Determination of levels of LDH and its isoforms in estimating the risk for the development of hypertension and associated conditions

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Abstract

Lactate dehydrogenase (LDH) catalyses the reversible transformation of pyruvate to lactate in glycolytic pathway. Activity of LDH and its isoenzymes is elevated in conditions like cancers, myocardial infarction etc., However the role of LDH in essential hypertension (EHT), a risk factor for myocardial infarction (MI) has not been reported. Hence the present study tested the association of serum LDH levels and isoenzyme proportions with EHT and associated conditions like Ischaemic heart disease (IHD) and Diabetes mellitus (DM). 230 hypertensive cases including 164 EHT, 41 IHD and 25 with DM were studied for LDH levels and 156 cases including 115 EHT, 23 IHD and 18 with DM for its isoforms. Significant increase in the mean levels of serum LDH was found in EHT ($P<0.0001$) and hypertension associated IHD ($P<0.0001$) and DM ($P<0.0001$) as compared to controls. Considering isoforms, LDH-1 was elevated in EHT ($P = 0.0061$) and hypertension associated IHD ($P < 0.0001$) as compared to controls. Since EHT is one of the predisposing factors to IHD and MI, variation in LDH-1 levels may help in monitoring and diagnosing the onset and progress of IHD and MI.

KEYWORDS: Lactate dehydrogenase, Hypertension, Ischaemic Heart disease, Glycolytic Pathway

Introduction

Lactate dehydrogenase (LDH) is an important enzyme that catalyses the reversible transformation of pyruvate to lactate (Holbrook et al, 1975) which occurs virtually in all the tissues. It is widely distributed throughout the body cells especially in cardiac and skeletal muscle, liver, kidney and red blood cells. Markett and Moller (1959) introduced the term isoenzyme to describe the existence of different molecular forms of an enzyme which catalyse the same biochemical reaction but differ in their electrophoretic mobility. In mammalian cells, LDH is expressed as five common isoforms (LDH 1, 2, 3, 4 and 5), in a tissue specific manner (Drent et al, 1996; Sevince et al, 2005; Le et al, 2010). The enzyme is a tetramer made up of 4 subunits. The subunits are of two kinds viz., M and H corresponding to skeletal muscle and heart muscle respectively and are under the control of two separate genes LDH-A and LDH-B which are located on chromosome 11 and 12 respectively (Boone et al, 1972; Chen et al, 1973). A third isoform, *LDHC* or *LDHX*, is expressed only in the testis and the gene concerned is also located on the chr 11. It is considered as a duplicate of *LDHA*. The subunits differ in their aminoacid sequence. All the five isoforms have the same molecular weight and contain four polypeptide chains each with molecular weight of 33,500daltons. Mutations of the M subunit have been linked to the rare

disease exertional myoglobinuria and mutations of the H subunit have been described but do not appear to lead to disease.

Physiological functions of the LDH isoenzyme fractions depend on the metabolic characteristic of a given tissue. LDH-1 is found to be abundant in cardiac muscle and LDH-5 in the liver tissue (Latner, 1961). Nilson et al, (1967) reported that oxidative metabolism is associated with LDH-1 while LDH-5 is associated with anerobic glycolysis.

Skeletal muscle and embryonic tissue tend to utilize glucose anerobically and break it down to form lactate during the process of glycolysis. Lactate dehydrogenase catalyses the last step in glycolysis i.e the reduction of pyruvate to lactate. Thus the skeletal muscle or M_4 isozyme of LDH converts pyruvate rapidly to lactate. On the other hand, heart muscle does not normally form lactate from glucose but it oxidizes pyruvate to carbon dioxide aerobically without going through an intermediate formation of lactate. The H_4 isozyme is well adapted for this different route of glucose metabolism reducing pyruvate to lactate. Heart muscle does not ordinarily involve lactate dehydrogenase in the oxidation of glucose. In emergencies when the oxygen supply is low the presence of lactate dehydrogenase allows the heart to obtain its energy from the conversion of glycogen to lactate (Abraham et al, 1984; Dube and Pande, 1984).

Isoenzymes are of great importance in the diagnosis of heart and liver diseases. LDH-1 fraction was found to be elevated in myocardial infarction while LDH-5 in liver diseases (Varley, 1969; Rajgopal et al, 1982). Further in septicaemia LDH-5 was found to be elevated whereas in leukaemia LDH-3 predominates (Abraham et al, 1984). LDH1 (LDH B) was found significantly up-regulated in lung cancer (Chen at al, 2006), while LDH5 (LDH A) is recently shown to be involved in both tumor initiation as well as for its maintenance (Le at al, 2010). LDH 5 or LDH A is found to be related with colorectal cancer metastasis and prostate cancer (Koukourakis et al, 2006; Karan et al, 2002). The increased expression of LDH 5 in various cancers, melanoma, leukemia, testicular cancer and certain solid tumors, led to suggest it to be a useful prognostic marker in these pathogenesis (Schneider et al, 1980; Pravonen et al, 1991; Castaldo et al, 1991; Lossos et al, 1999). Activity of lactate dehydrogenase was also observed to be increased in conditions like muscular dystrophies and myoglobinuria (Varley, 1969). However, the activity of this enzyme in hypertension which is one of the risk factors to Ischaemic heart disease (IHD) and myocardial infarction (MI) has not been reported so far. Hence, in the present study an attempt was made to test the association of serum lactate dehydrogenase levels and isoenzyme proportions with essential hypertension and its associated conditions like Ischaemic heart disease (IHD) and Diabetes mellitus (DM).

Materials and Methods

Study Population

A total of 230 hypertensive cases which included 164 cases with essential hypertension, 41 with ischaemic heart disease and 25 with diabetes mellitus were analysed for their association with serum lactate dehydrogenate levels. For isoenzyme proportions, 156 hypertensives including 115 cases with EHT, 23 with IHD and 18 with DM were studied. All the cases with confirmed diagnosis were recruited from the cardiology unit of Nizams Institute of Medical sciences, Hyderabad, India. 180

normotensive subjects (without diabetes and any other associated disorders) whose blood pressure was within normal limits and who were on routine health check up were collected at randomn from the same population for comparison with the patient group. The diagnosis of hypertension and its associated conditions in all the cases was made by the cardiologists, after subjecting the patients to various diagnostic and laboratory investigations such as ECG, rapid sequence intra venous pyelogram, echocardiogram, angiogram and determination of blood sugar, blood urea, serum cholesterol, serum creatinine, serum potassium and sodium level etc., These estimations were also done in controls to eliminate the individuals with higher levels of these parameters from the study. Detailed information relating to epidemiological and genetic factors like blood pressure measurement, sex, age, body mass index, age at onset, duration of the condition, habits like smoking, family history and pedigree information covering 2-4 generations were recorded from all the patients and controls in a specified proforma. Approval by the institutional ethical committee and informed consent from all the subjects for their participation in the study was obtained.

Methodology

3 ml of the blood was collected from all patients and controls in a dry test tube without any anticoagulant for obtaining serum. Serum was used for the quantification of serum lactate dehydogenase within 8hrs after the collection of blood samples. LDH was assayed according to the method of King (1965). To 1.0 ml of the buffered substrate (lithium lactate in 0.1M glycine buffer, pH 10), 0.1 ml of enzyme preparation was added and the tubes were incubated at 37.8^{o}C for 15 min. After adding 0.2 ml of NAD+ solution, the incubation was continued for another 15 min. The reaction was arrested by adding 0.1 ml of DNPH (2, 4-dinitrophenyl hydrazine), and the tubes were incubated for a further period of 15 min at 37.8^{o}C after which 7.0 ml of 0.4N NaOH was added and the color developed was measured at 420 nm using spectronic-70. LDH isoenzyme patterns were determined by electrophoresis using commercially available cellogel strips (Chemetron, Italy). Isoenzyme fractions were scanned using densitometer (Toshniwal, India) for their quantities.

Results

In the present study a significant increase in the mean levels of serum lactate dehydrogenase was found in essential hypertension cases ($\bar{X}$ = 278.07 ±5.67; t=14.51; P<0.0001) and its associated conditions i.e ischaemic heart disease ($\bar{X}$ = 292.24 ±9.57; t=10.32; P<0.0001) and diabetes mellitus ($\bar{X}$ = 252.48 ±14.79; t=5.63; P<0.0001) as compared to controls ($\bar{X}$=162.44 ± 5.59). Mean level of this enzyme was slightly elevated in hypertension associated with ischaemic heart disease ($\bar{X}$ =292.24 ±9.57) in comparison to hypertension ($\bar{X}$ = 278.07 ±5.67). No significant difference in the mean levels of serum lactate dehydrogenase was found when comparisons were made between males and females, familial and non-familial cases, smokers and non-smokers and different periods of duration of the condition in EHT and associated conditions.

Considering isoenzymes, the present study revealed a significant increase in the levels of LDH-1 in essential hypertension (X=24.22 ± 0.96; t=2.77; p = 0.0061) and hypertension when associated with ischaemic heart disease (X=29.98 ± 1.65; t=4.52; P < 0.0001) as compared to controls (X = 20.42 ± 0.92). Other fractions of this enzyme did not show any significant difference in their quantities in EHT and associated conditions when compared to controls.

Discussion

Lactate dehydrogenase enzyme catalyses the reversible conversion of pyruvate to glycolate in the glycolytic pathway. It is widely distributed throughout the body cells especially in cardiac and skeletal muscle, liver, kidney and red blood cells. Several authors have proposed that cellular damage may be responsible for the release of lactate dehydrogenase in to the blood stream from the damaged cells of the organs concerned as in case of ischaemic heart disease, liver damage, malignancies etc., (Latner, 1961; Lott and Stang, 1980; Abraham et al, 1984; Dube and Pande, 1984; Robbins et al, 1984; Joblonsky et al, 1985). The increase in the mean levels of serum lactate dehydrogenase as found in the present study can also be explained on the basis of vascular tissue damage that is likely to occur in hypertensive cases which is considered as one of the major risk factors in the causation of IHD and MI. This interpretation is supported by Prabha et. al., (1987) who studied the same patients and reported significantly reduced levels of haptoglobin as compared to controls providing evidence of haemolysis and vascular tissue damage. Haptoglobin is a serum protein that binds with free haemoglobin liberated into serum due to haemolysis and tissue damage. The level of this protein produced varies with the extent of tissue damage. When the damage and haemolysis occur at higher rate, this protein is utilised in binding with liberated haemoglobin thus resulting in the reduction of its levels.

Similar observation suggesting the role of vascular damage was made by Tanuja et al., (1993, unpublished) from our laboratory in 200 patients of pre-eclampsia who showed significantly elevated levels of LDH (251.05±8.1) as compared to 200 pregnant controls (238.46±7.4; t=1.15; <0.05) and 100 non-pregnant controls (157.32±4.7; t=7.87, p<0.001). Recently another study by Vanishree et. al., (2014, unpublished) on 50 preclamptic toxaemic patients showed higher levels of LDH and GGT (gama glutamyl transferase) as compared to 50 controls that was correlated with the possibility of endothelial vascular damage in the patients.

Study of isoenzyme fractions of lactate dehydrogenase is considered to be important in diagnosing diseases related to heart, liver, certain malignancies etc., Elevation of LDH-1 in myocardial infarction (Latner, 1961; Wright et al, 1966; Lott and Stang, 1980; Abraham et al, 1984; Jablonsky et al, 1985) and LDH-5 in liver diseases was explained on the basis of release of these isoenzymes by leakage through altered cell membrane occurring probably due to cellular injury or necrosis (Dube and Pande, 1984).

Elevation of LDH-1 and LDH-2 in pernicious anaemia was reported to be due to intravascular haemolysis (Wright et al, 1966). Vesell and Bearn (1958) from their study on the red cell lactate dehydrogenase isoenzyme patterns in haemolytic anaemia and haematological diseases proposed that erythrocyte LDH-5 is found exclusively in younger erythrocytes which are nucleated while LDH-1 is found predominantly in matured erythrocytes. Starkweather et al (1965) further supported the above statement suggesting that the early nucleated erythrocytes contain predominantly LDH-5 indicating that the gene responsible for M peptide synthesis is active in dividing cells. As the erythrocytes reach maturation the requirement for LDH-5 diminishes and the synthesis of M subunit decreases with subsequent increase in H subunit that is LDH-1.

Increase in the proportion of serum LDH-1 and LDH activity in the present study can be explained in two ways: (a) elevation in LDH activity and proportion of LDH-1 in relation to other fractions in essential hypertension and associated conditions could have resulted due to the damage of vascular tissue arising as a consequence of high blood pressure. Robbins et al, (1984) suggested that when there is cellular damage, the enzymes and other components may be thrown out in to the blood stream from damaged cells of the organs concerned causing elevation in the levels of the respective LDH components. There is possibility for the existence of similar phenomenon causing elevation of LDH in hypertension which is associated with vascular damage and thus making it a major risk factor for the onset of ischaemic heart disease. (b) Presence of elevated LDH-1 fraction among cases with hypertension and associated conditions (IHD) found in the present study may be correlated with occurrence of haemolysis due to vascular damage- a feature found in hypertensive casesand evidenced by the study of elevated haptoglobins (Prabha et. Al, 1987, . In light of the investigation carried out by Starkweather et al, (1965) and Vesell and Bearn, (1985) on red cell lactate dehydrogenase isoenzyme patterns in haematological diseases, it can be stated that the mature red blood cells in which LDH-1 is predominant may be breaking down as a consequence of high blood pressure resulting in elevation of of LDH-1 in the serum of hypertensive cases.

Studies on the lactate dehydrogenase activity and its isoenzyme have not been reported in hypertension so far. Since hypertension is one of the predisposing factors to ischaemia and MI, the present results showing variation in LDH-1 may help in monitoring and diagnosing the onset and progress of ischaemic heart disease and myocardial infarction. Further studies to substantiate the presence and extent of vascular tissue damage and intravascular hemolysis in hypertensive cases using parameters other than LDH may help in developing panel of markers to monitor the onset of complications associated with EHT.

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Table 1: Mean levels of lactate dehydrogenase in essential hypertension and associated conditions

Condition	Total Cases Studied		
	No.	Mean	SEM
Control			
Males	119	177.83	6.99
Females	61	132.43	8.04
Total	180	162.44	5.59
Essential Hypertension			
Males	90	271.73	7.75
Females	74	285.65	8.24
Total	164	278.07	5.67**
Associated Conditions			
Ischaemic heart disease			
Males	29	288.76	11.79
Females	12	300.64	15.75
Total	41	292.24	9.57**
Diabetes Mellitus			
Males	17	250.56	16.94
Females	8	256.59	28.90
Total	25	252.48	14.79**

****P<0.001**

t-value and P-value :

EHT Vs Controls – 14.5004; P<0.0001
IHD Vs Controls – 10.3195; P<0.0001
DM Vs Controls – 5.6342; P<0.0001

Table 2: Relative Proportions of Serum lactate dehydrogenase isoenzyme fractions in essential hypertension and associated conditions

Condition	Total no. Of cases	LDH isoenzyme fractions									
		LDH-1		LDH-2		LDH-3		LDH-4		LDH-5	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Control	84	20.42	0.92	34.98	0.77	20.48	0.94	13.23	0.61	11.01	0.54
Essential Hypertension	115	24.22	0.96*	31.43	0.84	20.61	0.77	13.59	0.63	10.15	0.63
Associated Conditions											
Ischaemic heart disease	23	29.28	1.65*	30.03	1.06	20.55	1.07	11.67	0.72	8.14	0.83
Diabetes Mellitus	18	21.63	2.15	31.71	2.39	22.85	2.28	13.22	1.50	10.56	1.27

***P<0.05**