ADJUSTMENTS OF SERUM LACTATE DEHYDROGENASE ISOENZYMES AND THEIR SIGNIFICANCE IN MONITORING THE TREATMENT IN PATIENTS WITH TUBERCULAR PYOTHORAX

Riaz Ahmad, Mumtaz Alam*, M Faisal Siddiqui and Absar-ul Hasnain

Section of Genetics, Department of Zoology, Faculty of Life Sciences, AMU, Aligarh-02 (UP) INDIA

*Department of Surgery, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh-02 (UP) INDIA

ABSTRACT

The adjustments and diagnostic significance of polyacrylamide gel electrophoretic (PAGE) profiles of lactate dehydrogenase isoenzymes (LDH: 1.1.1.27) was evaluated in the sera and pleural fluid of patients with tubercular pyothorax. Sera and pleural fluid samples were randomly collected from 72 and 18 patients respectively at two different timings; first, when patients were admitted to the Hospital and second, after an intensive phase of treatment. Sera of 20 healthy individuals served as control. Our results demonstrate significant differences in sera LDH (sLDH) and pleural fluid LDH (pfLDH) isoenzymes. In patients the order of LDH isoenzyme in sera and pleural fluid followed: LDH-5>-4>-2>-3>-1 and LDH-5>-4>-3>-2>-1 respectively. The ranking of activity levels in control was LDH-2>-1>-3>-5>-4. In the second phase of sampling from 31 patients, values of sLDH isoenzymes showed recovery and resembled profiles of controls. Therefore, the sLDH zymograms of patients can be used as the prognostic marker, since they tend to reach the normal level during recovery signifying the effect of chemotherapy in hospitalized patients. Moreover, according to the present findings on LDH-PAGE profiles, the levels of LDH-5 and -4 rise in pyothorax patients significantly ($P<0.05$). This elevation along with the rise in total LDH activity may, therefore, be used in the diagnosis and monitoring of tubercular pyothorax.

KEY WORDS

Lactate dehydrogenase, Sera LDH profiles, Polyacrylamide gel electrophoresis patterns, Pleural effusion, Tubercular pyothorax.

INTRODUCTION

Pyothorax is one of the common pleural diseases in which collected pus in pleural space limits lung expansion. As a result, toxins released into the blood stream causes further stress finally ending in empyema. The pathological description of empyema includes the combination of a thick cortex of fibrin and coagulum over the lung. Etiology of pleural effusions is quite varied (1-3). Pleural effusions are classified as either transudates or exudates based on fluid protein and lactate dehydrogenase (LDH) concentration. Transudative effusion

occurs as the result of a change in fluid balance in the pleural space. An effusion is considered exudative if it meets any one of the criteria described elsewhere (4). Lymphomas are an important cause of malignant effusion and account for 10% to 14% of all malignant pleural effusions. Pyothorax-associated lymphoma (PAL), a non-Hodgkin's lymphoma is a clinical complication consequent to a long-standing history of pyothorax develops in the pleural cavity (5). A few studies have been carried out where the correlation of interleukin-6 (IL-6) or interleukin-1 beta (IL-1 beta) concentrations in the pleural fluid of pyothorax patients with its pathophysiology has been demonstrated. Some workers have compared common biochemical parameters such as cholesterol, total protein and albumin (6-7).

Clinically, a number of enzymes (or isoenzymes) have for long been recognized for their value as biochemical markers (6,8,11,14-15,20). One such enzyme is Lactate

Address for Correspondence :

Dr. Riaz Ahmad

Section of Genetics,
Department of Zoology,
AMU, Aligarh-02 (UP) INDIA.
E-mail: riazool@rediffmail.com

dehydrogenase (LDH: 1.1.1.27), which is a tetrameric glycolytic enzyme that interconverts lactate to pyruvate in the presence of NAD or *vice versa* but with NADH as cofactor. Typically, homotetramer M4 is muscle and H4 heart specific isoenzyme. The variations in these two homotetramers and three random heterotetramers are remarkably sensitive to changing human physiological and clinical conditions (8-12). The published reports on pleural effusions or sera either in benign or malignant conditions have so far dealt with total LDH (TLDH) activity only (5,7,9). Defensins (antimicrobial peptides present in the azurophil granules of human neutrophils) and adenosine deaminase (ADA) activity are other markers, which have been evaluated in the differential diagnosis of specific pleural diseases and effusions (13-15).

Reports on the significance and contribution of individual isoenzyme band to changes in TLDH activity are scarce. The present investigations were therefore, aimed at: 1) assessing the contribution of individual isoenzymes to TLDH activity and adjustments in their PAGE profiles, 2) comparison of sera LDH (sLDH) and pleural fluid LDH (pLDH) PAGE profiles, and 3) significance of sLDH zymograms as a preferred diagnostic marker of tubercular pyothorax when the pleural fluid dries up. The present report is the first attempt where electrophoretically discernible changes in sLDH isoenzymes have been utilized as marker in the diagnosis and monitoring of tubercular pyothorax and placed at par with the PAGE profiles of pleural fluid.

MATERIALS AND METHODS

Patients were admitted to the Jawaharlal Nehru Medical College and Hospital (JNMCH) Aligarh, India for therapeutic as well as research purpose. Patients represented mixed populations of the nearby districts. They were diagnosed to suffer with tubercular pyothorax on the basis of the presence of *Mycobacterium tuberculosis* in sputum smear using Ziehl Neelsen staining (16) and the total protein assay and leukocyte counts by microscopic examination. Patients having complaint of $\sim 180 \pm 15$ days with no sign of comorbidity were randomly approached. Blood from 72 and pleural fluid from 18 patients in the age group, 52 ± 4 was collected twice, first at the time when the diagnosis was confirmed and patients admitted to the Hospital and second, after two months of completing the intensive phase of treatment (recovery time). Prior to taking the blood (to collect sera) and pleural fluid samples, patients were well informed regarding the purpose of sampling. Following their consent, blood was drawn out with the help of a 1ml sterilized disposable syringe (graduated, 0.01ml; needle gauge#27), whereas pleural fluid was taken with 5ml

disposable syringe with all aseptic measures. The samples of the patients were analyzed while fresh. Control groups were comprised of 20 healthy individuals in the mean ages 23 ± 3 . Analyses of control sera were also performed on the same day.

Protein concentration in the samples was estimated essentially according to the standard protocol of Lowry et al (17). Appropriate dilutions of sera were made to permit loading of the same amount of protein in each lane.

Polyacrylamide gel electrophoresis (PAGE) was performed on 7.5% gels as described previously (18). Freshly obtained sera were subjected to electrophoresis. In each PAGE lane approximately 15-18 μ g of protein was loaded. Each sample was run in triplicate and following quantitation by densitometry their mean $\pm$ standard deviation was calculated.

LDH zymograms were developed at 37°C with the histochemical staining protocol outlined by Shaw and Prasad (19). Gels were destained and fixed in 7% acetic acid (v/v). Gels with background aberrations were discarded.

Densitometric analyses of the LDH gel scans and quantitative estimates of isoenzymes within a single PAGE lane were made by Scion Imaging and GelPro (Media Cybernetics, USA) software programs, respectively. Total densitometric estimate (in arbitrary units) of all the LDH tetramers in a specific PAGE lane was taken as 100% LDH activity. Mean and standard deviations were calculated as usual. Mean value of individual PAGE lane estimate of each LDH isoenzyme was converted to its percent fraction of total LDH activity and the bars were plotted (Fig1). Student's 't'-test was applied between the obtained mean values and the differences were considered significant at $P < 0.05$.

RESULTS

Fig 1A displays the activity levels of LDH isoenzymes in sera of controls. A significant rise in the level of muscle specific (anaerobic) homotetramer LDH-5 and a heterotetramer with H-subunit LDH-4 (Fig1B) was recorded in tubercular pyothorax patients ($P < 0.05$). As compared to controls, in LDH-5 and -4 nearly four and five folds increase was noted in sera of patients, while decrease was observed in the heart specific (aerobic) homotetramer LDH-1 and heterotetramers LDH-2 and -3. In the patients, this decrease in LDH-1 and -2 isoenzymes accounted to $\sim$ six and two folds respectively. Whereas in heterotetramer LDH-3, the decrease of lower magnitude that is of approximately one and half fold was measured ($P < 0.05$).

Fig.1. Bars showing the quantitative changes (arbitrary units) in lactate dehydrogenase isoenzymes of patients with tubercular pyothorax in; (A): sera of healthy individuals (controls); (B): sera of patients, (C): pleural fluid of patients and (D) in sera of recovered patients after intensive phase chemotherapy. Value of each bar (representing an isoenzyme) is plotted as Mean±SD of three PAGE profiles.

Bars from left to right are: LDH-5, LDH-4, LDH-3, LDH-2 and LDH-1.

The changes in the intensity of sLDH isoenzymes were of the order: LDH-5>4>2>3>1 (Fig. 1A and B).

The software analyses of LDH electrophoretic pattern from pleural fluid of pyothorax patients also showed the predominance of LDH-5 and LDH-4 isoenzymes (Fig.1C). Similar to sLDH-PAGE profiles, samples of pleural fluid from patients were also characterized by a low value of LDH-1 isoenzyme.

Second sampling of sera collection was carried out from 31 patients at the end of intensive phase of treatment (short course chemotherapy) after two months. The duration of intensive phase of treatment is considered as recovery period for the patients in whom the symptoms of fitness were observed. Post intensive phase analyses of LDH electrophoretic profiles demonstrated the elevated level of LDH-1 and LDH-2 similar to that in controls (Fig.1D), with a concomitant decrease in the levels of LDH-5 and LDH-4 ($P<0.05$) rather than an increase or decrease in the levels of LDH-3.

DISCUSSION

Sera lactate dehydrogenase polyacrylamide gel electrophoretic profiles (LDH-PAGE) of control, observed in the present study were similar to the reported 5-banded electrophoretic profiles in human and other mammals (8-10,20-22). Same number of LDH isoenzyme bands was also a characteristic of LDH-PAGE profiles of the sera of 72 and pleural fluid of 18 tubercular pyothorax patients. A significant rise in the activity of pLDH isoenzymes as compared to controls ($P<0.05$) has been previously reported (6,8,23). Our results demonstrate a parallel pattern of changes in isoenzyme bands of sLDH, which is a novel finding that also indicates that sLDH profiles can serve as alternative markers (Fig1B). No abnormal or extra isoenzyme bands were detected in the patients with tubercular pyothorax as reported previously in certain malignant diseases (9,24). Thus, the absence of such extra bands indicates that the patients included in the present investigations were not having those malignant diseases.

The present findings also differ from previous reports in view of the contribution of individual tetrameric isoenzyme to the elevation of TLDH activity, which reflects the changes and adjustments in subunit constitution of isoenzyme bands (Fig1). Homotetramer LDH-1 is mainly associated with aerobic tissues like heart and has high pyruvate optimum, while LDH-5, which is restricted to muscle and other anaerobic tissues has high lactate optimum (25). Both isoenzymes are known to respond

to anaerobic stress (25). Previously, high levels of pfLDH-5 and pfLDH-4 have been correlated with infection related effusions (10,26). Our findings also show a trend of LDH-5>4>3>2>1 in the pleural fluid of patients at the time of admission (an increase of nearly four times in the levels of pfLDH-5 and pfLDH-4 with respect to control sLDH isoenzymes) and support the earlier investigations where a parallel ranking of LDH isoenzymes was obtained for patients with tuberculous pleural effusion (8).

No previous study had shown either the PAGE profiles of sera of pyothorax patients or correlation of sLDH isoenzymes with pfLDH isoenzymes. Our results demonstrate the activity level of sLDH isoenzymes is of the order: LDH-5>4>2>3>1. It is significant that the rise in sLDH-5 and sLDH-4 activity in patients (up to 38.66 ± 2.08 and 29.66 ± 1.52) as compared to controls (10.33 ± 1.52 and 6.66 ± 1.32 , respectively) fit in the data obtained for pleural fluid. Serum levels of LDH isoenzymes represent their spillage from damaged cells (8-9). Thus, specific isoenzymes elevations reflect the tissue source of the damaged cells, regardless whether the damage was induced by inflammation or any other mechanism. In pyothorax the most affected tissue is the lung muscle and also the most probable source of isoenzymes leakage. These isoenzymes leak via the pleural space into the blood stream. It has been proposed that the infection or inflammation damages the thoracic muscles of pyothorax patients that causes increased release of granulocytes or polymorphonuclear leukocytes at the site of inflammation (27). Therefore, it is likely that similar to other pathological states of sLDH elevation (28), the higher levels of granulocytes or polymorphonuclear leukocytes might have contributed to the increase in sera isoenzymes LDH-5 and LDH-4 observed during the present study.

The patients with tuberculous pyothorax were advised isoniazid, rifampicin and pyrazinamide supplemented by streptomycin or ethambutol. After two months of short course chemotherapy further investigation were carried out on 31 patients. Their x-rays showed reduced pleural fluid volume as was evident by blunting of cardiophrenic angle (CPA). Therefore, sera were taken to carry out further investigations on recovery of patients. Continued intake of the above medicines for two months did not affect the sLDH-PAGE profiles and the existence of all the five bands was detected (Fig. 1D). Activity of sLDH-5 and sLDH-4 isoenzymes significantly goes down to meet the level of control values from 38.66 ± 2.08 and 29.66 ± 1.52 to 21.53 ± 3.78 and 14.6 ± 2.08 respectively (Fig. 1D). An opposite trend was followed by the other three isoenzymes *i.e.* LDH-1, LDH-2 and LDH-3 where the activity levels goes up indicating

the positive effect of therapy and recovery of patients within the intensive phase of treatment. So far as we know, this is the first report that describes the diagnostic significance of sLDH isoenzymes PAGE profiles in pyothorax patients during the intensive phase chemotherapy.

Therefore, the present findings suggest that sLDH isoenzymes, which were chosen as the parameter because of their higher reproducibility may serve as an additional or alternative tool to conventional source, the pleural fluid; specifically during the recovery when the amount of pleural fluid declines. The follow up of LDH-5 isoenzyme level in the sera appears to be an accurate diagnostic marker of the evolution and cure of tuberculous pyothorax. It is also noteworthy that PAGE technique requires less time, low amount of sample and simultaneous screening of 40 or more duplicate samples; hence, it may prove a better routine biochemical laboratory test.

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