

ORIGINAL ARTICLE

Observation and identification of lactate dehydrogenase anomaly in a postburn patient

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Objective: Lactate dehydrogenase (LDH) anomaly is one of the macroenzymes. Macroenzymes are enzymes in serum that have formed high molecular mass complexes, either by self polymerisation or by association with other serum components. The aim of this study was to identify the properties of LDH anomaly and observe the changes from admission to discharge in a postburn patient with LDH anomaly in his serum.

Methods: LDH isoenzymes of the serum were electrophoretically fractionated with terylene cellulose acetate supporting media; LDH anomaly was identified by counter immunoelectrophoresis.

Results: An abnormal LDH-4 band and an extra band on the cathode of LDH-5 were observed in the serum of this patient and were found to be part of an LDH-IgG complex. As his symptoms improved, the patient's LDH anomaly gradually disappeared. The appearance and disappearance of the anomaly seemed to be related to the progression of the patient's burns.

Conclusion: In clinical practice, it is important to keep in mind the possibility of an LDH anomaly in patients when the LDH level is abnormally high or does not seem to be related to the clinical state. Early discovery of an LDH anomaly in a patient's serum may be useful for diagnosis and treatment.

Macrolactate dehydrogenase (LDH) complex is one of the macroenzymes. Macroenzymes are complexes of serum enzymes with a plasmatic protein. They have a higher molecular weight and more prolonged serum half life than those of unbound enzymes. Many enzymes in serum that are measured in clinical chemistry laboratories can occur in the form of a macroenzyme.¹⁻³ Macroenzymes are important to the clinician because they frequently cause falsely increased total serum enzyme levels by interfering with common clinical laboratory testing methods, and as a result can cause diagnostic and therapeutic errors.

LDH-immunoglobulin complex is known to often occur in certain diseases such as neoplasms, liver damage, and cardiovascular accidents,⁴ but the biological role of this complex remains unclear. We found an LDH anomaly in the serum of a postburn patient, and to better understand its background and character we aimed to identify its properties and observe its change in this patient from admission to discharge.

METHODS

Instruments and reagents

The Beckman Synchron CX-7 automatic biochemistry analyser (Beckman, USA); Paragon electrophoretic system and appraise densitometer system (Beckman Instruments, Fullerton, CA); and anti-IgG antiserum, anti-IgA antiserum, anti-IgM antiserum were from Beckman. The terylene cellulose acetate membrane was from the Siqing Biochemical Material Factory, Zhejiang, PRC; and the LDH kit reagent was from Shanghai Long March Company, PRC.

Methods

Serum and plasma specimens were collected from the postburn patient. Sodium heparin was chosen as an anti-coagulant in the plasma. LDH activity was determined at 37°C by a spectrophotometric assay in an automated analyser (CX-7 automatic biochemistry analyser) with use of an LDH test kit. The assay conditions were largely based on the method described by Wroblewski and LaDue.⁵ LDH isoenzymes were

electrophoretically fractionated at 150 V for 30 min with Tris-HCL-barbital-EDTA buffer (pH 8.6) on terylene cellulose acetate membrane supporting media. Activity bands were made visible by use of D, L-lactate and NAD as substrate, phenazine methosulphate as intermediate, and methyl thiazolyl tetrazolium as the final hydrogen acceptor. The final concentration of the single reagent mixture for LDH isoenzyme staining was as follows: 120 mmol/l D, L-lactate, 5 mmol/l NAD, 2 mmol/l methyl thiazolyl tetrazolium, 0.08 mmol/l phenazine methosulphate, and 50 mmol/l Tris-HCL (pH 7.4). The LDH isoenzyme staining reaction was performed at 37°C for 25 min (Sandwich method).

The properties of LDH anomaly were identified by counter immunoelectrophoresis using terylene cellulose acetate as the supporting media. First, we immersed membranes in the electrophoretic buffer until they were completely covered. The membranes were blotted on filter paper to remove excess buffer and stretched on the frame. We dispensed 5 µl of serum with an applicator to the one end of the membrane, and then applied 5 µl of antiserum to the positive position, that is, about 3 mm next to the serum sample strip. We placed the membrane in the Paragon cell with the edges of the membrane extending into electrolyte reservoirs for five minutes. The electrophoretic condition was the same as that for LDH isoenzyme electrophoresis. When the electrophoresis was over, we placed the membrane in physiological saline overnight to wash the protein off. The LDH isoenzyme staining procedure was performed the next day.

The activities of aspartate aminotransferase (AST), LDH, α-hydroxybutyrate dehydrogenase (HBDH), creatine kinase (CK), and CK-MB were also determined by a CX-7 automatic biochemistry analyser.

RESULTS

Figure 1 shows the densitometric patterns of LDH isoenzymes in the serum of the postburn patient. Figure 2 shows the result of the counter immunoelectrophoresis. Table 1 shows

Abbreviations: AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; HBDH, α-hydroxybutyrate dehydrogenase

Table 1 Changes of serum enzymes activities (U/l) of the postburn patient

Enzymes	Admission	Day 7	Day 14	Day 22	Reference range
AST	130	65	57	42	5–45
LDH	309	407	213	165	110–240
HBDH	212	331	168	–	74–120
CK	689	569	517	–	25–175
CK-MB	75.3	31.4	25.9	–	0–25
MB/CK	10.9	5.50%	5.00%	–	–

Table 2 Changes of serum LDH activity (U/l) and isoenzymes (%)

Time	LDH	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	LDH-5'
Day 7	407	11.8	15.8	17.6	42.5	5.4	6.8
Day 14	213	10.6	19.2	21.6	38.4	6.3	4
Day 22	165	20.8	28	27.8	19.1	4.2	0

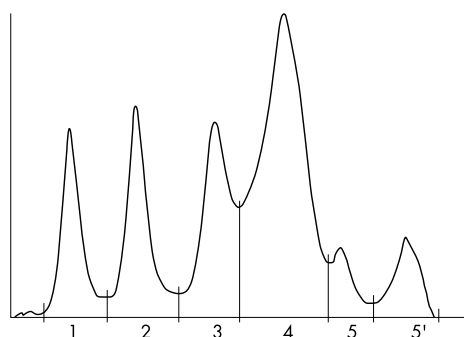
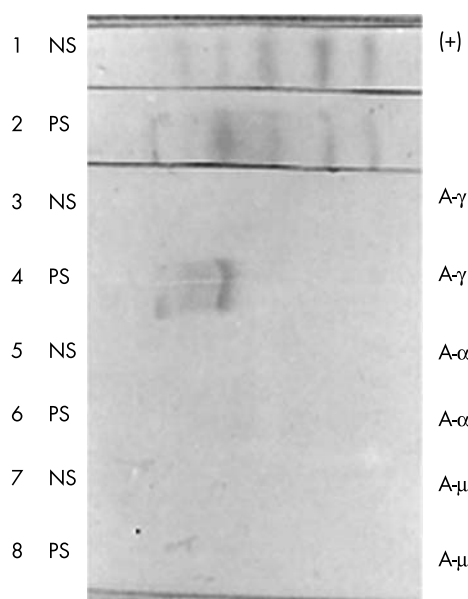
the changes in serum enzyme activities of the postburn patient; table 2 shows the LDH activity and isoenzymes of the patient from the time of admission to discharge.

DISCUSSION

The LDH-immunoglobulin complex has been reported in various diseases.⁶ We describe circulating LDH-IgG complex in a postburn patient; the complex appeared to be related to the progression of burn in this case.

The patient was admitted to our hospital because of 20% burns and his laboratory findings on admission showed high levels of LDH activity (309 U/l, normal 110–240 U/l). After seven days the LDH activity increased to 407 U/l and LDH isoenzyme analysis showed abnormal bands (LDH-1, 11.8%; LDH-2, 15.8%; LDH-3, 17.6%; LDH-4, 42.5%; LDH-5, 5.4%; and extra band, 6.8%).

A broad band around the region of the LDH-4 isoenzyme characterised the patient's LDH isoenzyme pattern, with the apparent appearance of an extra band on the negative side of LDH-5 (fig 1). In fig 2 it can be seen how we identified the extra band. The precipitate was observed only in the lane for anti-IgG antiserum and the patient's serum; in the other lanes no precipitate was seen. In a non-substrate test, we used a substrate without lactate but with NAD, methyl thiazolyl tetrazolium, and phenazine methosulphate. After the staining procedure, no colour band was observed on the membrane. Therefore there was no alcohol dehydrogenase present in the sample.⁷ When antibodies to albumin were used no precipitate was observed. The patient's erythrocyte LDH isoenzyme patterns were also normal, therefore it was not a genetic mutation that caused this anomaly because a genetic mutation changes erythrocyte and serum LDH

**Figure 1** Densitometric pattern of LDH isoenzymes.**Figure 2** Electrophoretic LDH isoenzyme patterns (1–2) and counter immunoelectrophoresis (3–8). NS, normal serum; PS, patient's serum; A- γ , anti-IgG antiserum; A- α , anti-IgA antiserum; A- μ , anti-IgM antiserum.

isoenzyme patterns.⁸ These results suggest that the anomaly was an LDH-IgG complex. With his symptoms improved, the patient's serum LDH activity and isoenzymes gradually returned to normal. When he was discharged from hospital, his serum LDH level had normalised to 165 U/l, and LDH isoenzymes were almost within the normal limit (LDH-1, 20.8%; LDH-2, 28.0%; LDH-3, 27.8%; LDH-4, 19.1%; LDH-5, 4.2% and no extra band). The LDH-immunoglobulin complex might be associated with the pathophysiology of this case.

From table 1 we can see that the activities of myocardial enzymes, AST, LDH, HBDH, CK, CK-MB, and MB/CK were all increased at the time of admission, indicating cardiac muscle was injured. Two weeks after admission, the activities of LDH, HBDH, and AST decreased to nearly normal ranges. Although CK was still high, the MB/CK ratio (5%) was less than the standard for myocardial injury (6%).⁹ Therefore, the increased CK activity was caused mainly by muscle injury after the burn. If we consider the changes in LDH and isoenzyme in the first week after admission: LDH activity was at its highest (407 U/l),

LDH-4 was 42.5%, and LDH-5' appeared (6.8%). In the second week, LDH activity returned to normal, LDH-4 was 38.4%, and LDH-5' was 4%. In the third week, LDH activity was 165 U/L, LDH-4 was 19.1%, and LDH-5' disappeared. These results indicate that it takes some time for the abnormal band of LDH to return to normal unlike LDH activity. The appearance and disappearance of abnormal LDH bands may be relative to the progression of the patient.

There have been many reports on abnormal isoenzyme patterns of plasma LDH in various diseases.^{4-6,10} However, there are few reports on LDH-immunoglobulin complexes in postburn patients. Further studies are required to determine the precise significance of the LDH anomaly in the serum of these patients. In clinical practice, it is important to keep in mind the possibility of an LDH anomaly in patients when the LDH level is abnormally high or does not seem to be related to the clinical state.

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IF ONLY I'D KNOWN

Ten things I wish I'd known when I was 25

- (1) Working full time and having three children is a constant challenge.
- (2) Always listen to the patients because they often know more about life than you do.
- (3) Being proactive and managing time effectively is a more useful skill than the ability to learn lists of medical facts.
- (4) Good communication skills are the key to effective medical practice.
- (5) Colleagues in professions allied to medicine give invaluable advice and opinions.
- (6) Whatever disasters befall you life goes on.
- (7) It doesn't matter if you are not the cleverest SHO on the patch as long as you get on with your colleagues.
- (8) Most conditions are not curable.
- (9) Doing endless investigations is a waste of time.
- (10) Don't be afraid to ask if you don't know something.

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