ORIGINAL ARTICLE

JAK 2 V617 Mutation with Myeloid Hyperplasia and Blast Formation in Idiopathic Myelofibrosis

FATIMA KHANUM, SUHAIB AHMAD, AMAN UR REHMAN

ABSTRACT

Objectives: The myeloproliferative disorders refer to clonal disorders of haemopoiesis that lead to an increase in the number of one or more mature blood cell progeny. Idiopathic myelofibrosis is a clonal myeloproliferative disorder of the pluripotent haemopoeitic stem cell in which a clonal proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis. JAK-2 mutation has been identified as the first genetic marker that is directly associated with the pathogenesis of myeloproliferative disorder (polycythaemia vera, essential thrombocythaemia and idiopathic myelofibrosis). The purpose of this study is to document the total leukocytes count and circulating blast of idiopathic myelofibrosis in JAK2 positive and JAK2 negative patients.

Design: Comparative and cross sectional study.

Place and duration of study: The study was carried out from January 2004 to December 2008, at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi.

Method: A total number of 35 patients were studied in which 19 patients were JAK2 positive and 16 patients were JAK2 negative. Sample collection technique was purposive non-probability sampling. Variations were observed among the studied JAK2 positive and JAK2 negative patients regarding total leukocytes count and the presence of circulating blast cells.

Results: The total leukocytes count and the presence of blast cells were compared in between JAK2 positive and JAK2 negative patients. Patient's positive for JAK2 mutation had higher total leucocytes counts (a mean of 20.4 x 10^9/l) and less circulating blasts 7/35 (36.8%) as compared to patients negative for JAK2 (a mean 12.4 x 10^9/l) and circulating blasts 12/35 (75.0%).

Conclusion: The JAK2 mutation is associated with expansion of the myeloid lineage and less tendency to blasts transformation.

Key words: Myeloproliferative disorder, Idiopathic myelofibrosis, Total leucocytes counts, Blast count.

INTRODUCTION

Myeloproliferative disorders are clonal disorders of haemopoiesis that lead to an increase in numbers of one or more mature blood cell progeny. All the myeloproliferative disorders arise as a somatic mutation of pluripotent haemopoietic stem cell[1,2]. In the myeloproliferative disorders the proliferative capacity of neoplastic stem cell is not properly controlled and excessive haemopoiesis occurs initially[3]. Idiopathic Myelofibrosis is a Ph negative clonal myeloproliferative disorder of the pluripotent haemopoietic stem cell (HSC), in which a clonal proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis[4]. Idiopathic myelofibrosis is characterized by anaemia, splenomegaly, immature granulocytes, erythroblast, tear drop red cells in the blood and bone marrow fibrosis. In the year 2005, several researcher groups reported a single, acquired point mutation in the Janus kinase 2 genes in the majority of patients with Ph-negative myeloproliferative disorders. JAK2 mutation plays a vital role in the pathogenesis of Ph negative myeloproliferative disorders and hematological malignancies[1,5]. A number of studies carried out show that STATS play a critical role in variety of tissues functions. Janus kinase signaling is activated in haematological malignancies by a number of mechanisms including the down regulation of negative regulators of JAK-STAT pathways, amplification of the JAK2 locus, and involvement of JAK2 in chromosomal translocations and by identification of an activating point mutation in JAK2. In the mutation of JAK2 there is a substitute of bulky phenyalanine for a conserved valine at position 617. Mutation in the JAK2 causes activation of STATs in the absence or in the presence of only trace quantities of haemopoietic growth factor[6,7]. The objective of this study was to observe and compare the total leukocytes count and circulating blast in between JAK2 positive and JAK2 negative Idiopathic myelofibrosis patients.

Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi
Sheikh Zayed Hospital, Lahore
Correspondence to Dr Fatima Khanum, Email: doctorfatima77@hotmail.com

MATERIAL AND METHOD

This study was conducted at the Armed Forces Institute of Pathology, Rawalpindi. It was a comparative and cross sectional study and sampling was done by purposive non-probability technique. The study was conducted from January 2004 to December 2008. The project was approved by the ethical committee of AFIP, Rawalpindi. An informed consent was taken from the patients who were studied prospectively. In return, the patients received their JAK2 result free of cost. A total of 35 patients of idiopathic myelofibrosis at the Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi, were studied. The patients were included irrespective of age, sex and socio-economic status. The patients of idiopathic myelofibrosis diagnosed by conventional criteria were included in this study. Idiopathic myelofibrosis patients who were on treatment and patients of secondary myelofibrosis i.e., Hairy cell leukaemia, acute leukaemia, metastatic carcinoma, disseminated tuberculosis and lymphoma etc. were excluded from this study. Their blood samples were collected for JAK2 mutation analysis and blood complete picture.3ml of blood sample was taken under aseptic condition in CP bottles containing EDTA anticoagulant. Then the blood count, including the white cell count, haemoglobin, mean cell volume, mean cell haemoglobin and platelets counts were carried out on haematology analyser Sysmex KX-21.Circulating blast were also noted in peripheral blood picture. Gene analysis for JAK2 mutation was carried out as follow:

1. DNA extraction
2. PCR
3. Electrophoresis

DNA Extraction: Extraction of DNA from the whole blood was carried out using the genomic DNA purification kit by Gentra as per manufacturers' instructions.

PCR (by Amplification Refractory Mutation System): PCR method known as amplification refractory mutation system (ARMS) (Newton et al, 1989) was used to detect the JAK2 mutation. The target DNA was amplified using the primer complementary to the JAK2 mutation. A set of three primers was used. JAK2 mutant allele was amplified by a common reverse primer (5'-CTGAATAGTCCTACAGTGTTTTCAGTTTCA) and a forward specific primer (5'-AGCATTTGGTTTTAAATTATGGAGTATATT) producing 203bp amplified product. The common reverse primer and a forward control primer (5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAAG) was used to amplify a 364bp product that served as PCR internal control.

Electrophoresis: The amplified products were run by polyacrylamide gel electrophoresis and the gel was stained with silver nitrate.

RESULT

The data was entered in statistical package for social sciences – SPSS (version 12.0) and the same software was used for statistical analysis. Mean±SD was given for the normally distributed quantitative variables. P value < 0.05 was considered statistically significant. A total of 35 patients with idiopathic myelofibrosis were studied. Out of 35 patients, 19(54.3%) were JAK2 positive and 16 (45.7%) patients were JAK2 negative. The total leucocytes count and circulating blast were compared in between JAK2 positive and JAK2 negative patients. In JAK2 positive patients mean total leucocytes counts were 20.4 x109/l and in the JAK-2 negative patients mean total leucocytes counts were 12.4 x109/l. JAK2 positive patients with circulating blast were 7/35 (20%) In contrast, JAK2 negative patients with circulating blasts were 12/35 (34.3%).

	JAK2 Positive	**JAK2 Negative**	**Significance Value**
Number of patients n = 35	n = 19	n = 16	
TLC ($x10^9$/l) Mean value	20.4	12.4	0.13
Patients with circulating blasts	7 (36.8%)	12 (75.0%)	0.23
Circulating blasts (Mean)	1.2	3.0	

DISCUSSION

Myeloproliferative disorders are clonal disorders of haemopoiesis. The myeloproliferative disorders can be either Ph positive or Ph negative. The Ph positive myeloproliferative disorder is chronic myeloid leukaemia while the Ph negative myeloproliferative disorders are polycythaemia rubra vera, essential thrombocythaemia and idiopathic myelofibrosis[8]. Idiopathic myelofibrosis is a chronic myeloproliferative disorder characterized by anaemia, splenomegaly, immature granulocytes, erythroblast, tear drop red cells in the blood and bone marrow fibrosis. The haematological features (like total leukocytes count and circulating blast) of idiopathic myelofibrosis have not been studied in Pakistan before. The JAK2 mutation plays a significant and independent influence on the disease phenotype that is correlated with the expansion of clonal haematopoietic cells. Some previous studies have

also focused on the phenotype of JAK2 positive and JAK2 negative patients and have concluded that phenotypically, the JAK2 mutation positive patients are different from the JAK2 negative patients[9]. In a multivariate analysis, a comparison of total leukocytes count was made in between JAK2 positive and JAK2 negative patients. In the patient's positive for JAK2 mutation, the total leukocytes count was higher as compared to JAK2 negative patients.

The same results were shown in the previous study. Patient's positive for V617F mutation had a significantly higher white cell count than patients negative for V617F mutation, suggesting that the mutation is associated with expansion of the myeloid lineage. A significant difference in blast count was observed between the V617F positive and V617F negative patients. JAK2 negative patients had more circulating blasts as compared to V617F positive patients suggesting that JAK2 negative patients have more tendencies to blasts transformation as compared to JAK2 positive patients[10].

CONCLUSION

The JAK2 mutation is associated with expansion of the myeloid lineage and less tendency to blasts transformation.

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