Bahar, B.; Dargahi, H.; Alimoghaddam, K.; Ghavamzadeh, A. and Ghaffari, S.H. (2009). JAK2-V617F Mutation and Philadelphia Positive Chronic Myeloid Leukemia. *IJHOSCR*.3 (3): 43-45.
10. Scott, L.M.; Campbell, P.J.; Baxter, E.J.; Todd, T.; Stephens, P.; Edkins. S.; Wooster, R.; Stratton, M.R.; Futreal, P.A. and Green, A.R.(2005). The V617F JAK2 mutation is uncommon in cancers and in myeloid malignancies other than the classic myeloproliferative disorders. *Blood*. 106(8):2920-2921.
11. 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 $JAK2^{V617F}$ Mutation and BCR/ABL Translocation without Chronic Myelogenous Leukemia Phenotype Acquisition during Hydroxyurea Treatment. *Ann Lab Med*. 33(3):229-32.
12. Véronèse, L.; Tchirkov, A.; Richard-Pebrel, C.; Ledoux-Pilon, A.; Fleury, J.; Chaleteix, 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.
13. Kim, Y.K.; Shin, M.G.; Kim, H.R.; Yang, D.H.; Cho, SH.; 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.
14. Bee, P.C.; Gan, G.G.; Nadarajan, V.S.; Latiff, N.A.and Menaka, N. (2010). A man with concomitant polycythaemia vera and chronic myeloid leukemia: the dynamics of the two disorders. *Int J Hematol.* 91(1):136-139.
15. Mirza, I.; Frantz, C.; Clarke, G.; Voth, A.J. and Turner, R. (2007). Transformation of polycythemia vera to chronic myelogenous leukemia. *Arch Pathol Lab Med.* 131(11):1719-1724.
16. Hummel, J.M.; Kletecka, M.C.; Sanks, J.K.; Chiselite, M.D.;, Roulston, D.; Smith, L.B.; Czuchlewski, D.R.; Elenitoba-Johnson, K.S. and Lim, M.S. (2012). Concomitant BCR-ABL1 translocation and JAK2 (V617F) mutation in three patients with myeloproliferative neoplasms. *Diagn Mol Pathol.* 21(3):176-183.
17. 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.
18. 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.
19. Yoku, O.; Kurnaz, F.; Balçık, Ö. Ş.; Uz, B. and Albayrak, M. (2010). Coexistence of JAK2V617F mutation and BCR/ABL translocation in one patient. *J Clin Exp Invest.* 1(2): 138-140.