Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

Research Paper

Hydroxyurea with Thalidomide Combination therapy in a Rare Case of JAK2 mutation (JAK2V617F) and BCR-ABL Positive Myelofibrosis

Medical Science

KEYWORDS : JAK2 mutation, Myelofibrosis, BCR-ABL translocation

Swati Dasgupta	M.Sc., Netaji Subhas Chandra Bose Cancer Research Institute, 16 A Park Lane , Kolkata-700016
Ashis Mukhopadhyay	M.D,DNB, DCH. Netaji Subhas Chandra Bose Cancer Research Institute, 16 A Park Lane , Kolkata-700016
Soma Mukhopadhyay	Ph.D. Netaji Subhas Chandra Bose Cancer Research Institute, 16 A Park Lane ,Kolkata-700016

ABSTRACT

Myelofibrosis is a fatal disease that accounts for 1% of all cancers and 10% of hematological malignancies. In this report coexistence of JAK2 V617F mutation with BCR ABL positive myelofibrosis was noticed. Hydroxyurea therapy along with Thalidomide treatment induced normalization of the patient's blood parameters (Complete haematological response) was seen after six months including cytogenetic and molecular remission where as Imatinib did not show the expected outcome. No detectable BCR-ABL fusion transcript in the bone marrow was found and spleen came to its normal condition. Hydroxyurea is a tyrosine kinase inhibitor that's why it shows a good response to inhibitions of Janus kinase 2 mutation because JAK2 and ABL both are kinase domain mutations . We can conclude that hydroxyurea with Thalidomide is a good alternative of JAK 2 inhibitor in patients suffering from Myelofibrosis.

Introduction

Myelofibrosis (MF) is a kind of myeloproliferative disorder (MPD), resulting from a pluripotent haematopoietic progenitor cell that acquires a clonal proliferative advantage and induces deposition of fibrin and collagen in bone marrow via the release of angiogenic factors. The myeloproliferative disorders (MPD) are a group of haematological conditions where there is a primary disorder at the level of the multipotent haematopoitic stem cell leading to increased production in one or more blood cell types (McLornan D et.al,2006) .

In 2005 four different groups independently identified a single, valine-to-phenylalanine somatic mutation at position 617 (V617F) in the Janus Kinase 2 or JAK2. This mutation is widespread in patients with chronic acquired MPDs. JAK2-V617F is present in a fraction of the myeloid cells of virtually every patient with polycythemia vera (PV), and about half of patients with essential thomnocythaemia (ET) and Idiopathic Myelofibrosis (MF) (Baxter EJ et.al, 2005; Levine RL et.al, 2005, James C et.al, 2005, Kralovics R et.al, 2005). The incidence of JAK2V67F in MPDs has been confirmed in a number of subsequent studies, which have also verified its absence in normal condition and rarity in other hematological disorders (Baxter EJ et.al. ,2005; Levine RL et.al, 2005; JJ, Moore S et.al.,2005; James C et.al. , 2005; Kralovics R et.al, 2005; Kralovics R et.al. ,2005; Scott LM. , 2005; Nelson ME and Steensma DP ,2006; Kaushansky K,2007).

But generally the above three major MPDs lack the abnormally short Philadelphia Chromosome or BCR-ABL translocation which distinguishes Chronic Myeloid Leukemia (CML). So the presence of both JAK2 and BCR-ABL in a MPD patient is really very rare and the treatment regimen is very difficult for a haematologist to decide, specially in a developing country like India.

In the present study we have presented a case report of MPD with both JAK 2 as well as BCR-ABL mutations.

Case Report: In August, 2009, a 46 year-old man came to the hospital with spleenomegaly (21.0cm) along with fever, weakness, weight loss, swollen leg and chronic diarrhoea. His leucocyte concentration was 5500/cu.mm, haemoglobin 10.6 g%, and platelets 6.0 lakhs/cu.mm. A differential blood count showed 84% neutrophils, 04% lymphocytes, 02% myelocytes, 02% metamyelocytes, and band cell 06%. A subsequent bone marrow biopsy revealed a hypercellular marrow with myeloid and megakaryocytic hyperplasia with fibrosis. Reducing iron store was suggestive of Myelofibrosis (MF). Cytology report of bone marrow revealed that myelocyte 5%, metamyelocytes 8%, neutrophils 56 %, eosinophils 4 %, basophils 2%, lymphocytes 2%, monocytes 1%, normoblasts 22%. By performing Nested Real Time Polymerase Chain Reaction and Sanger's sequencing method JAK 2V617F mutation was detected (Fig 1).

Simultaneously BCR ABL fusion protein was also detected by FISH method using BCR and ABL specific probes. The karyotype of this patient was 20% 46, XY, t (9; 22) (q34; q11.2) in all 20 metaphase plates, analyzed.

Treatment was started with Imatinib (400mg/day) but no haematological response is observed after 3 months. Then the treatment is switched to Hydroxyurea 500mg /day and Thalidomide 100 mg /day and observed for next three months. Significant changes were found in leucocyte concentration and platelet count respectively 2600/cu.mm and 1.90 lakhs /cu.mm.

For next six months patient was treated with Hydroxyurea (500/day) and Thalidomide (100 mg /day) and no transfusion was required. Complete haematological response was recorded after six months and JAK2 mutation was not detected also. BCR ABL fusion protein was also absent in RT PCR.

Hematological parameters of patient were studied for 36 months follow up. Collected data is presented in tabular (Table:1) and graphical (Fig:2) form below.

Discussion and Conclusion:

More than 100 published Philadelphia chromosome positive cases of chronic myeloid leukemia (Ph CML) were investigated for the 1849 G>T mutation of the Janus kinase 2 (JAK2V617F). The mutation causes a valine to phenylalanine amino acid substitution at codon 617(V617>F) of JAK 2 which turns the kinase switched on. The mutation can be found in 99% of Polycythemia Vera patients and about half of essential thrombocythemia / myelofibrosis cases. But JAK2 mutation 1849G T is rare in acute leukemias and CML though it can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia (Jelinek J et al., 2005; Bock O et.al,2006; Cortelazzo S et.al,1995).

Hydroxyurea (HU) is widely used as a first line myelosuppressive therapy for patients with PV and ET (James C et.al. , 2005; Cortelazzo S et.al,1995) . In a study conducted by Anne Ricksten et.al rapid decrease in the JAK2V617F levels was seen in 72% of the patients and this effect was statistically significant after four months of Hydroxyurea therapy in 18 patients. Only one of the

Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

patients had a higher level of JAK2V617F at four months compared with the level at start of Hydroxyurea therapy. The mean JAK2V617F levels in the patients decreased into 55% during the first four months. There was no significant change in the mean level of JAK2V617F between 4 to12 months of therapy (Ricksten A et.al,2008).

According to Garber K (2009) four research groups separately reported a point mutation in Janus kinase (JAK 2) in most patients with MPDs. The mutation activates the JAK2 protein, which normally transmits proliferation and survival signals to blood cells from cell surface receptors. It leads to sustained signalling, similar to the effect of the BCR-ABL fusion protein, a product of the Philadelphia chromosome in CML. In early trials, JAK2 inhibitors have reduced myelofibrosis symptoms, often substantially, but so far don't appear to reverse disease. Though six JAK2 inhibitors are already manufactured (INCBO18424 by Incyte, TG101348 by TargeGen, Lestautinib by Cephalon, AZD1480 by AstraZeneca, XLO19 by Exelixis, CYT-387 by Cytopia), still they are in clinical trial phase (Garber K; 2009).

In this report coexistence of JAK2 V617F mutation with BCR ABL positive myelofibrosis was noticed. Imatinib treatment shows no remission while Hydroxyurea therapy induced normalization of the patient's blood parameters was noticed after six months including cytogenetic and molecular remission. No detectable BCR-ABL fusion transcripts in the bone marrow were seen and spleen came to its normal condition. Follow-up biopsies of the bone marrow one after nine months of initial diagnosis and other on onset of treatment, revealed an increasing accumulation of megakaryoctes. Consequently, JAK2V617F mutation was analyzed with highly sensitive nested Real Time Polymerase Chain Reaction and Sanger,s sequencing methods. After the six months of follow-up with HYdroxyurea therapy, red blood cell counts, Hb, platelets, and WBC counts remained stable and within normal range (Fig 2 and Table 1).

Thalidomide is a putative antiangiogenic and immunomodulatory agent with significant antitumour activity in several haematological malignancies (Singhal S et.al, 1999; Raza A et.al , 2001) . It is a novel investigational option for the treatment of myelofibrosis and it inhibits the activity of VEGF, bFGF and TNFα in several *in vitro* and *in vivo* models (D'Amato RJ et.al, 1994; Kenyon BM et.al 1997; Sampaio EP et.al, 1991) . Several clinical trials of Thalidomide in the treatment of MF have been conducted and overall response rate had ranged from 30-40%. Our patient did not respond in imatinib, therefore started with Hydroxyurea 500mg/day and Thalidomide 100 mg/day and surprisingly showed haematological as well as molecular response after a period of six months of continuous therapy.

We know that Janus kinase 2 (commonly called JAK2) is a non-receptor tyrosine kinase. In case of BCR ABL translocation the *ABL1* proto-oncogene encodes a cytoplasmic and nuclear protein that is also tyrosine kinase . In this study patient was treated by Hydroxyurea (500mg /day) as any JAK2 inhibitor was not available at that time and Hydroxyurea responded very well. After six months we found negative result in JAK2 V617F mutation. Complete cytogenetic remission was seen (no Ph chromosome in 20-25 metaphase cells) by treatment with Hydroxyurea which is rare in CML patients with BCR ABL translocation.

Hydroxyurea is a tyrosine kinase inhibitor, that's why it shows a good response to inhibitions of Janus kinase 2 mutation because JAK2 and ABL both are kinase domain mutations . Though Myelofibrosis is a disease with multiple dimensions and various patients respond in various combinations of therapies, here we also can conclude that Hydroxyurea with Thalidomide may be a good alternative of JAK 2 inhibitor in patients with BCR-ABL positive and suffering from the most serious form of MPD, Myelofibrosis.

Acknowledgement: At first we owe our sincere acknowledgement to a patient, without his cooperation this study could not be succeeded. We are also thankful to Mr. Priyabrata Das, Mr. Abhijit Chakraborty and Mrs. Nabamita Pal for their extensive support. We are also grateful to West Bengal University of Health Sciences under with our Institute is affiliated.

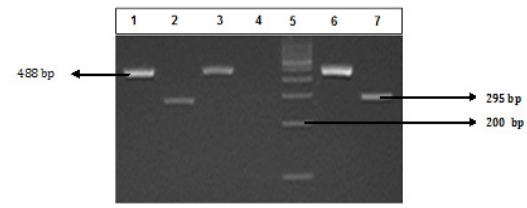

Fig 1: JAK2 Exon 14 V617F mutation analysis: Lane 1: Internal Control for patient sample, lane2: JAK2 mutation for patient sample, lane 3: Internal Control for Negative case, lane 4: JAK2 for Negative case, lane 5: 100bp DNA ladder, lane 6: Internal Control for Positive case, lane 7: JAK2 mutation for Positive case.

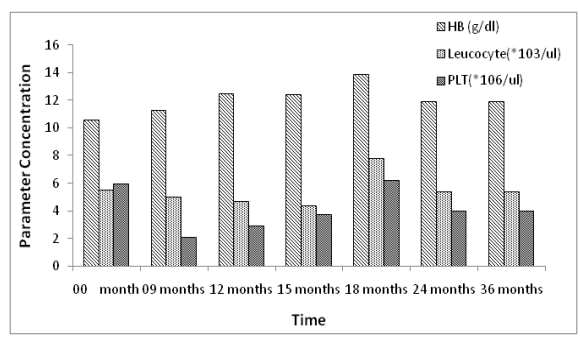

Fig2: Total count, Hemoglobin concentration and Platelet count of peripheral blood of patient from onset of disease to 36 months follow up treatment with Hydroxyurea and Thalidomide .

Differential Count	0Month (%)	6 Months (%)	12 Months (%)	15 Months (%)	18 Months (%)	24 Months (%)
Neutrophil	84	80	91	60	76	78
Lymphocyte	04	16	05	34	20	19
Myelocyte	02	-	-	-	-	-
Metamyelocyte	02	-	-	-	-	-
Band cell	06	-	-	-	-	-
Monocyte	01	02	02	04	03	02
Eosinophil	01	02	00	02	01	01
Basophil	-	00	02	00	00	00

Table 1: Differential count of peripheral blood of the patient from onset of disease to 24 months follow up treatment with Hydroxyurea and Thalidomide.

REFERENCE

1. McLornan D, Percy M and McMullin MF. (2006)." JAK2V617 F:A Single Mutation in Myeloproliferative group of disorders. "The Ulster Med J ,2, 112-119. | | 2. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN and Green AR (2005). "Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders." Lancet, 365, 1054-1061. | | | 3. Levine RL, Wadleigh M, Cools J, Ebert BL , Wernig G,Huntly BJP, Boggon TJ, Wlodarska I, Clark JJ, Moore S, Adelsperger J, Koo S ,Lee JC, Gabriel S, Mercher T, Andrea AD, Fröhling S, Döhner K, Marynen P, Vandenberghe P, Mesa RA, Tefferi A, Griffin JD, Eck MJ, Sellers WR, Meyerson M, Golub TR, Lee SJ and Gilliland DG. (2005)."Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis." Cancer Cell,7, 387-397. | | 4. James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N and Vainchenker W.(2005). "A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera." Nature, 434,1144-1148. | | 5. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt BSR, Passweg JR, Tichelli A, Cazzola M and Skoda RC.(2005)." A gain-of-function mutation of JAK2in myeloproliferative disorders." N Engl J Med,52, 1779-1790. | | | 6. Jelinek J, Oki Y, Gharibyan V, Ramos CB, Prchal JT, Verstovsek S, Beran M, Estey E, Kantarijan HM and Issa JPJ.(2005). "JAK2 mutation 1849G > T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia." Blood,106,3370-3373 | | 7. Kralovics R, Teo SS, Buser AS, Brutsche M, Tiedt R, Tichelli A, Passamonti F, Pietra D, Cazzola M and Skoda RC.2005. "Altered gene expression in myeloproliferative disorders correlates with activation of signaling by the V617F mutation of Jak2".Blood,106,3374–3376. | 8. Scott LM, Campbell PJ, Baxter EJ, Todd T, Stephens P, Edkins S, Wooster R, Stratton MR, Futreal PA and Green AR. 2005. "The V617F JAK2 mutation is uncommon in cancers and in myeloid malignancies other than the classic myeloproliferative disorders." Blood ,106, 2920–2921. | | 9. Nelson ME and Steensma DP. 2006. "JAK2 V617F in myeloid disorders: what do we know now, and where are we headed?" Leuk Lymphoma, 47, 177–194. | | 10. Kaushansky K. 2007. "The chronic myeloproliferative disorders and mutation of JAK2:Dameshek's 54 year old speculation comes of age."Best Pract Res Clin Haematol, 20, 5–12 | 11. Cortelazzo S, Finazzi G, Ruggeri M, Vestri O, Galli M, Rodeghiero F, and Barbui T. 1995. "Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis." N Engl J Med,332,1132-1136. | | | 12. Bock O, Busche G, Koop C, Schroter S, Buhr T and Kreipe H. 2006 "Detection of the single hotspot mutation in the JH2 pseudokinase domain of Janus kinase 2 in bone marrow trephine biopsies derived from chronic myeloproliferative disorders."J Mol Diagn,8, 170-177. | | 13. Ricksten A, Palmqvist L, Johansson P and Andreasson B. 2008. ; 93(8)Rapid decline of JAK2V617F levels duringhydroxyurea treatment in patients withpolycythemia vera and essentialthrombocythemia. Haematologica, 93, 1260-1261. | | 14. Garber K. 2009. "JAK 2 inhibitors: not the next imatinib but researchers see other possibilities." J Natl Cancer Inst., 101, 980-982. | | | 15. Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, Munshi N, Anaissie E, Wilson C, Dhodapkar M, Zeddis J, Barlogie B. (1999)" Antitumor activity of thalidomide in refractory multiple myeloma". N Engl J Med.341, 1565–1571. | | 16. Raza A, Meyer P, Dutt D, Zorat F, Lisak L, Nascimben F, du Randt M, Kaspar C, Goldberg C, Loew J, Dar S, Gezer S, Venugopal P, Zeldis J (2001) "Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes" Blood. 98,958–965. | | | 17. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. (1994) "Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A.91, 4082–4085. | | 18. Kenyon BM, Browne F, D'Amato RJ.(1997) Effects of thalidomide and related metabolites in a mouse corneal mode of neovascularization. Exp Eye Res. 64,971–978. | | | 19. Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G.(1991) " Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes." JExp Med. 173(3),699-703 |