Clinical Relevance of JAK2V617F Mutation with Lactate Dehydrogenase Activity in Patients with Myeloproliferative Diseases

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Summary

Clinical significance of the JAK2V617F mutation in patients with a myeloproliferative disease has been the target of intensive research in recent years. There is a need for development of newer molecular parameters for detection of myeloproliferative diseases. Therefore, aim of the present study was to identify the effect of JAK2V617F mutation on clinical phenotypes in patients with myeloproliferative diseases. Eighty eight patients with myeloproliferative diseases were included in the present study. Blood samples were collected by venipuncture from the subjects and DNA isolation was carried out. Real-Time Polymerase Chain Reaction (RT-PCR) method was carried out for analysis of JAK2V617F mutation status. Lactate Dehydrogenase (LDH) and routine hematological parameters were analyzed by autoanalyser. Statistical analysis was carried out using SPSS statistical software (version 15). JAK2V617F mutation was detected in 45.5% of patients with myeloproliferative diseases. In which 64.3%, 50.0% and 68.0% positivity of JAK2V617F mutation showed in polycythemia (PV), essential thrombocythemia (ET) and primary myelofibrosis(PMF) respectively. Present study was observed that mean LDH activity were higher in patients with PMF compared to ET and PV. Furthermore, it was also noted that patients with JAK2V617F mutation had higher mean LDH activity as compared to patients without JAK2V617F mutation. Concluding remarks from present study is that the integration of laboratory testing for JAK2V617F mutation and LDH activity is necessary to improve the diagnosis and screening of the myeloproliferative diseases. In Furtherance, isoforms of LDH activity would provide better understanding for correlation of JAK2V617F mutation with LDH activity in myeloproliferative diseases.

Keywords: Essential Thrombocythemia, JAK2V617F Mutation, Lactate Dehydrogenase, Myeloproliferative Diseases, Polycythemia Vera, Primary Myelofibrosis

Introduction

Myeloproliferative diseases (MPDs) are clonal disorders characterized by excessive production of mature blood cells.[1] in the most recent World Health Organization (WHO) classification of hematologic malignancies, this group of diseases was renamed from "myeloproliferative diseases" to "myeloproliferative neoplasms". This reflects the underlying clonal genetic changes that are a salient feature of this group of disease.[2] All myeloproliferative neoplasm (MPNs) are clonal disorders with an initial hit in the HSCs resulting in an excessive production of blood cells in some combination in the bone marrow, peripheral blood, and body tissues because of hypersensitivity or independence from normal cytokine regulation.[3] In 1951, the hematologist William Dameshek was first introduced concept of myeloproliferative diseases and also described four different diseases with clinical and biologic similarities: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF).[3] Some MPNs are the result of a genetic event leading to the constitutive activation of a tyrosine kinase that mimics the intracellular signalling pathways induced by hematopoietic growth factors. The risk of thrombosis is increased in some types of MPN. [3,5-6]

The most commonly recognized mutation in the remainder of the Philadelphia chromosome-negative MPNs is Janus kinas 2 (JAK2V617F), which is located on chromosome 9p24.[7] This mutation substitutes phenylalanine for valine at position 617 in the JH2 domain (Val617Phe, V617F) of exon 14, leading to constitutive activation of the JAK-STAT and other pathways resulting in uncontrolled cell growth. In the western population, this mutation is found in almost all PV cases and nearly half of PMF and ET cases.[8] various studies have recommended that analysis of JAK2V617F allelic burden is useful for discriminating between the different MPNs subtypes.[9]

Characterization of a malignant disease by molecular marker is expected to improve understanding of variations in the clinical course of individual patient and help to estimate their prognosis. Moreover, molecular marker that is linked to malignant transformation may provide a non-surgical therapeutic approach by targeting these molecules. The body of literature on molecular markers of malignancy in general is huge, but there is fewer reports have been shown association of JAK2V617F mutation with LDH activity in myeloproliferative diseases. Therefore, the aim of the study was to

Table 1: Clinical characteristics of patients with myeloproliferative diseases

Characteristics	No. of Patients
Gender	
Male	44
Female	44
Age (year)	
Range	17-87
Median	38
Diagnosis N	
PV	14
ET	22
PMF	25

Table 2: Frequency of JAK2V617F mutation in different types of myeloproliferative diseases

	JAK2V617F negative patients N (%)	JAK2V617F positive patients (%)
PMF	8(32.0)	17 (68.0)
ET	11 (50.0)	11 (50.0)
PV	5 (35.7)	9 (64.3)

Table 3: Correlation of hematological parameter with JAK2V617F mutation status in myeloproliferation diseases

	Negative JAK2V617F Mutation Status (Mean Values)	Positive JAK2V617F Mutation Status (Mean Values)
WBC count (×103/cmm)	19.42	35.79*
Hemoglobin (gm/dl)	11.09	11.26
Platelet count (×103/cmm)	454.71	558.71

*p=0.001 compared with negative status of JAK2V617F mutation

correlate JAK2V617F mutation with LDH activity and haematological parameters in myeloproliferative diseases.

Materials and Methods

The study was conducted in The Gujarat Cancer & Research Institute and prior consent was obtained from all the subjects to participate in the study. A total of eighty eight patients with myeloproliferative diseases were enrolled in the study, in which, fourteen cases were diagnosed with PV, twenty two cases as ET and twenty five cases as PMF. The patient group represented a median age of 38 years with an age range of 17 to 87 years (Table 1). Blood samples were collected for JAK2V617F mutation analysis. Genomic DNA from peripheral blood was extracted using QIAamp DNA blood Kit (Qiagen, Germany) as per manufacturer instructions.

Figure 1: Frequency of JAK2V617F mutatioNin myeloproliretaive diseases patients

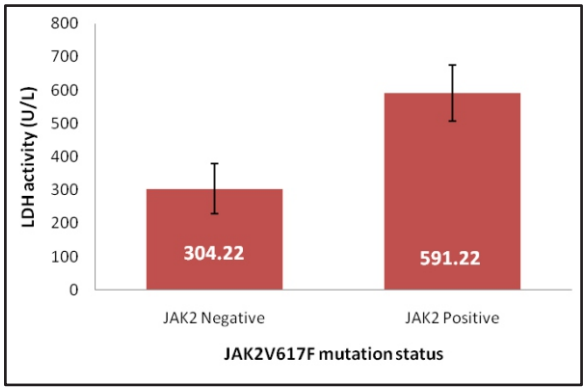

Figure 2: Correlation of mean LDH activity with JAK2V617F mutations status in myloproliferative patients

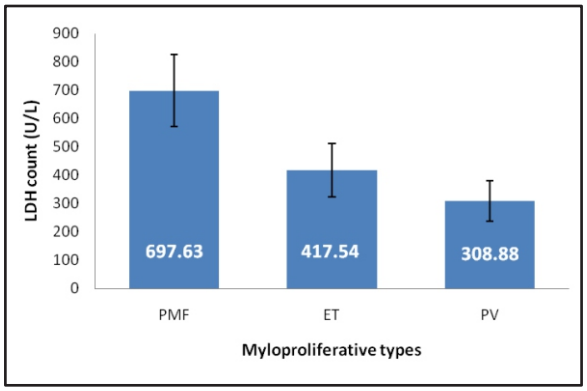

Figure 2: Correlation of mean LDH activity with JAK2V617F mutations status in myloproliferative patients

Further, JAK2V617F mutation were analysed from genomic DNA by real time PCR method. Routine haematological parameters and LDH activity were carried out by auto analyzer. Statistical analysis was performed using SPSS software version 15. Student's independent't' test was performed to analyze the significance between different groups.

Results:

JAK2V617F mutation status in patients with myeloproliferative diseases

PV, ET and PMF are different types of myeloproliferative diseases. Therefore, present study evaluates overall status of JAK2V617F mutations in myeloporlierative diseases. Figure 1 shows JAK2V617F mutation status in patients with myeloproliferative disease. Among these patients, 45.5% were JAK2V617F positive and 54.5% were JAK2V617F negative. It is clear from results that there was no difference in JAK2V617F mutation status of myloprolierative diseases. Present study also evaluated JAK2V617F mutation in myeloprolierative disease like PMF, ET and PV. Table 2 depicts the positivity and negativity of JAK2V617F mutation in PMF, ET and PV. It was observed that JAK2V617F allele burden was higher in PMF (68.0%) and PV (64.3%) patients as compared to the ET (50.0%).

Association of JAK2V617F status with hematological parameters in myeloproliferative diseases

Present study also observed association of hemoglobin, WBC and platelet counts with JAK2V617F mutations in myloproliferative diseases (Table 3). JAK2V617F positive patients with myeloproliferative diseases had significantly higher WBC counts as compared to JAK2V617F negative patients with myeloproliferative diseases. But there was no statically significant correlation of JAK2V617F mutation status with hemoglobin and platelet count in myeloproliferative diseases.

Correlation of JAK2V617F mutation status with LDH activity in myeloproliferative diseases

In present study, correlation of JAK2V617F mutation status with LDH activity in myeloproliferative diseases was also analysed. Figure 2 depicts correlation of JAK2V617F mutation status with LDH activity in myeloproliferative patients. Mean LDH activities in patients with positive and negative JAK2V617F mutation were 591.06 and 304.22 U/L, respectively. LDH activity was lower in patients with no JAK2V617F mutations as compared to patients with JAK2V617F mutations. Figure 3 depicts that LDH activity was higher in PMF patients as compared to patients with ET and PV. However, it was not statistically significant. Mean value of LDH activity in PMF, ET and PV were 139.90, 731.18 and 440.75 IU/L, respectively.

Discussion

Myeloproliferative diseases are currently increasing in general population and the incidence per year is approximately 0.55%. The annual incidence rate per 100,000 populations was 2-2.53% for PV and ET.10 The JAK2V617F mutation has a prevalence of 0.1-0.2% in the general population, but its clinical implications are still unknown for those individuals who are harboring the mutation without overt signs of a myeloproliferative disease.[11] The hallmark of myeloproliferative disease is over production of mature blood cells which initiate myeloproliferative diseases.[12] The impact of JAK2V617F mutation burden on a number of clinical parameters such as WBC counts, hemoglobin concentration, platelet counts and thrombosis especially for thrombotic events had been demonstrated in patients with myeloproliferative diseases.[13] In present study, JAK2V617F mutation was observed in 45.5% of myeloproliferative diseases. Similar results were also observed by Baxter et al[14] It was also observed that among all myeloproliferative diseases, 26.6% patients were PV, 36% patients were ET and 33.3% patients were PMF.[14]

It has been previously suggested that the JAK2V617F mutation in PV patients can be detected approximately in 95% of patients.[15] The JAK2V617F allele burden was significantly different among patients with PV and ET. Moreover the burden of JAK2V617F was highest in primary myelofibrosis (63.64%). In patients with PV, 50% of the patients showed JAK2V617F positive mutation. JAK2V617F mutation is found in approximately 55% of patients with ET, and these represents by World Health Organization diagnostic criterion. In present study, it was observed that about 50% patients with ET showed JAK2V617F positivity. Similar results were found in Chinese patients suggested by Zhao et al[16] Currently, JAK2V617F mutation is considered as a genetic diagnostic criterion for myeloproliferative diseases.

Numerous studies indicate that the JAK2V617F allele has been variably associated with higher indices of erythropoiesis, decreased platelet count, older age, and longer duration of disease in PMF.[17-18] Present study observed that platelet count was decreased in PMF patients than the patients with ET and PV. The platelet count was significantly higher in patients with ET and PV. Additionally, patients with WBC count had higher proportion of JAK2V617F mutation positive patient's burden than negative.

A number of studies suggested leukocytosis as a novel marker for vascular risk.[19-20] In present study, neither WBC counts nor JAK2V617F mutation burden appeared to be a risk factor for thrombosis in patients with PV, ET and PMF patients. V617F allele burden progressively increases alongside changes in phenotype, with lower allele burden inducing isolated thrombocytosis and higher levels being accompanied by increases in hemoglobin level, leukocytosis, and spleenomegaly. As expected, erythrocyte volume fraction increased during follow up in individuals with a myeloproliferative disease. Lower JAK2V617F allele burden has been reported in women with MPN compared with men. Several

studies reported that JAK2V617F allele burden was higher in PV than that in ET. [21]

In a study by Zhao et al., it was observed that PV patients with leukocytes had higher JAK2V617F allele burden and in ET with high hemoglobin level, the mean JAK2V617F mutation burden was more than those without elevated hemoglobin level.16 Controversial results were found in our study that higher leukocytes counts were found in PMF. LDH activity exceeded control level among individuals diagnosed with essential thrombocythemia; but present study had observed higher LDH activity in PMF patients. However, difference was observed in JAK2V617F mutation positive and negative patients. But present study also observed higher LDH activity in patients with myeloporlierative disease with positive JAK2V617F mutation as compared to patients with negative JAK2V617F mutation status. These results indicated significant association of LDH activity with frequency of JAK2V617F mutation in patients with myeloproliferative disease. It was indicated from present study that association of isoforms of LDH enzymes with JAK2V617F mutation would give better understanding for higher LDH activity in myeloproliferative diseases.

It can be concluded from the present study is that the detection of JAK2V617F allele burden is a simple and easily accepted in clinical set up. It was also observed that the clinical and hematological phenotypes of myeloproliferative diseases were associated with JAK2V617F mutation. JAK2V617F mutation was also associated with LDH activity. Therefore, the integration of laboratory testing for JAK2V617F mutation with LDH activity is necessary to improve the diagnosis and screening of myeloproliferative diseases In Furtherance, isoforms of LDH enzymes with JAK2V617F mutation would give better understanding of role of LDH activity in different types of myeloproliferative diseases.

Conflict of Interest: Nil

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Get a little bit better every single day. "
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