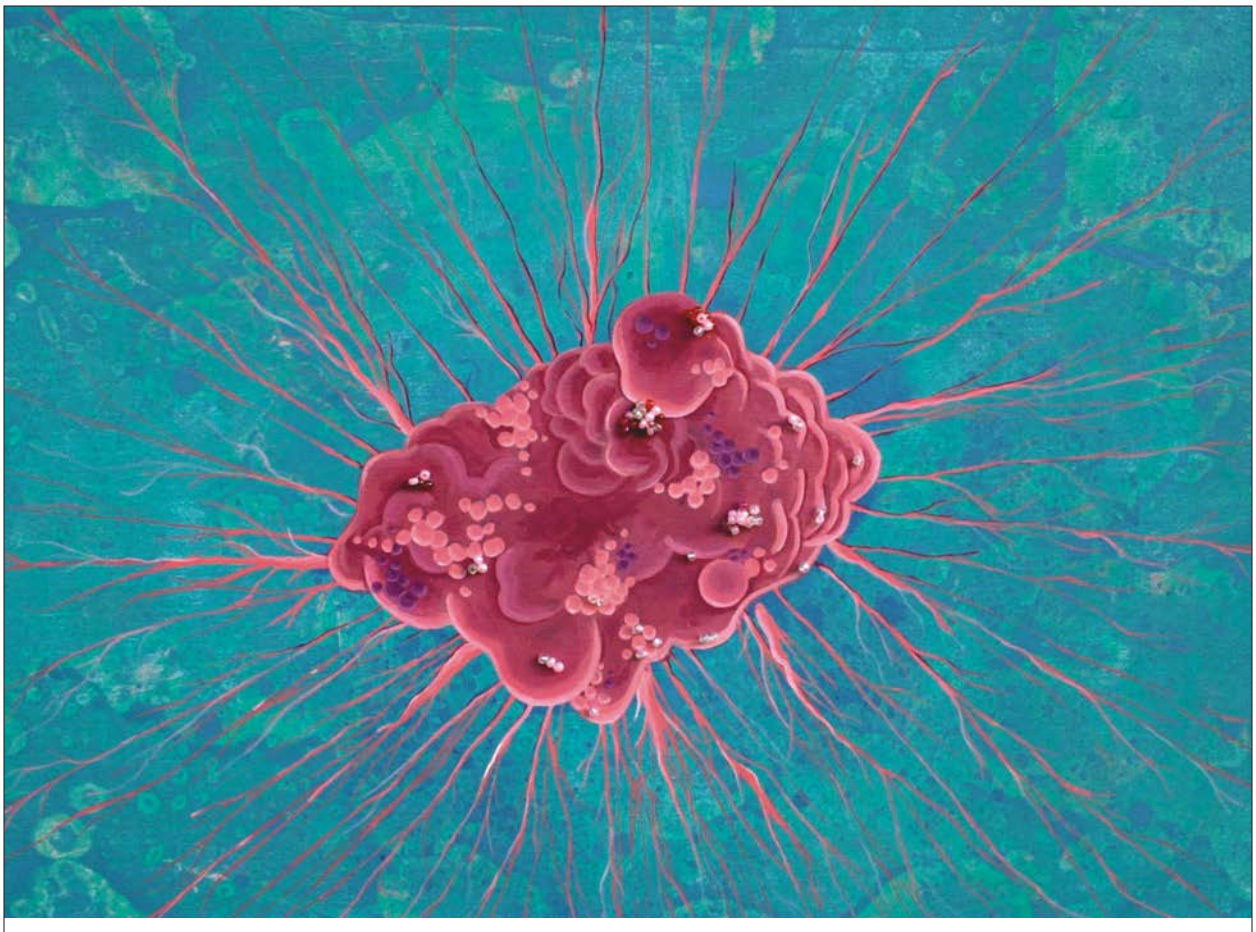

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

International Journal of Cancer Research 2 (4): 383-389, 2006
ISSN 1811-9727

Role of Serum Lactate Dehydrogenase as a Bio-Marker in Therapy Related Hematological Malignancies

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Abstract: Lactate dehydrogenase (LDH) is a general indicator of the existence and severity of acute or chronic tissue damage. The present study aims to determine the LDH levels in patients with hematological malignancies to evaluate its role in the diagnosis and prognosis especially in Indian population. We have analyzed the serum LDH levels of 265 patients and the diagnosis included, 50 Acute Lymphocytic Leukemia (ALL) patients, 40 Non-Hodgkin's Lymphoma (NHL) patients, 35 Chronic Myeloid Leukemia (CML) patients, 30 Acute Myeloid Leukemia (AML) patients, 15 Hodgkin's Lymphoma (HD) patients, 20 Chronic Lymphocytic Leukemia (CLL) patients, 25 Multiple Myeloma (MM) patients and the remaining 50 are normal serum samples, taken for control group. Among these 215 blood cancer patients, 160 patients (74.41%) had shown abnormal levels of serum LDH compared to control group samples. Out of the 50 control group, only 02 (04%) people had shown elevated serum LDH levels. When serum LDH levels of 50 diagnosed patients were observed against their survival rates, patients with higher LDH levels were exposed to early relapse of their disease. Hence we can conclude from our results that serum LDH can be used as an important biomarker in diagnosis and prognosis of hematological malignancies.

Key words: LDH, hematological malignancies, bio marker, univarient analysis

Introduction

Serum Lactate dehydrogenase (LDH) plays a major role in hematological malignancies. As cells die, their LDH is released and finds its way into blood. Hence almost all hematological malignancies show elevated levels of serum LDH. It is also useful in the assessment of tissue breakdown in general (Copur *et al*., 1968; Shipp *et al*., 1994). Lactate dehydrogenase is an oxido-reductase, which catalyses a critical step in glycolysis, the reversible transformation between pyruvate and lactate. LDH is a tetramer composed of two types of monomers H (for Heart) and M (for Muscle). The five iso-enzymes of LDH can be distinguished by gel electrophoresis and correspond to various combinations of these monomers, LDH (H4), LDH2 (MH3), LDH3 (M2H2), LDH4 (M3H) and LDH5 (M4) (Dimopoulos *et al*., 1991; Patel *et al*., 1991). The forms with high H content are found in tissues with steady oxygen supply (heart, brain); where as tissues which produce large amounts of lactic acid such as muscle are rich in M monomer. The ratio of H and M monomers has been reported by the state of hypoxia in tissues (Bouafia *et al*., 2004). Increased levels of LDH-1 are seen in myocardial infarction, red blood cell diseases like hemolytic anemia, kidney disease including kidney transplantation rejection and testicular tumors. Increased levels of LDH-2 are found in lung diseases such as pneumonia and congestive heart failure, as well as in lymphomas and other tumors. Elevations of LDH-3 are significant in lung disease and certain tumors. Elevations of LDH-4 are greatly increased

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in pancreatitis. High levels of LDH-5 are found in liver disease, intestinal problems and skeletal muscle disease and injury, such as muscular dystrophy and advanced solid-tumor cancers also cause significant elevations of LDH isoenzymes at the same time. According to International Prognostic Index (IPI), patients with increased LDH levels in NHL, CML, ALL and Multiple myeloma had a 90% risk of early deaths. As tumor aggressiveness is directly proportional to serum LDH levels (Dumontet *et al*., 1999), it would be worthwhile to determine the diagnostic significance and prognostic value of serum LDH in hematological malignancies.

Hematological malignancies are a group of neoplasm's that arise through malignant transformation of bone marrow derived cells. Leukemia; lymphoma and myeloma are different hematological malignancies that originate in the bone marrow or lymphatic tissues as a result of genetic injury. Serum LDH levels were also found to be significant in shorter survival groups than in longer survival groups (Itoyana *et al*., 2001).

Although Serum LDH is elevated in many diseases like myocardial infarction (MI), heart failures, pulmonary infraction and jaundice and in some muscular disorders etc., serum LDH levels have a major role in hematological malignancies (Henry, 1984). Currently, the main use for LDH is as a general indicator of the existence and severity of acute or chronic tissue damage and, sometimes, as a monitor of progressive conditions. LDH isoenzymes may also be used in differential diagnosis to help determine which organs are likely to be involved.

The aim of this study was to determine the role of serum LDH levels in hematological malignancies in order to find out if this could be used as a diagnostic as well as prognostic marker and whether it could be correlated with univarient analysis data. Devita (2005) has reported the prognostic value of LDH in hematological malignancies. This is a useful hypothesis to be tested in Indian populations; hence this study was under taken.

Materials and Methods

Study Group

The study group was of Indian origin; of a particular geographic region (Andhra Pradesh). The procedure to enroll the study group was in accordance with ethical standards of responsible committees of the institutes and the hospitals. Two hundred and fifteen diagnosed patients of blood cancer and 50 normal patients were enrolled from June 2004-June 2006. The patients were identified through an extensive search of blood and bone marrow reports. Blood and bone marrow results were carried out at the pathology department for the clinical evaluation of diagnosis or prognosis.

Univarient Analysis for Diagnosis of Hematological Malignancies

Clinical, bio-chemical and hematological characteristics were analyzed for all the individuals enrolled in the study. The characteristics recorded in the Proforma were age, sex and history of previous malignancies, hemoglobin level and platelet and WBC counts, serum LDH levels.

Estimation of Serum LDH Levels

Whole blood was collected from the patients and centrifuged for 10-15 min at 1500-2500 rpm. The separated supernatant serum was used for LDH estimation. Serum LDH activity was determined by Photometric method by using Bio-systems (Spain; 1×50 mL pack size) kit.

$$\text{Principle involved: Pyruvate + NADH} \xleftrightarrow{\text{LDH}} \text{Lactate + NAD}^{+}$$

Statistical Analysis

Comparison of serum LDH activity against Hematological malignancies was done according to age, sex, anatomic stage and aggressive histology. Statistical representation of serum LDH activity

against hematological malignancies was done on percentage basis and shown in graphical representations. Serum LDH thus estimated in hematological malignancy cases and controls were statistically analyzed by using student t-test of equal variance and the survival periods were calculated from the time of diagnosis to time of disease recurrence.

Results

Patients' Profile and Disease Characteristics

All the patients were suffering from different hematological malignancies (blood cancers). The diagnosis of patients was confirmed through hematology and bone marrow investigations. The serum samples of 215 blood cancer patients and 50 control group cases were analyzed for serum LDH levels. Among these 215 patients 160 patients (74.41%) had shown increased levels of serum LDH and the remaining 55 (only 25.59%) had shown normal serum LDH levels, though they were suffering from different types of blood cancers. Out of 50 control samples, only 02 (4%) had shown slightly elevated serum LDH levels. It is seen from the results presented in Table 1 that hematological malignancies are more prevalent as ALL in the age group of 2-14 years and other malignancies more prevalent in the age group of 24-50 years. From the results presented in Table 2, it is clear that Mean LDH levels can be presented in the following decreasing order in various hematological malignancies.

AML>ALL>CML>NHL>HD>MM>CLL.

Table 1: Characteristics of the patients at the time of diagnosis

	Total No. of patients	Male	Female	Age groups (years) 2-14	14-23	24-50	>50
ALL	50	32	18	25	12	12	01
AML	30	18	12	04	10	06	10
CML	35	23	12	03	02	27	03
CLL	20	02	18	00	00	02	18
NHL	40	30	10	08	02	12	18
HL	15	06	09	02	02	11	00
MM	25	20	05	00	00	10	15
Total	215	131	84	42	28	80	65

Table 2: Serum LDH levels in various hematological malignancies (including control group)

Parameters	Control group	ALL	AML	CML	CLL	NHL	HD	MM
No. of samples	50	50	30	35	20	40	15	25
Min. conc. (IU L^{-1})	150	140	317	490	182	162	110	150
Max. conc. (IU L^{-1})	480	6310	7600	2666	477	4000	1330	1000
Mean±SE (IU L^{-1})	304±19.5	1259± 244	1395± 460	1251± 190	345± 65	927± 149	539± 121	385± 66
CV	0.321	1.241	1.399	0.608	0.378	0.899	0.675	0.663

Table 3: Serum LDH elevated cases in various hematological malignancies

Hematological Malignancies (HM'S)	No. of patients	No. of increased LDH patients	Percentage of elevated LDH
Control group	50	02	4.00
Total HM cases	215	160	74.41
ALL	50	39	78.57
AML	30	28	94.44
CML	35	33	93.75
CLL	20	05	25.00
NHL	40	34	89.00
HD	15	11	77.77
MM	25	10	40.00

Table 4: Serum LDH levels in control group and hematological malignancies

Serum LDH	Control group	Hematological malignancies
No. of samples	50	215
Minimum Concentration (IU L^{-1})	200	110
Maximum Concentration (IU L^{-1})	450	7600
Mean±SE (IU L^{-1})	330±19.53	1026±108
CV	0.675	1.224

(p-value<0.001; Student t-value>$t_{0.05}$)

The results presented in Table 3, show the number of cases with increased LDH levels in this order.

AML>CML>NHL>ALL>HD>MM>CLL.

i.e., more number of cases with high serum LDH levels were seen in AML and lowest numbers of cases were seen in CLL.

When serum LDH levels were compared between the control group and total hematological malignancy cases, it was found that the malignancy cases had significantly higher values compared to control group ($p<0.001$ and $t>t_{0.05}$). Hence there is a significant difference between Control Mean group and Hematological Malignancy Mean group was observed (Table 4).

Serum LDH Findings in Diagnosis

Among 225 patients, 167 patients (74.22% had shown elevated serum LDH levels. In ALL, out of 50 patients 39 (78.57%) patients had shown abnormal levels of serum LDH. Among these 50 patients, 32 patients were males and 18 patients were females. The elevated serum LDH levels ranged from 140 to 6310 IU L^{-1}. Out of 50 patients, 25 patients were categorized under the age group 2 to 14 years. This revealed that the young children below 14 years were the most susceptible age group to ALL (Table 3).

In AML, out of 30 patients, 28 (94.44%) patients had elevated serum LDH levels. The frequency of susceptibility to AML was shown more in adults than children.

In CML, out of 35 patients 33(93.75%) had shown increased levels of serum LDH. Adults, age group between 24 to 50 years were most susceptible to CML. Out of 35 patients 27 patients were categorized under this age group.

In CLL, out of 20 patients only 5 (25%) cases had shown elevated levels of serum LDH and they were all of above 50 years age. Hence LDH plays very little significance in CLL diagnosis.

In NHL, among 40 patients, 34 (89%) patients had shown increased serum LDH levels and only rest of the 6 (5.1%) patients had with normal levels of LDH in serum.

In Hodgkin's disease (HD), 11(77.77%) patients had shown elevated serum LDH levels, out of 15 HD patients.

In Multiple Myeloma only 10 (40%) patients had shown increased serum LDH levels, out of 25 Multiple Myeloma patients (Table 3).

The control group observed has no significant abnormal LDH levels. Among the control group cases, only 02 (4%) cases had shown elevated serum LDH levels (Table 3). This could be because of cardiac complaints or myopathies. Figure 1 shows the LDH ranges in different hematological malignancies and the dispersion of LDH levels in various hematological malignancies were presented in Fig. 2.

At the time of diagnosis, most of these patients showed low hemoglobin levels (<10 mg dL^{-1}), leucocytosis (>11000 cells/cubic mm) and prominence of blasts in peripheral blood and marrow of acute leukemia patients. Simultaneously the diagnosed cases of multiple myeloma showed plasma cell percentages of more than 30% in marrow when compared to control group samples.

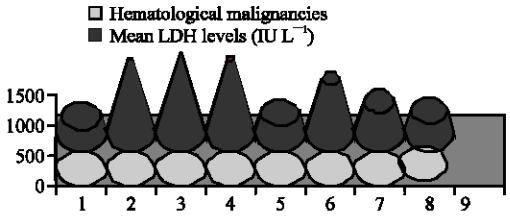

Fig. 1: Mean LDH ranges in various hematological malignancies. 1: Control, 2: ALL, 3: AML 4: CML, 5: CLL, 6: NHL, 7: HD, 8: MM

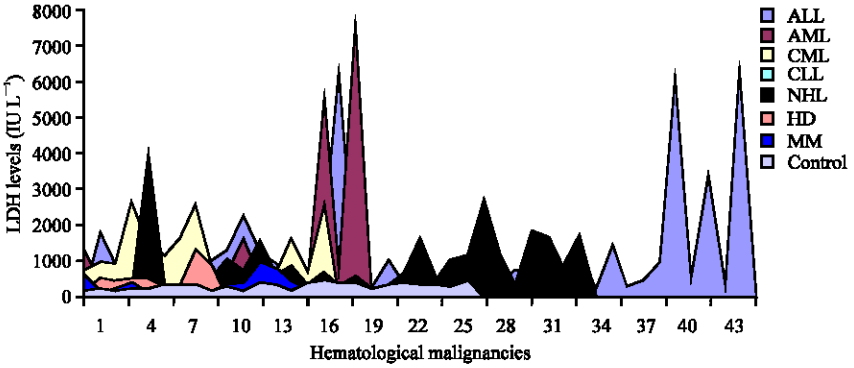

Fig. 2: Dispersion of serum LDH levels in hematological malignancies. CML: Chronic myeloid leukemia, CLL: Chronic lymphocytic leukemia, NHL: Non-Hodgkin's lymphoma, HD: Hodgkin's Lymphoma, MM: Multiple Myeloma

Table 5: Survival period studies in LDH elevated hematological malignancies

Hematological malignancies	Average survival period in months (patients with normal LDH levels)	Average survival period in months (patients with elevated LDH levels)
ALL (N = 15)	6-24	3-18
CML (N = 10)	7-12	6-8
AML (N = 10)	6-18	6-10
NHL (N = 10)	4-12	4-10
MM (N = 05)	6-7	5-6

N = Number of patients taken for prognosis

The serum LDH levels were found to correlate with other parameters also. Therefore we can conclude that serum LDH levels play an important role in the diagnosis of hematological malignancies.

Serum LDH Findings in the Prognosis

To evaluate the prognostic role of serum LDH levels in hematological malignancies clinical and hematological characteristics were corroborated with serum LDH levels for their association with survival. The inclusion criteria were based on parameters like age, sex, performance status, hemoglobin level, platelet and WBC counts, bone marrow blast cell percentage (Jane Gau, 2004).

Univariate Analysis of Serum LDH

The serum LDH levels of 50 diagnosed patients were observed against their survival rate. It was observed that patients with higher LDH levels were exposed to early relapse of their disease when compared to the cancer patients who had normal serum LDH levels. The average survival time was

observed as 3 to 18 months in ALL, 6 to 8 months in case of CML and in NHL the median survival time was 4 to 10 months. A poor prognostic response was observed in cases of Multiple myeloma and Hodgkin's lymphoma. The prognostic response of various blood cancer patients, who had elevated serum LDH levels, is shown in Table 5.

Itoyana (2001) has reported the clinical significance of Cytogenetic analysis in adult T-cell leukemia/lymphoma along with elevated levels of serum LDH as prognostic markers, in patients with survival period of less than six months.

Discussion

Increased total serum LDH is commonly interpreted as reflecting high tumor burden or tumor aggressiveness. High total serum LDH carries a poor prognosis in Multiple myeloma, CLL, melanoma, lung adeno-carcinoma and colorectal carcinoma. Increased serum LDH has a major prognostic as well as diagnostic significance in patients with NHL and total serum LDH is one of the parameters of International Prognostic Index (IPI) used in patients with NHL (Bouafia *et al*., 2004). Molica *et al*. (2004) have reported serum angiogenin in patients with B-cell chronic lymphocytic leukemia may not be elevated like serum LDH but also it is a prognostic factor for disease progression. LDH can help in distinguishing progress of the disease toward recovery, as it is indicated by normal serum LDH levels. Also the exacerbation of disorder is suggested by increased serum LDH levels. Hence sequential determinations of serum LDH levels are useful in diagnosis and prognosis of hematological diseases (Horecker *et al*., 1975; Onida *et al*., 2004). Elevated levels of LDH and changes in the ratio of the LDH isoenzymes usually indicate some type of tissue damage. Usually LDH levels will rise as the cellular destruction begins, peak after some time period and then begin to fall.

Present investigation reveals that Serum LDH can be routinely measured in patients with hematological malignancies, without much concern regarding its iso-enzyme content in these patients. Increased values of serum LDH directly reflects the tumor mass in patients with NHL, ALL, AML. Etc. Hence it is suggested that serum LDH can be used as one of the important bio-marker of survival in patients with hematological malignancies. Patients of NHL, CML, ALL, AML and Multiple myeloma, who have increased serum LDH levels than others had a 90% risk of death in the first five months. Serum LDH has been shown as a simple and useful bio-marker directly associated with hematological malignancies in the blood cancer patients. Porrata *et al*. (2001) have also predicted survival after analogous hemopoietic stem cell transplantation in Multiple Myeloma and NHL.

We have shown that the abnormalities in total serum LDH levels have a good diagnostic significance, apart from the prognosis. Table 3 also shows that among the hematological malignancies ALL cases were highest; Hodgkin's disease and CLL cases were lowest. But survival periods were highest for both ALL, CML cases and lowest for multiple myeloma cases. With some chronic and progressive conditions and some drugs, moderately elevated LDH levels may persist. Low and normal levels of LDH do not usually indicate a problem. Low levels of LDH are sometimes seen when a patient ingests large amounts of ascorbic acid (vitamin C).

Conclusions

It can be inferred from this study that disease aggressiveness correlates with serum LDH levels. Also, LDH had a sensitivity score of about 75% i.e., 160 cases out of 215 and the control samples had no significant abnormal serum LDH levels. From our results it is concluded that the serum LDH plays an important role, both in the diagnosis as well as prognosis of hematological malignancies.

Acknowledgments

We are grateful to both the hospitals (Indo-American Cancer Institute and Research Centre and Bhagwan Mahavir Medical Research Centre, Hyderabad, India.) for their cooperation and help extended to us for this study, without which it would not have been possible to carry out this work. We are also thankful to the study group for their cooperation and understanding. Our special thanks to Mr. Indel Viswakarma for providing statistical assistance to this work

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