Original Research Article

Study of LDH isoenzyme patterns in different Hematological malignancies

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Abstract

Background: Lactate Dehydrogenase (LDH) isoenzymes have been studied in a wide range of diseases and in different malignancies. They form an important prognostic marker in certain malignancies. In present study we aimed to assess the isoenzyme patterns of LDH, in various hematological malignancies, to see the preponderance of the type of LDH isoenzymes with hematological malignancy. **Material and Methods:** The present study was conducted in diagnosed patients of hematological malignancies who were on treatment, to assess the isoenzyme patterns of LDH. The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. Statistical analysis was done using descriptive statistics. **Results:** 100 hematological malignancy patient samples were studied by electrophoresis. Most common age group was 61-70 years (23%) and 67 % patients were males. Patients with acute myeloid leukemias (AML) were most common (36 %) followed by multiple myeloma (MM) (31 %). 56 patients had LDH values less than 480U/L and 44 patients had LDH values more than 480U/L. Isoenzyme 1 shows maximal distribution in 58% patients. 90 % of the patients had an increase in isoenzyme pattern and 5% had decrease in pattern. The rest 5% of the patient's isoenzyme patterns lie in the normal range. LDH isoenzyme 1 has maximum value among all the malignancies. Isoenzyme 4-5 had lesser representation. **Conclusion:** LDH 1 isoenzyme is most prominent to be raised in hematological malignancies. LDH 2 isoenzyme forms the major subgroup of LDH enzyme levels and it's decrease is more evident in hematological malignancies.

Key Words: Lactate Dehydrogenase, LDH isoenzyme, AML, Multiple myeloma, hematological malignancies

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INTRODUCTION

Lactate Dehydrogenase (LDH) is a tetramer which exists in different isoenzyme forms formed by varying combination of H and M monomers. These monomeric subunits are coded for by different genes located on chromosome number 11 and 12 respectively.[1,2] These different isoenzymes reversibly catalyze the same reaction but vary in their physical and chemical properties and can be distinguished on the basis of pH, cold liability, resistance to heat, sensitivity to inhibitors, reactivity to substrates, buffer concentration and their electrophoretic mobility. Enzyme molecular heterogeneity allows electrophoretic fractionation of the enzyme into at least five isoenzymes.[3] LDH 1and 5 is controlled by the action of two separate genes; the intermediate forms (LDH 2, LDH 3, LDH 4) represent hybrids of genes for LDH 1and 5 assembled by random association determined by the activity of the two alleles controlling LDH 1 and LDH 5.[4] Following maturation and development the adult LDH isozyme patterns in human tissue become organ specific.[5] The various isoenzyme forms are expressed by different tissues as follows,

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Sr. No.	LDH isozyme	Specific tissue
1.	LDH1-(H4)	Heart.
2.	LDH2-(H3M)	Reticuloendothelial system.
3.	LDH3-(H2M2)	Lungs.
4.	LDH4-(HM3)	Kidney, placenta, pancreas.
5.	LDH5- (M4)	Liver, striated muscle.

LDH isoenzymes have been studied in a wide range of diseases and in different malignancies. They form an important prognostic marker in certain malignancies. LDH is one of the enzyme systems preferentially produce and is retained by cancer cells as it is necessary to maintain tumor growth. A Study done on Acute Myeloid Leukemia patients on chemotherapy to find factors of prognostic importance, it was found that the only variable of statistical importance was LDH levels ≥ 400 U/ml which was significantly related to mortality rate.[6] Serum LDH is an important prognostic factor in patients with Non-Hodgkin's lymphoma (NHL). High LDH levels is one among other factors like age, staging etc, which is included in the International Prognostic index of aggressive NHL which can predict the 5 year survival of patients.[7] In present study we aimed to assess the isoenzyme patterns of LDH, in various hematological malignancies, to see the preponderance of the type of LDH isoenzymes with hematological malignancy.

MATERIAL AND METHODS

The present study was conducted in the Department of Biochemistry and the Department of Clinical Hematology and Bone Marrow Transplant Unit, Christian Medical College and Hospital, Ludhiana. Study design was prospective and observational. Study approval was obtained from institutional ethical committee. Diagnosed patients of Hematological Malignancies who were on treatment were considered for present study. Clinical details such as symptoms, duration of illness, clinical diagnosis and associated complications were collected from the medical records of the respective patients. The blood samples for the serum LDH and its isoenzyme pattern were collected under complete aseptic condition from the antecubital vein. The samples were transported to the laboratory immediately and serum was separated after centrifugation of the sample. Samples were stored at 4°C after their total LDH levels is estimated. They were later processed by electrophoresis to detect the isoenzyme patterns by Barnett H method 9 with barbiturate buffer at pH 8.6. The serum LDH levels were estimated on Modular P-800 auto-analyzer, using kits supplied by ROCHE. The method was by UV-assay, based on the formulation described by the German Society for Clinical Chemistry, Deutsche Gesellschaft für klinische Chemie. (DGKC) in 1972.26. LDH isoenzymes were separated and quantified by agarose gel electrophoresis using the method described by Barnett H method with barbiturate buffer at pH 8.6 .[8] The data obtained was subjected to statistical analysis using computer software (SPSS version 20; Chicago Inc., USA). The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. Statistical analysis was done using descriptive statistics.

RESULTS

100 hematological malignancy patient samples received in the Biochemistry laboratory were collected and their isoenzyme patterns were studied by electrophoresis using the Barnett H method [8] with barbiturate buffer at pH 8.6. Patients were in the age range of 6 to 90. Most common age group was 61-70 years (23%), followed by 51-60 years (18%) and 41-50 years (17%). Mean age in present study was 61.8 ± 10.2 years. 67 % patients were males and 33 % patients were females.

Table 1: General characteristics

Characteristics	Number of cases	Percentage (%)
Age (in years)		
0-10	2	2
11-20	12	12
21-30	11	11
31-40	7	7
41-50	17	17
51-60	18	18
61-70	23	23
71-80	9	9
81-90	1	1
Gender		
Males	67	67
Female	33	33

Patients with acute myeloid leukemias (AML) were most common (36 %) followed by multiple myeloma (MM) (31 %). 480 U/L was considered as the upper limit cutoff for LDH. 56 patients had LDH values less than 480U/L and 44 patients

had LDH values more than 480U/L. Both patients of CLL had values less than 480U/L. 50% of patients with ALL had values less than 480U/L and the other half of patients had values more than 480U/L.

Table 2: Distribution of malignancies and LDH enzyme values in patients

Malignancies	No. Of Patients	Patients with LDH value <480	Patients with LDH value >480
Acute Myeloid Leukemias (AML)	35	12 (34 %)	23 (66 %)
Multiple Myeloma (MM)	31	27 (87 %)	4 (13 %)
Lymphomas	18	10 (56 %)	8 (44 %)
Acute Leukemoid Leukemia (ALL)	8	4 (50 %)	4 (50 %)
Chronic Myeloid Leukemia (CML)	6	1 (17 %)	5 (83 %)
Chronic Leukemoid Leukemia	2	2 (100 %)	0

Isoenzyme 1 shows maximal distribution in 58% patients. 90 % of the patients had an increase in isoenzyme pattern and 5% had decrease in pattern. The rest 5% of the patient's isoenzyme patterns lie in the normal range.

Table 3: Distribution of isoenzymes in study patients.

Isoenzyme	Isoenzyme pattern		Range of LDH enzyme levels in the respective isoenzymes (U/L)
	Increase	Decrease	
1 (n = 58)	55	1	102-5720
2 (n =15)	9	3	232-8599
3 (n =15	14	1	55-1638
4 (n = 6)	6	0	255-620
5 (n = 6)	6	0	314-679
Total	90	5	55-8599

LDH isoenzyme 1 has maximum value among all the malignancies. Isoenzyme 4-5 had lesser representation.

Table 4: Distribution of isoenzymes in different malignancies

ISOENZYMES	ACUTE LEUKEMIAS (n=43) %		CHRONIC LEUKEMIAs (n=8) %		LYMPHOMA (n=18) %		MULTIPLE MYELOMA (n=31) %		TOTAL
1	28	65.1%	5	62.1%	7	38.8%	18	58.1%	58
2	6	14.0%	1	12.5%	3	16.7%	5	16.1%	15
3	3	7.0%	2	25%	4	22.2%	6	19.3%	15
4	3	7.0%	0	0%	1	5.6%	2	6.5%	6
5	3	6.80%	0	0%	3	16.7%	0	o%	6
TOTAL	**43**	**100%**	**8**	**100%**	**18**	**100%**	**31**	**100%**	**100**

LDH 1 is the most prominent isoenzyme in NHL and non NHL-Diffuse large B-cell lymphoma (DLBCL) and in other lymphomas, and HL had increase LDH 3. LDH 2 had maximum decrease in NHL and non NHL-Diffuse large B-cell lymphoma (DLBCL).

Table 5: Isoenzyme pattern in the subgroups of lymphoma patients.

Lymphomas (n= 18)	Number of increase in isoenzyme level					Number of decrease in isoenzyme level				
	Iso 1	Iso 2	Iso 3	Iso 4	Iso 5	Iso 1	Iso 2	Iso 3	Iso 4	Iso 5
NHL (N=5)	3	1	2	2	1	0	3	2	0	0
NON NHL DLBCL (N=5)	3	1	2	0	2	1	4	2	2	1
HL (N=4)	1	1	3	2	1	2	2	1	0	0
Others (n=4)	3	0	1	0	1	1	3	2	3	1

DISCUSSION

LDH is the key enzyme in Lactic Acid production and degradation which is an end product of anaerobic glycolysis. Leakage of the LDH from the damaged tissue increases the levels of LDH in the serum, which is the basis of using it as a diagnostic marker to tissue damage.[2] LDH appears to have a good correlation with disease activity and tumor mass. LDH levels correlated with number of blast during remission and relapse.[8] The prominent isoenzymes can be indicative of the disease tissue, since different organs contain characteristic proportions of different isoenzymes. Therefore the pattern of isoenzymes found in plasma serves to identify the site of tissue damage. LDH is a strong pretreatment prognostic factor in these patients

and it correlated with disease and survival status.[2] In the present study we investigated the levels of LDH and the percentage increase of its isoenzymes in different hematological malignancies. In present study male patients were more as compared to female patients. Similar findings were noted by Kornberg A *et al...*[9] Mean age in present study was 61.8 ± 10.2 years. Simiar results were noted by Dumontet C *et al...*[10] and Bouafia F *et al...*[11] Kornberg A *et al...*[9] studied the LDH values in different hematological malignancy patients and found that LDH in acute non lymphoblastic leukemia the range was 126-684 U/L, in acute lymphoblastic leukemia it was 402-3582 U/L, in patients with CML in blast crisis had levels of 970-1940 U/L. Similar findings were noted in present study. Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly integrated, hence the cells tends to utilize from about five to ten times as much glucose as do normal tissues, converting most of it into lactate. Hence increase the need for high levels of LDH. A relationship between neoplasia and increased LDH levels has been reported by many in human tumors. LDH appears to have a good correlation with disease activity and tumor mass. High levels of serum LDH have been observed in patients with solid tumors, leukemia, in non-Hodgkin's Lymphoma, particularly Burkitt's lymphoma, small cell lung cancer and testicular neoplasm.[12,13] Bouafia *et al...*,[11] studied the prognostic values of isoenzymes in hematological malignancies by agarose gel electrophoresis. On analysis the LDH isoenzyme profiles showed increased percentages of isoenzyme 2 in patients with NHL, CLL and myeloproliferative syndromes, but not in samples from patients with myeloma or Hodgkin's disease. LDH 1 values were found to be frequently increased in patients with NHL and myeloproliferative syndromes. LDH 3 values were increased in more than 50% of patients with NHL, myeloma, CLL and myeloproliferative diseases. In chronic leukemia 87.5% patients had increase in LDH 1. Of which CLL 100% and CML 83.3% had increase in LDH 1. Similar findings were observed by Drexler HG *et al...*[14] and Chirulescu Z *et al...*[15] for CML and Bouafia F *et al...*[11] for CLL patients. Whereas Muller CP *et al...*[16] and Buchsbaum *et al...*[17] observed an increase in LDH 5 and LDH 3 respectively in CML patients. In present study 15% had increase percentage of LDH 2. In acute leukemia 4.6% patients had increase LDH 2. In ALL 12.5% and in AML 2.9% had increase in LDH 2. Pandit MK *et al...*[18] observed similar findings in ALL chemotherapy responders. 15% patients had increase percentage of LDH 3. This is in corroboration with Bouafia F *et al...*[11] study who had observed increase in LDH 3 in MM, CLL and NHL. 6% had increase percentage of LDH 4. In acute leukemia 55.8% and in chronic leukemia 25% had increase in LDH 4. Which is in agreement with Patel *et al...*[19] 6% had increase percentage of LDH 5. In our study are there was increase in LDH 5 in ALL, AML, lymphomas and CML with no increase CLL which is contrary to study by Rambotti P *et al...*[20] Various study noted following findings.

Bouafia F *et al...*[11]	LDH 3 increased LDH 1-2 increased	NHL with poor prognosis CLL
Mizobe T *et al...*[1]	LDH 3 increased	Angioimmunoblastic T cell lymphoma
Giatromanolaki *et al...*[21]	LDH 5 increased	DLBCL
Lin Na *et al...*[22]	LDH 1-4 increased	Myeloma kidney disease

William BM *et al...*,[23] studied the levels of LDH in DLBCL patients who relapsed after complete remission and compared it with patients who did not relapse. They concluded that a 1.5 fold increase in LDH levels over 3 months is associated with increased likelihood of relapse in DLBCL patients. In present study, blood samples for LDH estimation were processed irrespective of the stage of the illness. Studies with specific time interval of diagnosis, start and response to treatment are required to ascertain the changes in isoenzymes with regard to clinical condition and stage of disease. Study of pattern at start of treatment and with remission could give a better picture of isoenzymes. Further studies in this area may suggest the role of LDH as a prognostic factor and help in correlating with survival status.

CONCLUSION

From this study we conclude that LDH 1 isoenzyme is most prominent to be raised in hematological malignancies. LDH 2 isoenzyme forms the major subgroup of LDH enzyme levels and it's decrease is more evident in hematological malignancies. All hematological malignancies had increase in LDH 1 with the exception of AML patients who had increase in LDH 4.

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