Clinical Correlation of Lactate Dehydrogenase Activity with JAK2V617F and FLT3 Mutations in Myeloproliferative Disorders and Acute Myeloid Leukemia: Indian Population

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Abstract

Background: Lactate dehydrogenase (LDH) play main role in the cell damage.Previous study has shown elevated level in acute leukemia and myeloproliferative disorders. Molecular markers specifically for JAK2V617F mutation in myeloproliferative disorders (MPDs) and FLT3 mutations in acute myeloid leukemia patients (AML) are well known for clinical practice. But, still only few reports have shown the correlation of LDH activity with molecular markers in Hematological Malignancy. Therefore, aim of study is to correlate LDH activity with established specific molecular markers in MPDs and AML. **Methods:**10 healthy individuals, 50 patients with MPDs and 50 patients with AML were included for the study. Serum LDH was performed by autoanalyser. The JAK2V617F mutations is analyse from blood by Real time PCR and FLT3 ITD/D835 mutations are analysed from blood by PCR-RFLP respectively. Statistical analysis was done by SPSS statistical software. **Results:** LDH Activity were higher in MPDs and AML as compared with healthy individual. Mean LDH activity was higher in FLT3 ITD/D835 positive mutation and JAK2V617F positive mutation in AML and MPDs respectively was observed. **Conclusion:** High LDH activity with Positive frequency of FLT3 ITD/D835 mutation in Acute Myeloid Leukemia and Positive frequency of JAK2V617F mutation in Myeloproliferative Disorders were observed.

Keywords: Acute Myeloid Leukemia, FLT3 ITD/D835, JAK2V617F, Lactate Dehydrogenase, Myeloproliferative disorders

Introduction:

Lactate Dehydrogenase (LDH) is an enzyme, which is required during the process of turning sugar into energy from the cells. The conversion of lactate into pyruvic acid, which is located in cytoplasm and various organs such as heart, muscles, liver and brain. There are five different isoenzymes found in different organs. The two isoenzymes i.e., LDH-1 and LDH-2 mainly expressed on red blood cells. LDH, which is a glycolytic enzyme, mainly found in liver and represent for gluconeogenesis process. LDH test is mainly for the cell damage. The increased level of LDH was found in acute leukemia. [1] Range of LDH activity has been used as a diagnostic tool in patients with malignant diseases, haemolysis, myocardial infarction and liver diseases.[2] Myeloproliferative neoplasms (MPNs) are clonal disorders characterized by excessive production of mature blood cells. In 1951, the hematologist William Dameshek was first introduced concept of myeloproliferative disease. Also described four different types of diseases with clinical and biologic similarities: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). [3] Janus kinase 2 (JAK2) is one of the well-known marker for myeloproliferative disorders. This acquired mutation is characterized by a G to T transversion at nucleotide 1849 in exon 14 of the JAK2 gene, leading to a substitution of valine to phenylalanine at amino acid

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position 617 (V617F) of the JAK2 protein.[4] JAK2V617F mutation was detected mostly in PV patients as compared with ET and PMF patients. The change in JAK2V617F alleles affects the disease course and its potential use as a prognostic marker.[5]

Acute Myeloid Leukemia (AML), which regulates the normal hematopoietic stem cells, which is a clonal disease of leukemic cells within the bone marrow niche. FMS like tyrosine kinase (FLT3) is one of the well-established marker for AML. Approximately, 30% of AML patients suffering from FLT3 mutations. FLT3 gene, which is located on chromosome 13q12, which plays an important role in cell proliferations, survival, differentiation and pathogenesis of AML and it is associated with drug resistance and its role in AML. FLT3 gene is divided into two categories: (A) Internal Tendom duplication (FLT3 ITD) mutations (B) point mutation or missense mutation (FLT3 D835). FLT3 ITD mutation is one of the most frequent bad prognosis mutation found in AML patients. FLT3 D835 mutation was found rare in AML patients.[6] Based on review of literature, it is well known that the characterization of molecular marker is expected to improve the understanding of variations in the clinical cause of individual patients and help to estimate their prognosis. Moreover, molecular marker that is linked to malignancy transformation may provide a non-surgical therapeutic approach by targeting these molecules. Therefore, in a light of this, the aim of the study is to correlate the clinical utility of LDH activity with JAK2V617F mutation and FLT3 ITD/D835 mutation in MPDs and AML patients respectively.

Methods:

Patients Collection:

In the present study, 50 patients with MPDs, 50 patients with AML and 10 healthy individuals were enrolled. Peripheral blood samples were collected from all the patients and healthy individuals in EDTA vaccutee. Serum from peripheral blood samples separated by serum vaccutee for LDH activity. Table 1 shows clinical characteristic of patients. The range of

Table 1: Clinical Characteristics of Patients with MPDs and AML

Characteristics	No. of Patients
Healthy Individual for LDH activity (n = 10)	
Gender	
Male	6
Female	4
Age(Years)	
Age range	19 - 55
Median	45
Myeloproliferative Diseases (n = 50)	
Gender	
Male	27
Female	23
Age(Years)	
Age range	4 - 78
Median	48
Sub Types	
ET	24
PV	13
PMF	13
Acute Leukemia Patients (n = 50)	
Gender	
Male	29
Female	21
Age(Years)	
Age range	1 - 68
Median	36
Stages	
M0	8
M1	7
M2	22
M3	3
M4	2
M5	8

age group is from 4 to 78 years in MPDs patients. The median age of MPDs patients is 48 years. Out of the 50 patients 27 were male and 23 were female. 24 cases of ET, 13 cases with PV and 13 cases with PMF subtypes in MPDs. The range of age group is from 1 to 68 years in AML patients The median age of AML patients is 36 years.

Estimation of LDH activity:

Serum sample is used for the analysis of LDH activity and it was carried out by Cobass 6000 Analyzer.

Analysis of FLT3 ITD and D835 mutations in Acute Myeloid Leukemia:

Qiagen DNA blood kit was used for the extraction of genomic DNA. Using semi-quantitative PCR method, DNA was amplified. 200ng of genomic DNA was used for FLT3 ITD in PCR. The primers for FLT3 ITD are: Forward 5´- GCAATTTAGGTATGAAAGCCAGC - 3´ and reverse 5´- CTTTCAGCATTTTGACGGCAACC -3´. The thermal condition for FLT3 ITD is denaturation at 95°C for 1 min, annealing at 60°C for 30 sec and extension at 72°C for 90 secs and total 35 cycles are performed. PCR products was separated on 2% agarose gel electrophoresis. Gel was analysed using Gel documentation system Alpha Innotech, Inc. The band above 329bp would be considered positive for FLT3 ITD mutation. For FLT3 D835 PCR-RFLP was carried out. 600ng of genomic DNA was used for FLT3 D835 in PCR. The primers for FLT3 D835 are Forward 5´- CCGCAGGAACGTGCTTG - 3´ and Reverse 5´- GCAGCCTCACATTGCCCC - 3´. The thermal condition for FLT3 D835 is Denaturation at 94°C for 1 min, annealing at 66°C for 1 min and Extension at 72°C for 90 sec and total 35 cycles were performed. PCR product was further digested by EcoRV endonuclease enzyme at 37°C for 3 hrs. PCR products was separated on 3% agarose gel electrophoresis. Gel was analysed using Gel documentation system Alpha Innotech, Inc. Wild type FLT3 D835 restriction digestion results into 68bp and 49bp products in the presence of EcoRV restriction enzyme. In heterozygous mutation, it produces 3 bands 114bp, 68bp, 49bp products while in homozygous mutation, it products one band corresponding at 114bp product. [7-12]

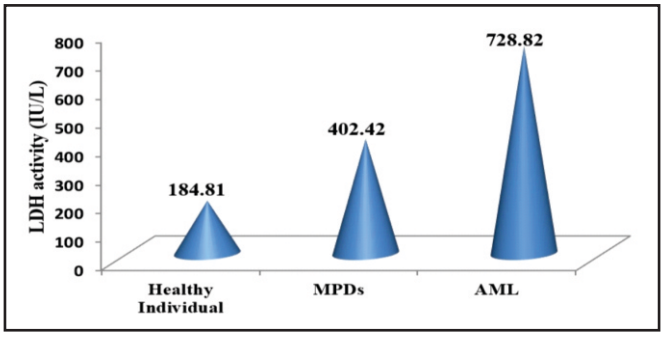
Analysis of JAK2V617F mutation in MyeloproliferativeDisorders:

Genomic DNA from peripheral blood was extracted from QIamp DNA blood kit manufacture by protocol. Semi-quantitative Real time PCR was carried out for the quantification of JAK2V617F mutation using commercial kit. A 25-fold dilution of the provided positive DNA control by transferring 2µL of positive DNA control to 48µL of DNase Free Water. From this mixture add 5µL of diluted JAK2 DNA control as positive control. Add 4µL of JAK2 PCR Master mix, 5µL of JAK2V617F Primers, add 50ng of genomic DNA and upto 20µL of DNase free water. Then place it into the real time PCR thermal cycler and run the profile. In the setup mean TaqMan reagents, FAM is to detect JAK2V617F mutation and HEX is for the internal amplification control. The thermal condition for JAK2V617F is denaturation at 95°C for 15 min and 1 cycle and amplification at 95°C for 15 sec and at 60°C for 1 min and total 50 cycles were performed. The JAK2V617F (FAM) shows <32.4 considered as Positive and≥32.4 should considered as Negative.

Statistical Analysis:

The association between two groups is carried out by Independent t-test using SPSS statistic software version 20 (SPSS Inc, USA). P-value ≤0.05 was considered to be significant.

Figure 1: Mean LDH activity in healthy individual, MPDs, AML



Results:

Figure 1 shows the LDH activity in healthy individual, MPDs and AML. The mean LDH activity is higher in AML and MPDs is higher as compared with healthy individual.

Figure 2 (a) displays mean LDH activity in subtypes of MPDs. Mean LDH activity is higher in patients with PMF as compared with patients with PV and ET. The specific significant correlation found in the group of PMF and PV (p=0.034), PMF and ET (0.001).

AML is classified in various subtypes according to FAB classification. Correlation of mean LDH activity with subtype of AML patients is shown in Figure 2 (b). Mean LDH activity is higher in patients with subtypes of M3 and M5 of acute myeloid leukemia as compared with subtypes of M0, M1 and M2. There is a specific significant correlation found between the subtypes of M2 and M3 (p=0.005).

Figure 2 (a): Mean of LDH activity in subtypes of MPDs

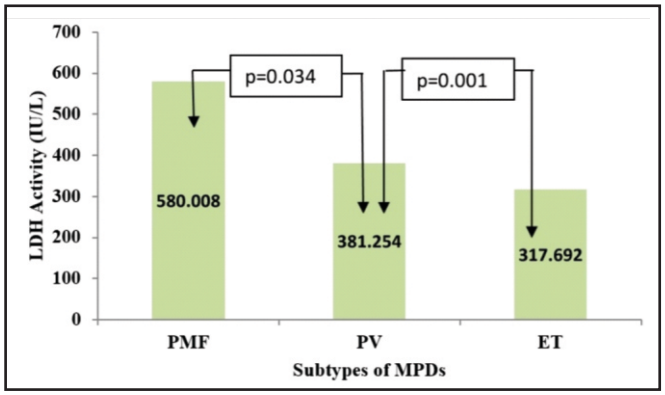


Figure 2 (b): Mean LDH activity in clinical subtypes of AML

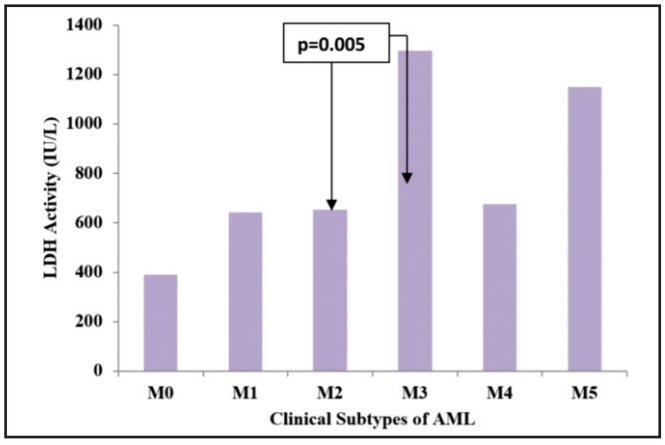


Figure 3: Frequency of FLT3 mutations in AML and JAK2V617F mutations in MPDs

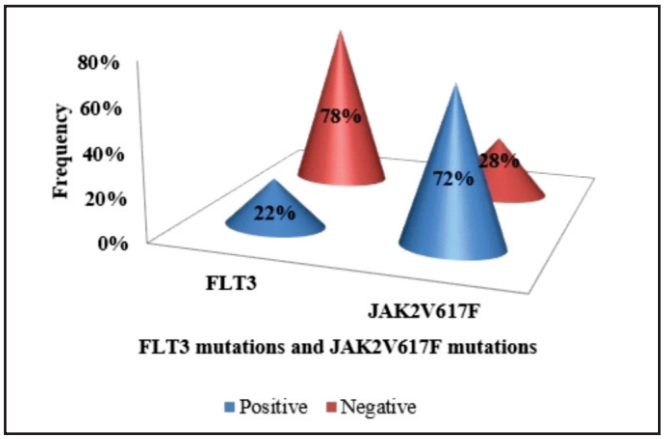


Figure 3 shows negative and positive frequency of FLT3 and JAK2V617F mutations in AML and MPDs respectively. 22% positive frequency and 78% negative frequency of FLT3ITD/D835 mutations were observed in AML. 72% positive frequency and 28% negative frequency of JAK2V617F mutations were observed in MPDs.

Table 2 shows that among AML, 9(18%) showed FLT3 ITD positive frequency and 41(82%) showed FLT3 ITD negative frequency, while FLT3 D835 positive frequency was observed in 3 (6%) and FLT3 D835 negative frequency was observed in 47 (94%).

Table 3 depicts the positivity and negativity of JAK2V617F mutation in PMF, ET, PV. It was observed that JAK2V617F allele burden was higher in PMF mutation (84.61%) and PV (70.84%) mutation as compared to the ET (61.53%).

Table 4 shows the mean LDH activity with molecular markers in AML and MPDs. Mean LDH activity was higher in those patients who had positive mutation FLT3 ITD/D835 as compared with negative mutation of FLT3 ITD/D835 even high mean LDH activity in those patients who had positive frequency of FLT3 ITD/D835 as compared with healthy individuals. It was also observed high mean LDH activity in those patients who had positive frequency of JAK2V617F as compared with negative mutation of JAK2V617F and healthy individuals.

Table 2: Frequency of FLT3 mutations in AML

	FLT3 mutation
Positive frequency of FLT3 ITD	9 (18%)
Negative frequency of FLT3 ITD	41 (82%)
Positive frequency of FLT3 D835	3 (6%)
Negative frequency of FLT3 D835	47 (94%)

Table 3: Frequency of JAK2V617F mutation in MPDs

	Positive frequency of JAK2V617F mutation N (%)	Negative frequency of JAK2V617F mutation N (%)
PMF	11 (84.61%)	2 (15.39%)
ET	8 (61.53%)	5 (38.47%)
PV	17 (70.84%)	7 (29.16%)

Table 4: Correlation of LDH Activity with Molecular Markers in Hematological Malignancy

	Frequency with LDH Activity	Mean ± S.D.
Acute Myeloid Leukemia	Positive Frequency of FLT3 ITD/D835 mutation	651.41 ± 702.7579
	Negative Frequency of FLT3 ITD/D835 mutation	303.273 ± 256.0
	Positive Frequency of FLT3 ITD mutation	662.9 ± 358/0.2560
	Positive Frequency of FLT3 D835 mutation	2312.4 ± 2822.2
Myeloproliferative Disorders	Positive Frequency of JAK2V617F mutation	446.058 ± 248.2967
	Negative Frequency of FLT3ITD/D835 mutation	290.207 ± 165.4871
Healthy Individual	No Mutation	184.810 ± 3.0856

Discussion:

LDH and lactate production are mainly involved due to invasive potential, tumor initiation, metastasis and recurrence. Serum LDH plays a key biomarkers role in many types of cancer and baseline LDH levels are become elevated in tissue injury or damage. Serum LDH levels are prognostic biomarkers for poor survival in multiple myeloma, hematological malignancy, prostate cancer. [13] In the present study, LDH activity was higher significantly in AML and MPDs patients as compared to healthy individuals. Previous reports also showed that LDH activity was higher in acute lymphocyctic leukemia patients as compared with AML.[1, 15] This, may be due to the higher the serum LDH in AML patients which reflects the function of leukemic cells. In the present study, LDH activity is higher in PMF as compared with PV and ET and there is no such work which co-relate to our study. Few studies showed that the LDH activity was higher in M4 subtypes but our study state that the LDH activity is higher in M3 subtypes.[1]

In our study, the frequency of FLT3 positive (22%) is lower, while some agreed our study. This may be due to differences in sizes of examined groups, population genetics, environmental factors, selected population studies.[16,17] The frequency of FLT3 ITD (18%) mutation is higher than the frequency of FLT3 D835 (6%) mutations was observed in acute myeloid leukemia patients in this present study. Previous study also showed that the overall incidence of FLT3 tandem mutations was 20.4% (200 of 979), which is consistent with numbers reported by the group. [18] One of the study also showed lower frequency of FLT3 ITD mutation (7.4%), this may be due to the small number of patients included in that study, or the intrinsic characteristics of the population studied.[14] JAK2V617F mutation is found in many patients of MPDs. This may be due to the JAK2V617F mutation status have been linked with a higher risk of thrombosis, fibrosis, splenomegaly and transformation to leukemia. [19] In present study, it was observed that JAK2V617F allele burden was higher in PMF mutation (84.61%) and PV (70.84%) mutation as compared to the ET (61.53%). Some results are contractor as, Horn et.al., detected 96% in PV mutations, 74% in ET mutation as compared to the PMF (62%). [20]

Concluding remark of the present study, LDH activity was higher in those acute myeloid leukemia who has positive mutation in FLT3 ITD/D835 mutation. It was observed that positive association of JAK2V617F mutations with high LDH activity in patients with myeloproliferative disorders. These results specify that positive onset of higher LDH activity with FLT3 ITD/D835 and JAK2V617F mutations in acute myeloid leukemia and myeloproliferative disorders as in Indian Scenario. Though, in patients with acute myeloid leukemia and myeloproliferative disorders more studies require to understand the LDH activity as it forms isotypes. [21] Concluding remark of the study is high LDH activity with Positive frequency of FLT3 ITD/D835 mutation in Acute Myeloid Leukemia and Positive frequencies of JAK2V617F mutation in Myeloproliferative Disorders were observed.

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