RESEARCH ARTICLE

Acquired JAK-2 V617F Mutational Analysis in Pakistani Patients with Essential Thrombocythemia

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Abstract

Background: Essential thrombocythemia (ET) is a clonal hemopoietic stem cell myeloproliferative neoplasm characterized by persistent thrombocytosis along with megakaryocytic hyperplasia. In the last decade following the identification of an acquired JAK2 V617F mutation, there has been acceleration in our understanding of this disease. The rational of this study was to determine the mutational profile of JAK2 V617F in Pakistan patients with ET. Materials and Methods: In this retrospective cross sectional study, 21 patients with ET were enrolled from January 2011 to December 2014. Patients were diagnosed based on WHO criteria for essential thrombocythemia. Complete blood count was done on an automated hematology analyzer, while JAK2 V617F expression was evaluated by polymerase chain reaction. Results: The mean age was 56.7±19.0 years (range 18-87) and the male to female ratio was 1:1.1. The frequency of JAK2 V617F positivity in our ET patients was found to be 61.9%. The mean hemoglobin was 11.7±2.4 g/dl with a total leukocyte count of 13.3±8.1x109/l and a platelet count of 1188±522x109/l. Positive correlations for JAK2 V617F mutation were established with high TLC count and raised LDH (P<0.05). No correlation of JAK2 V617F could be established with age and gender (P>0.05). Conclusions: JAK2 V617F mutation frequency in our ET patients was similar to those reported previously. Screening for the mutation in all suspected essential thrombocythemia cases could be beneficial in differentiating patients with reactive and clonal thrombocytosis.

Keywords: Essential thrombocythemia - JAK-2 V617F mutation - Pakistan

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Introduction

Essential thrombocythemia (ET) is an indolent hematopoeitic stem cell neoplasm that shares phenotypic and pathological affiliation with other myeloproliferative neoplasms, in particular polycythemia vera (PV) and primary myelofibrosis (IMF) (Sag et al., 2015; Yang et al., 2015). ET is a myeloproliferative disorder of unknown etiology in which distinct proliferation of the megakaryocytes in the bone marrow leads to elevated platelet count.

The reported annual incidence rates range from 1 to 3/100,000 population (Sag et al., 2015). The disease usually presents at a median age of 65-70 years with a second peak at around 30 years, when it is more pronounced in females (Briere, 2007; Kiladjian et al., 2013).

The diagnosis of ET has always been a controversial and challenging, owing to indistinct margins between various myeloproliferative diseases. Insight into the underlying molecular pathogenesis has been accompanied by the development of new diagnostic approach. In the last decade there has been escalation in our understanding of ET, after recognization of an acquired JAK2 V617F mutation in approximately 50% of ET patients (Zhang et al., 2014; Birgegard., 2015).

The janus associated kinase JAK2 V617F mutation represents a new diagnostic molecular marker for the patients with Ph-negative myeloproliferative neoplasms (Duangnapasatit et al., 2015). It has been identified in subsets of each Philadelphia chromosome-negative MPDs; in 23-55% of ET, 43-65% of IMF and around 96% in PV of patients (Levine et al., 2007; Sag et al., 2015).

Some studies have been conducted on the effects of JAK2 V617F mutation on the overall disease process. It has been found that presence of the JAK2 mutation, particularly if associated with leukocytosis, is a predictor of a subsequent thrombotic event and simultaneously it does not have a protective effect on haemorrhagic jeopardy (Ross et al., 2008; Tefferi., 2013).

Since, there is no study has hitherto been available from Pakistan; we sought to look for the prevalence of JAK2V617F mutation in Pakistani patients with essential thrombocytosis and also to determine any correlations with age, gender, hematological and different biochemical parameters.

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Materials and Methods

This descriptive cross sectional study, extended from January 2011 to December 2014. Twenty one patients with Essential thrombocythemia were enrolled in the present study. An informed consent was obtained from all the participating patients.

Patients were diagnosed to have ET according to the World Health Organization (WHO) criteria 2008 (Thiele et al., 2008). All patients included were subjected to the complete blood count, differential WBC count and blood smear examination. All included patients had platelets ≥450x10^9/l. Also patients had either demonstration of JAK2 mutation positivity or in negative case; all reactive causes of thrombocytosis were excluded. Bone marrow examinations were done on each patient. Those patients who met WHO criteria for Chronic myeloid leukemia (BCR-ABL translocation positivity), polycythemia vera, myelofibrosis, myelodysplasia or other myeloid neoplasm were excluded.

Hematological parameters including hemoglobin, MCV, WBC count and platelets count were determined by Cell Dyne (Abott, Diagnostics). Serum creatinine, lactate dehydrogenase and serum uric acid were detected by HITACHI 912 (Japan) by photometric assay. JAK2 mutational analyses were detected by Polymerase chain reaction (PCR). Bone marrow aspirate and trephine biopsy specimen were taken through Jamshidi needle and were reviewed by expert hematopathologist.

The ethical endorsement of the study was granted by research and ethical committee LNH taken prior to the study.

Data analysis

Data was compiled and analyzed using SPSS version 21. The results were expressed as mean±SD for quantitative variables and qualitative variables are presented as frequency & percentages. Student't' test was applied for the comparison of mean. Data were considered statistically significant at P value <0.05. Chi-square test was applied to assess the correlation.

Results

A total of 21 confirmed Essential thrombocythemia patients using the non probability consecutive sampling were included in this study.

Out of 21 patients 10 were males (47.6%) and 11 were females (52.4%) with male to female ratio of 1:1.1. The mean age at presentation was 56.66±18.98 (range 18-87) years.

Overall 61.9% of patients were diagnosed incidentally and were asymptomatic. In symptomatic patients, major symptoms were weakness (19%); burning sensation of extremities (14.2%), transit paraplegia of limbs (9.5%) and gastrointestinal bleed (4.7%).

Physical examination revealed splenomegaly as predominant finding detected in 3 patients (14.2%) followed by hepatomegaly in 2 patients (9.5%).

The mean hemoglobin level was 11.7±2.4 g/dl (range 8.3-15.6) with the mean MCV of 86.49±11.3 fl. The mean TLC count was 13.32±8.1x10^9/l (range 4.7-37.8) and the mean platelets count was 1188.76±522.2x10^9/l (range 448-2604). Serum lactate dehydrogenase, serum creatinine and uric acid were 454.3±127.8, 1.2±0.5 and 7.4±3.4 respectively. As per Myeloproliferative neoplasm working group, the risk stratification revealed 12 (57.1%) patients were in high risk; 5 (23.8%) patients in intermediate risk while 4 (19.1%) patients in low risk group.

Table 1. Comparative Analysis of JAK-2 Mutation Positive and Negative Patients With ET

Parameters	JAK-2 positive mean(SD) n=13	JAK-2 negative mean(SD) n=8	P- value
Age	55.15(20.6)	59.12 (16.9)	0.6
Hemoglobin	12.04 (2.3)	11.23(2.5)	0.4
MCV	85.08 (10.2)	88.71(13.3)	0.5
TLC count	15.82 (9.5)	9.88 (2.2)	0.01*
Platelets count	1179.84(499.9)	1203.25 (591.8)	0.9
Uric acid	7.97 (3.6)	5.15 (1.3)	0.3
LDH	602.0(30.7)	380.5 (33.7)	0.002*
Creatinine	1.37 (0.6)	0.75 (0.7)	0.03*

*P-value statistically significant

JAK2 V617F was found to be positive in 13 (61.9%) patients, while 8 (38.1%) were negative. The comparative analyses of JAK2 V617F positive and negative patients are shown in Table 1.

No correlation could be established between JAK2 V617F positivity with age and gender (P>0.05). However comparative analysis revealed significantly high total leukocytic count of 15.82±9.5 in JAK2 V617F positive patients group as compared with negative group that was 9.88±2.2 (P=0.01). Another important pertinent finding was positive correlation of JAK2 V617F expression with raised LDH and elevated serum creatinine (P≤0.05).

Discussion

Essential thrombocythemia is an uncommon hematopoietic malignancy with stable disease course (Zhang et al., 2014). Most patients do not need treatment, whereas some require treatment early after diagnosis or with disease progression. In the last decade a number of diagnostic molecular markers have been identified. Amongst them, the expression of JAK2 V617F in myeloproliferative neoplasms is increasingly being recognized as of paramount importance (Tefferi and Barbui, 2015).

In the present study, JAK2 V617F mutation was evaluated in Pakistani patients with ET. It was noted that the prevalence of JAK2V617F mutation in our patients was slightly higher (61.9%) than that was reported in the previous local study from Northern Pakistan that was 52.6% (Sadiq et al., 2013).

JAK2 V617F positivity in ET has been observed from various racial backgrounds ranging from 25% to 70% (Sazawal et al., 2010; Alshemmari et al., 2014). When compared with earlier reports, our results are intermediate with regional studies reported from India; ranging 38% to as high as 70% for JAK2V617F mutational expression (Sazawal et al., 2010; Varghese et al., 2013).

Other studies from Kuwait and Malaysia by Alshemmari, et al and Hamidah et al have reported the frequency of JAK-2 positivity in 25% and 52.9% of patients with ET respectively (Hamidah et al., 2012; Alshemmari et al., 2014).

Compared with data from developed countries outcome are more or less similar. Recent study from France also reported the similar prevalence (57.5%) in their patients that is comparable to our findings (Ben Said et al., 2015). Subsequently Gangat et al from USA also reported mutational frequency as 53% in a large cohort of patients (Gangat et al., 2015). Mutation frequency was determined as 61% for JAK2V617F in recent large series from the Italian group (Palandri et al., 2015). It was 68% in a Turkish study in their ET patients (Payzin et al., 2014).

In the present study we stratified the patients based on JAK-2 mutation positivity and negativity. We did not find any difference in age, gender, disease duration and patients symptoms. But significant correlations were observed with raised TLC, high LDH and creatinine in the present study.

It has been also reported that ET cases with JAK-2 positivity displays a higher leucocyte count and haematocrit value at diagnosis. These considerations suggest that JAK-2 expression in ET may reflect an activation state of the malignant clone that is associated with progressive disease. Thus, a laboratory test for JAK2V617F expression could be an important tool in the patient's diagnosis and disease progression monitoring.

One study from Egypt reported significant correlation between high TLC counts and JAK-2 mutation (Ayad and Nafea., 2011). In the present study, significantly high total leukocytic count of 15.82±9.5 in JAK2 V617F positive patients group is seen as compared with negative group that was 9.88±2.2 (P=0.01). In parallel to our findings, later study from India also demonstrated significant correlation (P<0.01) of JAK2V617F with raised total leukocytic count (Sazawal et al., 2010).

Raised serum lactate dehydrogenase was strongly correlated with JAK-2 mutation in our study. The mean LDH was 602.0±30.7 in JAK-2 positive patients compared with 380.5±33.7 in negative group (P=0.002). Yonal-Hindilerden et al, from turkey also disclosed a strong association of JAK2 mutation with high lactate dehydrogenase which is in concurrence to our results (Yonal-Hindilerden et al., 2015).

We could not determine any association of gender with JAK-2 mutation, similar was reported previously in one Korean study (Ahn et al., 2007). However, raised serum creatinine is determined as a significant marker correlated with JAK-2 mutation in the present study (P=0.03). In the available literature, we did not come across any study that has mentioned similar finding. Furthermore studies are definitely needed to substantiate this finding.

In summary, our findings are more or less similar to studies reported from various part of world. However high TLC and LDH levels indicate that our patients had biologically and clinically advanced disease. This is seen more consistently in JAK-2 positive group. It also supports the recommendation that mutational screening for JAK2V617F should be incorporated into the initial evaluation of patients with suspected ET.

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