JAK2-V617F Mutation Combined with Philadelphia Chromosome-Positive Chronic Myeloid Leukaemia: a Case Report

Mehrdad Payande[1], Mohammad Erfan Zare[1, 2], Saber Ghanbari Haji Shure[2], Farhad Shaveisi Zadeh[3]

[1]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Medical Genetics Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran

Corresponding Author: Mohammad Erfan Zare, BSC Student of Medical Lab Sciences
Medical Biology Research Center, Sorkheh Lizheh, Kermanshah University of Medical Sciences, Kermanshah, Iran
P.O.Box: 1568
Tel: +98 831 4276473
Fax: +98 831 4276471
E-mail: mezarelab@yahoo.com

Abstract

Myeloproliferative neoplasms (MPNs) such as polycythemia vera, essential thrombocythemia, primary myelofibrosis and chronic myeloid leukemia have too similar and accurate way to differentiate their is study of genetic disorders in these patients. Philadelphia chromosome is a sure way to definitively diagnose CML. Recently, JAK2V617F mutation introduced as a diagnostic marker for other Myeloproliferative neoplasms. Many studies show that the absence of the JAK2 mutation in chronic phase Philadelphia positive CML. In contrast with these reports, more recently, several cases with the coexistence of Philadelphia positive chromosome and JAK2V617F mutation in blood and bone marrow samples were reported. Here, we report a patient that have the Philadelphia chromosome disorder and JAK2V617F mutation in same time.

Keyword: Myeloproliferative neoplasms**,** chronic myeloid leukemia**,** JAK2V617F**,** JAK2

Introduction

Myeloproliferative neoplasms (MPNs) which include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PM) and chronic myeloid leukemia (CML) have almost similar clinical and laboratory features and most accurate way to differentiate them is detect genetic disorders in these patients.(1- 3) Detection of the Philadelphia chromosome in CML patients, causing a massive change in the diagnosis of this malignancy and differentiation that from other neoplasms. However, detection of common genetic disorders in other classic myeloproliferative neoplasms had long-term issue for scientist's research. Finally in 2005, common JAK2V617F mutation was detected by different research groups.(4- 8) JAK2 is a tyrosine kinase that has an important role in the signalling pathways of many haemopoietic growthfactor receptors. The single acquired point mutation V617F (1849G>T) in JAK2 occurs in 50–97% of patients with idiopathic myelofi brosis, essential thrombocytosis, and polycythemia vera.(4- 6, 8, 9) By contrast, the JAK2V617F mutation has never been identified in a patient with Philadelphia positive CML.(10) But recently, some cases of Philadelphia positive CML with concomitant JAK2V617F mutation have been reported.(11- 18) Here, we report a patient that have the Philadelphia chromosome disorder and JAK2V617F mutation in same time.

Case presentation

In February 2011 the 82-year-old male patient with leukocytosis, thrombocytosis, anemia and LDH level higher than normal (763U/L) was referred to us. Hematological parameters at presentation were as follows: White Blood Cells (WBC) 20×10^9/L, Hemoglobin 8.3 g/dL and platelets 958×10^9/L. Physical examination were normal with no palpable spleen enlargement. A bone marrow biopsy revealed marked hypercellularity (90%) composed of polymorphic population of hematopoietic cells and also Megakaryocytes are increased in number. The clinical symptom of the patient was suspected

ET and treatment with Hydroxyurea (HU) was started. For diagnosis, Philadelphia chromosomal disorder and JAK2V617F mutation testing was performed. Expression of B2A2 BCR-ABL mRNA (chimer protein 210 KD) was detected by a reverse transcriptase polymerase chain reaction (RT-PCR) in peripheralblood leucocytes.

We performed the JAK2 mutation test to detect this mutation on genomic DNA (ARMS-PCR) and RNA (ASO-PCR) which had been isolated from the whole peripheral blood of the patient. A diagnosis of BCR-ABL positive chronic myeloid leukaemia (CML) was made and treatment with hydroxyurea continued with the goal of lowering blood cell count. After 2 weeks, the platelet count returned to normal but white blood cell count had increased. But, the patient was suffering from severe anemia (Haemoglobin 7.7 g/dL) and we changed his drug by Imatinib. The patient showed allergy to this drug and suffered severe muscle spasms and bone pain and we changed his drug by Interferon α again. The last laboratory test after 4 months of initial diagnosis showed decrease levels of LDH. Hematological parameters at this test were as follows: White Blood Cells (WBC) 19.7×10^9/L, Haemoglobin 10 g/dL and platelets 375×10^9/L.

ARMS PCR for the detection of JAK2-V617F mutations: Genomic DNA was extracted by phenol/chloroform after proteinase K digestion, following standard techniques. The JAK2 V617F mutation was detected by ARMS-PCR with some modification.

The ARMS-PCR technique uses 4 primers as follows; a forward outer primer, a reverse outer primer, a forward inner wild type specific primer and a reverse inner mutant specific primer. 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 wildtype specific primer produce a fragment of 229-bp. PCR reaction was performed within a total volume of 25 µL containing approximately 25 ng DNA, 12.5 µL of TaqMan Universal PCR Master Mix 2X (Roche, Germany), 0.5 µl of each FO, RO and Fwt, and 1µl of Rmt primer.

The PCR program on the thermal cycler (Eppendorf) was as follows: an initial denaturation step at 94°C for 6 min, followed by 40 cycles of 40 sec. at 94°C, 45 sec. at 56°C, 45 sec. at 72°C, and a final extension step of 10 min at 72°C.(19)

Table- 1. ARMS PCR primers (MWG Biotech, Germany)

Forward Outer (FO): 5'- TCC TCA GAA CGT TGA TGG CAG - 3'
Reverse Outer (RO): 5'- ATT GCT TTCCTT TTT CAC AAG AT - 3'
Forward wild-type specific (FWt): 5'- GCATTTGGT TTTAAATTATGGAGTATATG - 3'
Reverse mutant-specific (RMt): 5'- GTT TTA CTT ACT CTC GTC TCC ACA AAA - 3'

Table- 2. ASO PCR primers (MWG Biotech, Germany)

Forward: 5′- GAA GAT TTG ATA TTT AAT GAA AGC CTT - 3′
Reverse: 5′- GTA ATA CTA ATG CCA GGA TCA CTA AGT T - 3′
Mutant: 5′- AGC ATT TGG TTT TAA ATT ATG GAG TAT ATT - 3′

ASO- PCR for the detection of the JAK2-V617F mutation: Total RNAs were isolated by using the TRIZOL reagent. First, strand cDNAs were prepared by reverse transcription of total RNAs with random primers. The ASO- PCR program was as follows: an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 40 sec. at 94°C, 40 sec. at 56°C, 45 sec. at 72°C, and a final extension step of 10 min at 72°C. The mutant specific forward amplified a 295-bp product from the mutated allele, whereas the internal control forward primer should have given a 488-bp product from both the mutant and wild-type alleles (Figure- 2). A total of 10 µl from the PCR product were electrophoresed on 3% standard agarose gels (Sigma, Germany) at 80 V for 15 min. The fragments were visualized by ethidium bromide under UV transilluminator.(19)

Discussion

Recently, a number of reports have described the coexistence of the JAK2V617F mutation in patients with Philadelphia positive CML.(11-18) There is a debate as to whether MPD and CML arise separately from each other, representing independent development from a normal stem cell, or whether the Philadelphia chromosome arises in a stem cell of a MPD clone.(16) In some cases, the mutated JAK2 was detected after therapy for CML with imatinib or other agents but retrospective analyses of the initial specimen in those cases demonstrated that the mutated JAK2 was present at the time of the initial diagnosis of CML.(13, 15- 16) Campiotti et al. have prospectively detected JAK2 mutation positivity in one patient among 13 new cases of Philadelphia positive CML. After the partial cytogenetic response to imatinib treatment, JAK2 mutation was disappeared. Therefore, they have firstly hypothesized that imatinib treatment

have caused the regression of CML clone, as well as JAK2 mutated cells, and that JAK2 mutation can be acquired by Philadelphia positive cells.(18) But in most cases, as described previously, imatinib mesylate therapy did not affect the coexisting and/or acquired JAK2 clone.(12- 17) In addition, a number of cases of CML have been reported in patients with well-established polycythemia vera, primary myelofibrosis, or ET with mutated JAK2.(11, 20- 23) The patient discribed in this study before refer to us, had not any hematologic disorder. Likely, it can be concluded that he has the Philadelphia chromosome and JAK2 mutated in the same time. Although some authors have postulated that the BCR-ABL1 fusion gene and the abnormal JAK2 occur in independent cell populations,(15) most evidence indicates that they occur simultaneously in the same cells and that the JAK2V617F mutation likely precedes the BCRABL1 abnormality.(11)

Conclusion

Even though the JAK2V617F mutation has been excluded in a large cohort of BCR-ABL patients;(10) the present report gives evidence that the coexistence of these two disease-specific mutations is possible. This is still a question that whether MPN and CML arise separately from each other, representing independent development from a normal stem cell, or whether the Philadelphia chromosome arises in a stem cell of a MPN clone. Further studies are required to determine the exact frequency and prognostic role of the JAK2V617F mutation in Philadelphia positive CML patients.

References

1. Bennett M, Stroncek D. Recent advances in the bcr-abl negative chronic myeloproliferative diseases. J Transl Med 2006; 4: 41.

2. Cervantes F. Modern management of myelofibrosis. Br J Haematol 2005; 128: 583-92.

3. Diez-Martin JL, Graham DL, Petitt RM. Chromosome studies in 104 patients with polycythemia vera. Mayo Clinic Proc 1991; 66: 287-90.

4. Baxter EJ, Scott LM, Campbell P, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005; 365: 1054–61.

5. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005; 434: 1144–48.

6. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005; 352: 1779–90.

7. Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz SB, et al. Identification of an acquired JAK2 mutation in polycythemia vera. J Biol Chem 2005; 280: 22788–92.

8. Levine RL, Wadleigh M, Cools J, Ebert BJ,Wernig G, Huntly BJP, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005; 7: 387–97.

9. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2V617F mutation in chronic myeloproliferative disorders. Blood 2005; 106: 2162–68.

10. Jelinek J, Oki Y, Gharibyan V, et al. JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood 2005; 106: 3370–73.

11. Krämer A. JAK2-V617F and BCR-ABŁ double Jeopardy? Leukemia Res 2008; 32: 1489–90.

12. Pardini S, Fozza C, Contini S et al. A case of coexistence between JAK2V617F and BCR/ABL. Eur J Hematol 2008; 81: 75–6.

13. Inami M, Inokuchi K, Okabe M et al. Polycthemia associated with the JAK2V617F mutation emerged during treatment of chronic mylogeneous leukemia. Leukemia 2007; 21: 1103–4.

14. Cambier N, Renneville A, Cazaentre T et al. JAK2V617F-positive polycythemia vera and philadelphia chromosome-positive chronic myeloid leukemia: one patient with two distinct myeloproliferative disorders. Leukemia 2008; 22: 1454–5.

15. Hussein K, Bock O, Seegers E et al. Myelofibrosis evolving during imatinib treatment of a chronic myeloproliferative disorder with coexisting BCR-ABL translocation and JAK2V617F mutation. Blood 2007; 109: 4106–7.

16. Krämer A, Reiter A, Kruth J et al. JAK2-V617F mutation in a patient with Philadelphia-chromosome-positive chronic myeloid leukemia. Lancet 2007; 8: 658–60.

17. Veronese L, Tchirkov A, Richard-Pebrel C et al. A thrombocytosis occuring in Philadelphia positive CML in molecular response to imatinib can reveal

an underlying JAK2V617F myeloproliferative neoplasm. Leuk Res 2010; 34: 94–6.
18. Campiotti L, Appio L, Solbiati F, AgenoW, Venco A. JAK2-V617F mutation and Philadelphia positive chronic myeloid leukemia. Leukemia Research 2009; 33. 212–3.
19. Nadali F, Ferdowsi Sh, Karimzadeh P, Chahardouli B, Einollahi N, Mousavi SA, Bahar B, Dargahi H, Alimoghaddam K, Ghavamzadeh A, Ghaffari SH. JAK2-V617F Mutation and Philadelphia Positive Chronic Myeloid Leukemia. IJHOSCR 2009; 3(3): 43-5.
20. Bornhauser M, Mohr B, Oelschlaegel U, et al. Concurrent JAK2 (V617F) Mutation and BCR-ABL Translocation within Committed Myeloid Progenitors in Myelofibrosis [letter]. Leukemia. 2007; 21: 1824- 1826.
21. Bocchia M, Vannucchi AM, Gozzetti A, et al. Insights into JAK2-V617F mutation in CML. Lancet Oncol. 2007; 8: 864-866.
22. Conchon MR, Costa JL, Novaes MMY, et al. Simultaneous detection of JAK2V617F mutation and BCR-ABL translocation in a patient with chronic myelogenous leukemia [letter] [published online ahead of print July 16, 2008]. Int J Hematol. 2008; 88: 243-245.
23. Jallades L, Hayette S, Tigaud I, et al. Emergence of therapy-unrelated CMO on a background of BCR-ABŁ negative JAK2V617F-positive chronic idiopathic myelofibrosis. Leuk Res. 2008; 32: 1608-1610.