Lactate Dehydrogenase Isoenzymes Pattern in Differential Diagnosis of Pleural Effusions.

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Abstract

Objectives: Total lactate dehydrogenase (LD) in the pleural fluid (PF) is of little value in the discrimination of various types of exudative effusions such as malignant from non-malignant effusions.
The aim of this study is to assess the diagnostic value of LD isoenzymes activity in serum & pleural fluid in the differentiation between various exudative pleural effusions.

Methods: Sixty-Six patients with pleural effusions were included in the study. Activity of total LD & isoenzyme were measured in pleural fluid & serum. Isoenzymes were separated by agarose gel electrophoresis & the quantity of each isoenzyme was measured by spectrophotometer.

Results: Exudative (inflammatory, neoplastic) effusions had a relatively high LD levels compared to transudates.
LD isoenzymes pattern was significantly different between transudates & exudates. PF LD isoenzymes pattern differs from that in serum. Our results showed that mainly the pattern of LD3 in pleural fluid & serum was helpful in discriminating inflammatory exudates from neoplastic exudates.

Conclusion: The LD isoenzyme pattern differed between pleural effusions of transudative and exudative origin. Moreover including the LD isoenzyne activities in the biochemical work up of pleural effusions reveal an additional discriminatory value in the separation between various exudative effusions, especially between inflammatory exudate & neoplastic exudates.

Keywords: Pleural effusion,lactate ehydrogenase isoenzymes

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Introduction

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in essentially all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death [1]. Due to its extraordinarily widespread distribution in the body, serum LD is abnormal in a host of disorders [2, 3].

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Therefore, LDH measurement is a sensitive, but rather non-specific test.
LDH activity has been extensively used in the analysis of pleural effusions, especially in distinguishing between transudate & exudate [4, 5]. However, total LDH activity in the pleural fluid (PF) is of little value in the discrimination of various types of exudative effusions such as malignant from non malignant effusions [4, 6].
Eventhough the total PF LDH activity is not useful in distinguishing among various exudative pleural effusions; one might suppose that LDH isoenzymes could be of additional value in the differentiation. Few studies reporting the analysis of LDH isoenzymes in pleural

effusions were found and the results were conflicting [7-9].

Patients and Methods:

Patients

From 1st of February 2000 to the end of October 2000, 66 pleural effusion fluids, as well as blood samples were obtained from 66 in patients admitted to the kahdemyia hospital. Patients were categorized into three groups (table 1).

Table (2) shows the age & sex distribution among patients presented with pleural effusions on all PF samples, the following analyses were performed: protein, LD, LD isoenzymes, bacterial culture, acid-fast bacilli smear and cytology. Simultaneously a sample of serum was obtained to measure biochemical parameters.

The aspirated PF, blood (10 ml each) were separated by centrifugation by 2000 xg for 10 minutes, then supernatant and serum were aspirated and dispensed into 0.2 ml tubes and stored at room temperature (20-25° c) for not more than 2-3 days.

Determination of LD activity

LD activity was determined according to the method of Wroblewski and La Due [10].

Separation & measurement of LD isoenzymes by electrophoresis

1- LD isoenzymes separated on agarose gels according to the method of Eleritch 1966 [11] with some modification: colorimetric determination of the relative amounts of each isoenzyme present is accomplished by the addition of substrate containing lactate (500 mM), nicotinamide adenine dinucleotide (NAD)(10 mg), nitroblue tetrazolium salt (NBT) (1 mg/ml), phenazine methosulphate PMS (1 mg/ml), Tris-Hcl buffer (0.057 M,PH 8.0).
2- After the isoenzyme have been separated by agarose gel. Cellulose acetate membrane was soacked in the above reaction mixture and then layered over the separation gel; the plate is incubated for 15-20 min. in 37° c oven. After incubation, the membrane is removed, fixed with 5% acetic acid and stored for elution.
3- To estimate the relative amount of each isoenzyme, the strips were cut into sections, each section was transferred to a test tube with tight cup and both the dye and the membrane were completely dissolved by solvent mixture (ethanol: chloroform).
4- The substance was read at 546 nm against a blank made by using part of cellulose strip with a similar area.
5- The absorbance of given fraction divided by the sum of all the absorbance, yield the fractional amount, in percent of the given isoenzyme. This fraction when multiplied by the total LDH activity gives the total amount of the fraction in U/L.

Statistical analysis

Student's t-test was used for comparison of pleural fluid and serum LD activity and ANOVA was used for comparison among different groups. The linear regression and the Pearson coefficient of correlation (r) were determined.

Results

Table (3) shows the mean PF LD isoenzymes activity. Among groups, LD1 activity in male patients did not show significant difference, while the mean LD isoenzymes activity from LD2 to LD5 were significantly high in group II and III as compared to group I ($P< 0.01$), but there was no significant difference in LD isoenzyme activities between group II & III.

In female patients, the pattern differes from that in male patients with a significant high LD1 activity in group II as compared to both group I & II ($P< 0.01$; $P< 0.05$, respectively).

LD3 activity was higher in group II as compared to both group I and group

III (P< 0.01), as well as group III as compared to group I (P< 0.01).

While the results of LD isoenzymes in serum of male patients revealed that the mean serum LD1, LD2 and LD3 activities were higher in group I as compared to group II (P< 0.01), and non significantly different as compared with group III (table 4). The mean LD4 and LD5 activities did not show significant difference between the three groups.

In female patients the isoenzyme pattern differs completely from that in male patients, LD1 was higher in group I compared to group II (P< 0.05), but non significantly different as compared to group III (P> 0.05). In addition LD2, LD3, LD4 and LD5 did not show significant difference among the three groups

Serum Vs pleural fluid LD isoenzymes activity:

Figure (1) illustrates the distribution of individual results for both PF and serum LD isoenzyme activities for both sexes.

LD3 isoenzyme activity had distinct pattern in the three groups. Since in group I, serum LD3 activity was significantly higher than that in PF (P< 0.01), and vice versa in group II, while there was no significant difference between PF and serum LD3 activity in group III patients (table 3,4). Figure (2) demonstrates a suggested scheme for separation of the three groups of pleural effusion patients according to their LD isoenzyme activities.

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Table 1: Classification of 66 pleural effusions.

Group I	No.	Group II	No.	Group III	No.
Transudate	12	Inflammatory exudates	54	Neoplastic effusion	23
CHF	6	Pulmonary TB	23	Lung CA	5
Renal disease	6	Pneumonia	4	Breast CA	3
		Empyema	4	Larynx CA	2
				Bronchial CA	1
				Bladder CA	1
				Thyroid CA	1
				Pancrease CA	1
				Lymphoma	4
				Unknown primary	5

*** CHF : Congestive Heart failure**
*** TB : Tuberculosis**
*** CA : Cancer**

Table 2: Age and sex distribution among patients with pleural effusions.

Group (No.)	Mean ± SEM (yr)	Range (yr)
Group I Transudate		
Male (7)	*53 ± 4.8 Years	40-80
Female (5)	47.4 ± 7.6 Years	29-72
Group II Inflammatory exudate		
Male (24)	34.6 ± 3.6 Years	12-70
Female (7)	34.3 ± 6.9 Years	15-65
Group III Neoplastic effusion		
Male (11)	**60.8 ± 3.13 Years	45-80
Female (12)	49.1 ± 6.3 Years	14-88

* P<0.05 versus group II

** P< 0.01 versus group II

Table (3): Lactate dehydrogenase (LD) and LD isoenzymes activity in pleural effusion fluids:

	Male (U/L)							Female (U/L)						
Groups	No	LD	LD_1	LD_2	LD_3	LD_4	LD_5	No	LD	LD_1	LD_2	LD_3	LD_4	LD_5
Group I	7	120±22**	31±5.4	26±5.0**	28±5.0**	20±3.2**	19.5±4.7**	5	105±9**	21±4.0	23±3.0**	23±3.0	20±1.6**	17±2.0**
Group II	24	342±23	50±3.6	63±5	72±6.5	78±6.5	79±7.7	7	318±47	59±12*#	68±10	85±15##	60±8	62±10
Group III	11	283±43	36±8.8	58±10	60±10	68±11	72±11	12	235±45	39±8	52±11	52#±11	50±10	44±9

- Date were expressed as (Mean ± SEM)

* P<0.05 versus group III

\# P<0.01 versus group I

\## P<0.01 versus group I and III

** P<0.01 versus group II and III

Table (4): Lactate dehydrogenase (LD) and LD isoenzymes activity in serum:

	Male (U/L)							Female (U/L)						
Groups	No	LD	LD_1	LD_2	LD_3	LD_4	LD_5	No	LD	LD_1	LD_2	LD_3	LD_4	LD_5
Group I	7	299±35	75±8.7**	74±10.7**	71±12.7**	43±4.6	36±3.4	5	263±38	75±11*	79±11.6	57±11.2	26±3.6	24±5.5
Group II	24	225±16	48±4.3	51±3.8	47±3.8	38±3	40±4.6	7	237±39	53±9	67±17	55±12	36±2.6	28±3.4
Group III	11	234±22	59±11.7	59±7.6	51±3	35±2.5	31±2.4	12	250±27	59±6.7	69±10.2	54±6.5	35±4.8	34±4.5

- Date were expressed as Mean ± SEM

* P<0.05 versus group II

** P<0.01 versus group II

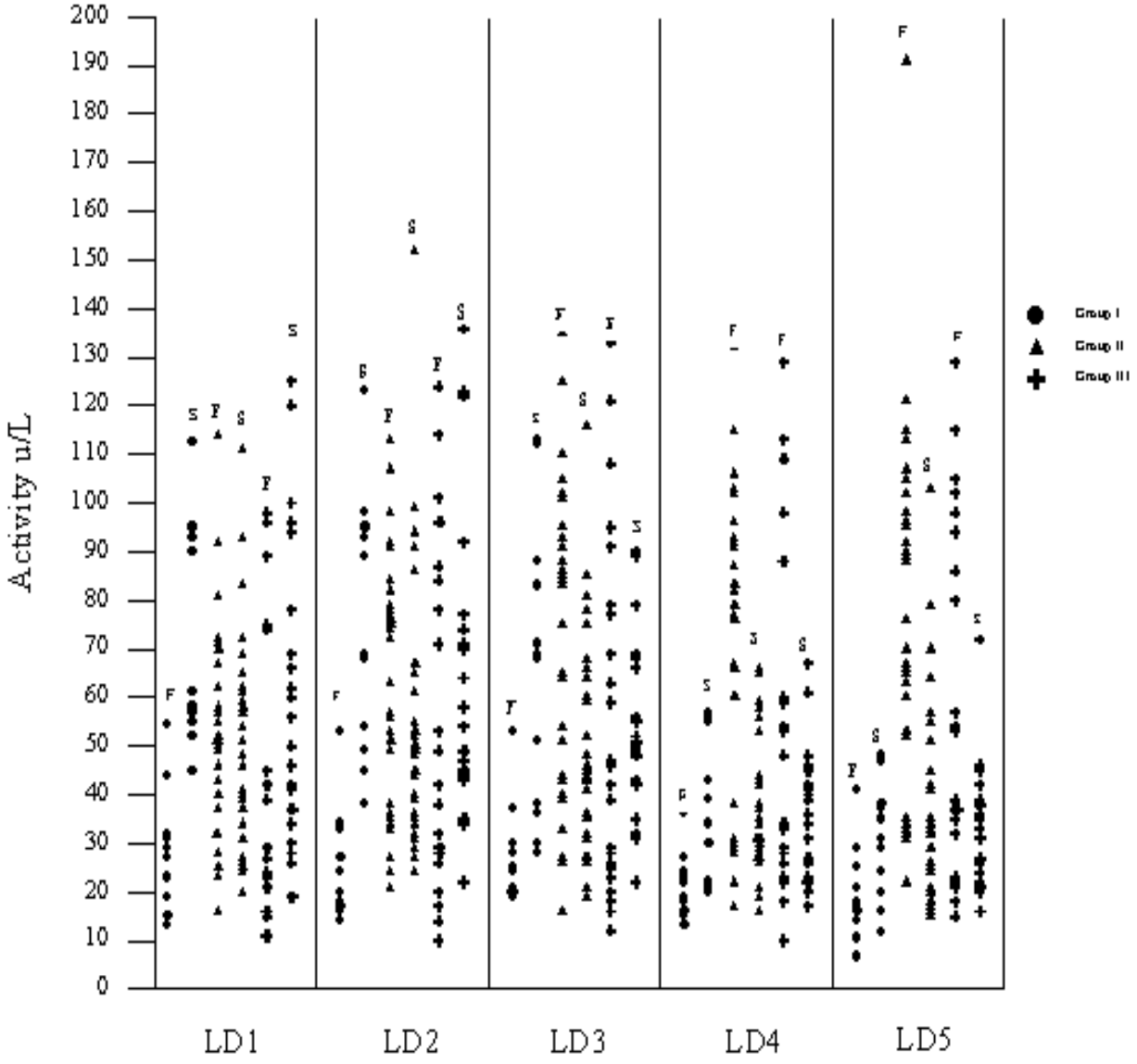

Figure (1): Distributions of pleural effusion fluids (F) and serum (S) Lactate dehydrogenase (LD) isoenzymes.

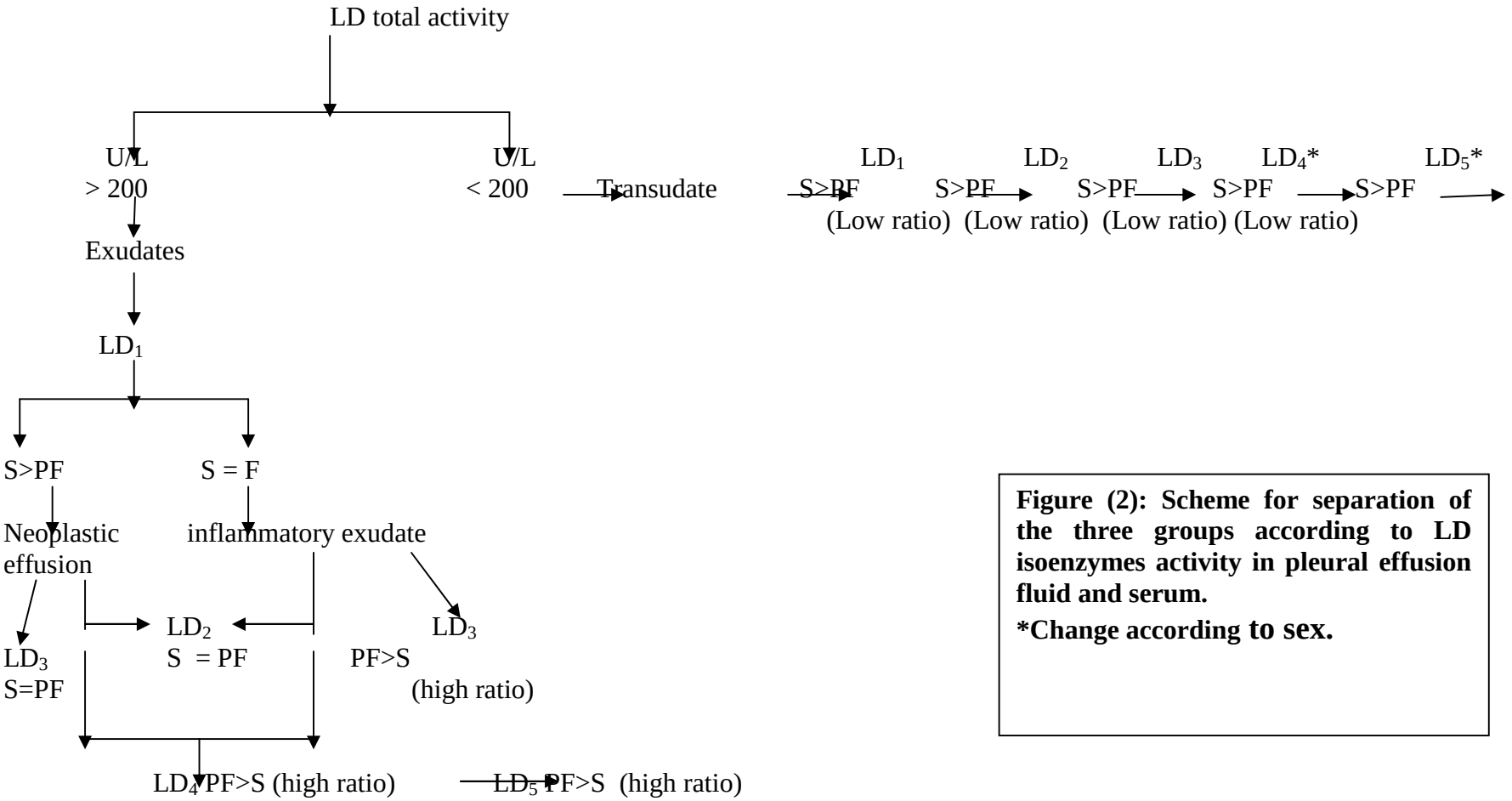

Figure (2): Scheme for separation of the three groups according to LD isoenzymes activity in pleural effusion fluid and serum.

***Change according to sex.**

Discussion

Pleural fluid LD activity has among others, been used in the analysis of pleural effusion especially, to discriminate transudates from exudates [3, 4].

The current study indicates that exudative (inflammatory, neoplastic) effusions have a relatively high LD levels compared to transudates, which is in agreement with other workers [5, 6, 7], and in contrast with others [10, 12, 13], who reported that an elevated PF LD was characteristic of malignant effusions and that nearly all benign effusions had low LD levels.

Cytoplasmic, cellular enzymes, such as LD in the extracellular space are suggestive indicators for disturbance of the cellular integrity induced by pathological conditions.

As LD is present in essentially all major organ system [14], LD measurement is a sensitive, but rather non specific test. The concentration of the PF LD is a reliable indicator of pleural inflammation even though, the total PF LD activity is not useful in distinguishing among various exudative PF, one might suppose that LD isoenzymes could be of additional value in the differentiation.

Reviewing the literature conflicting data were found and only the relative values of LDH isoenzymes, as percentage of total LDH were studied. The current study evaluates the absolute LDH isoenzymes activity in various PF as well as among different sex groups. In this study LDH isoenzymes pattern was significantly different between transudates and exudates (inflammatory & neoplastic) which is in agreement with other worker [15].

Moreover, in exudates the absolute activity of LD3 was higher in inflammatory than that in neoplastic effusions, although it was significant only in female patients. In the current study PF LD isoenzymes pattern differs from that in serum, a result was comparable to the result found by Paavonen and associates [16]. Most of the isoenzymes activity in transudates was lower than that in serum, which is in agreement with other workers [17].

All inflammatory exudates were characterized by higher activity of LD3, LD4, and LD5 than the corresponding serum isoenzymes. Similar results were obtained by other workers [7, 18].

The finding that inflammatory exudates effusions had a high LD4 and LD5 as compared to the corresponding serum may be explained by the following observations. Because a marked PF leukocytosis usually occurs in disease in which injury to the lung occurs, the LD4, and LD5 from PMN leukocytes probably contributes in the elevation of these isoemzymes in the PF [19.] Processes characterized by mesothelial proliferation would show mostly elevation of LD4 & LD5, since these isoenzymes predominate in mesothelial cells [20]. Moreover, the PF lymphocytes in disease states such as tuberculosis are probably immunologically stimulated, and although lymphocytes usually contain LD1 & LD2, immunologically stimulated lymphocytes contain mostly LD4 & LD5 (M), where as in neoplastic effusions only LD4, LD5 activities in effusions were higher than their corresponding serum activities. However, it was significant only in male patients. This result was in agreement with other worker [17] and in contrast to Frohlich & associates [18], who reported that neoplastic effusions were characterized by maximal enzyme activity in LD2, LD3 and LD4. The high activity of PF LDH4 and LDH5 in neoplastic effusions indicates that the origin of these isoenzyme in effusions is unlikely to be from the serum. Since no correlation has been found in the current study and local LD4, LD5

concentration exceeding those found in serum with a high fluid to serum ratio.

Our results showed that mainly the pattern of LD3 in PF & serum was helpful in discriminating inflammatory exudates from neoplastic exudates. The high LD3 activity in pleural inflammatory exudates indicate that the source of LD3 in effusion is unlikely to be from the serum since no correlation was observed, and probably LD3 contribution from the lung and from the inflammatory cells in the pleura cavity. In contrast to Cobben and associates [21], who reported that mainly the percentage of LD4 & LD5 are helpful in discriminating malignant effusions from benign exudative effusions (i.e parapneumonia effusions).

In the current study, neoplastic pleural effusion had variable LD isoenzymes pattern. This could be due to the various neoplastic tissues that secrete different LD isoenzymes. It has been shown that malignant lymphoma and small cell lung carcinoma differ from other malignancy by a low LD5 isoenzyme secretion. Alternatively, the extent of the pleural inflammatory response to malignancy and the variable degree of pleural PMN leukocytosis may determine the relative levels of LD4 & LD5 isoenzymes [8]. The marked heterogenity of malignant etiologies and the relative small number of patients with neoplastic effusions in the current study precluded separation between various LD isoenzymes pattern according to the cytopathologic diagnosis.

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