

The Impact of JAK2 V617 Mutation in Splenomegaly and Reticulin Grade of Idiopathic Myelofibrosis

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ABSTRACT

Objectives: To compare and document the sign (splenomegaly) and reticulin grade 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 spleen size and reticulin grade.

Results: Most of the JAK2 positive patients had massive splenomegaly (42.8%). On the other hand the JAK2 negative patients had mild splenomegaly and some of them had only spleen tip palpable. In contrast to the reticulin grade, there was a substantial interindividual variation in reticulin grade.

Conclusion: This suggests that many of the clinical manifestations e.g. splenomegaly and reticulin grade of idiopathic myelofibrosis are caused by factors unrelated to the V617F mutation.

Key words: Myeloproliferative disorder, Idiopathic myelofibrosis, splenomegaly, reticulin grade

INTRODUCTION

Myeloproliferative disorders are clonal disorders of haemopoiesis that lead to an increase in numbers of one or more mature blood cell progeny^{1,2}. In the myeloproliferative disorders the proliferative capacity of neoplastic stem cell is not properly controlled and excessive haemopoiesis occurs initially³. 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 fibrosis⁴. Idiopathic myelofibrosis is characterized by anaemia, splenomegaly and leucoerythroblastic blood picture. 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}. 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.

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In the mutation of JAK2 there is a substitute of bulky phenylalanine 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 splenomegaly and reticulin grade between JAK2 positive and JAK2 negative idiopathic myelofibrosis patients.

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. 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 were excluded from this study. Gene analysis for JAK2 mutation was carried out as 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 Genra as per manufacturers' instructions.

PCR (by Amplification Refractory Mutation System): PCR method known as amplification refractory mutation system (ARMS) was used to detect the JAK2 mutation. The target DNA was amplified using the primer complementary to the JAK2 mutation.

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

RESULTS

The data was entered in statistical package for social sciences – SPSS (version 12.0) and the same software was used for statistical analysis. A total of 35 patients with idiopathic myelofibrosis were studied. In 6/35 patients the spleen tip was palpable, in 7/35 there was mild splenomegaly and 22/35 had massive splenomegaly. Among JAK2 positive patients, 1/35 had spleen tip palpable, 3/35 had mild splenomegaly and 15/35 had massive splenomegaly. Most of the JAK2 positive patients had massive splenomegaly. On the other hand, the JAK2 negative 5/35 patients had spleen tip palpable, 4/35 had mild splenomegaly and 7/35 had massive splenomegaly. The degree of fibrosis as seen on reticulin stain varied widely between the JAK2 positive and the negative patients. 1/35 JAK2 positive patients had reticulin fibrosis grade I, 5/35 patients had reticulin fibrosis grade III, 2/35 patients had reticulin fibrosis grade II/III, 5/35 patients had reticulin fibrosis grade IV and 6/35 patients had reticulin fibrosis grade III/IV. 3/35 JAK2 negative patients had reticulin fibrosis grade II, 3/35 patients had reticulin fibrosis grade III, 1/35 patients had reticulin fibrosis grade II/III, 2/35 patients had reticulin fibrosis grade IV and 7/35 patients had reticulin fibrosis grade III/IV.

Table-1

	JAK2 +ve (n = 19)	JAK2 -ve (n = 16)
Splenomegaly (n=35)		
Tip	1	5
Mild	3	4
Massive	15	7

Table-2 (n=35)

	JAK2 +ve (n = 19)	JAK2 -ve (n=16)
Reticulin Fibrosis Grade		
I	1	-
II	-	3
III	5	3
II / III	2	1
IV	5	2
III / IV	6	7

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 myelofibrosis⁸. 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 splenomegaly and reticulin grade of idiopathic myelofibrosis have not been studied in Pakistan before. Most of the JAK2 positive patients had massive splenomegaly. On the other hand the JAK2 negative patients had mild splenomegaly and some of them had only splenic tip palpable. Previous studies also reported that JAK2 positive patients had significant spleen size than JAK2 negative patients⁹. In contrast to the reticulin grade, no significant difference was observed between the JAK2 positive and JAK2 negative groups as a whole. However, there was a substantial inter-individual variation in reticulin grade.

CONCLUSION

This suggests that many of the clinical manifestations e.g., splenomegaly and reticulin grade of idiopathic myelofibrosis are caused by factors unrelated to the V617F mutation.

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