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Qualitative studies of serum lactate dehydrogenase isoenzyme patterns in leprosy

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Summary Serum lactate dehydrogenase isoenzyme patterns were studied in polyacrylamide gel disc electrophoresis in 72 cases of leprosy to define correlation with clinical varieties and presence of specific pattern. The cases were classified on the basis of history, clinical examination, bacterial and morphological indices, lepromin test and biopsy according to Ridley and Jopling into lepromatous 41: 15 active, 23 regressing and 3 ENL, borderline lepromatous 5 cases, borderline tuberculoid seven cases, tuberculoid 18 and indeterminate, 1 case. Serum LDH zymograms showed a diversity of patterns but there was no correlation with the clinical form of the disease. The commonest abnormalities affected isozymes 4 and 5. Thus in 28 cases both isozymes were depressed or absent and in a further 18 cases one or other of these isozymes were depressed or absent. 4 cases showed other abnormalities and in 11 cases each the LDH zymogram was normal or showed a generalized increase in density of all 5 LDH bands. In 25 healthy controls, 23 showed a normal isozyme pattern and in 3 LDH 5 was absent.

Introduction

The term isoenzyme was coined to describe multimolecular forms of enzymes showing electrophoretic heterogeneity but having the same biological catalytic function.[1] Electrophoretic separations using starch or polyacrylamide gels became routine for the characterization of isoenzymes and the methods have found frequent application in diagnostic laboratories. Lactate dehydrogenases are perhaps the best studied. LDH catalyses the interconversion of pyruvic and lactic acids and is an essential enzyme in both anaerobic and aerobic glycolysis.[2] Human serum LDH shows 5 isoenzymes. These were designated by the numbers 1–5 in terms of decreasing electrophoretic mobility, 1 being the fastest moving in European system.[2] Human LDH consists of H or M

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polypeptide chains in varying ratios the individual isoenzymes being as follows: (1) H_4, (2) H_3M_1, (3) H_2M_2, (4) H_1M_3 and (5) M_4. The H unit typifies the cardiac variety and M the muscle though all tissues contain most of the LDH isozymes albeit in varying proportions. Qualitative and quantitative studies of LDH isoenzymes have been useful in diagnosing necrotic parenchymal diseases of the heart (raised 1 & 2) and liver and muscle[2] (raised 4 & 5).

A careful survey of the literature disclosed only two studies of serum LDH isoenzymes in leprosy.[3, 4] Isozymes 1 and 2 are prominent in well-oxygenated tissues and 4 and 5 in tissues capable of anaerobic metabolism. Leprosy is a unique example of host parasite interactions in which host immune reactions rather than parasite attributes determine the clinical spectrum of the disease. Moreover, in lepromatous leprosy the bacillary load is unique and enormous. We describe here a study of the serum LDH isoenzyme patterns in patients of leprosy.

Materials and methods

CASES

Seventy-two cases of leprosy attending the Sir J. J. Group of hospitals, Bombay, were each studied as follows: a detailed history and clinical examination were recorded; the bacterial (BI) and morphological (MI) indices determined;[5] a lepromin test with armadillo lepromin carried out; and, a biopsy of a cutaneous lesion studied for structural changes and for acid fast organisms. Cases were then carefully classified according to the schema of Ridley and Jopling.[6] Lepromatous leprosy (LL) was subdivided into active, regressing, and erythema nodosum leprosum (ENL) on the basis of clinical features, MI and therapeutic history.

CONTROLS

Sera from 25 normal healthy adults served as the control.

QUALITATIVE ASSAY OF SERUM LACTATE DEHYDROGENASE (LDH) ISOENZYMES

This was carried out in polyacrylamide gel disc electrophoresis[7] in a miniaturized system using gels cast in glass tubing with an internal diameter of 2 mm.[8] Briefly the details were as follows: a 5% monomer was the spacer gel and 7% the separator gel. The buffer was tris-glycine (pH 8.5, 0.1 M). A trace of 1% aqueous bromophenol blue was added to the serum sample and 5 μL of the coloured serum with an equal amount of 40% sucrose was layered on the spacer

gel. Electrophoresis was at a constant current of 1 mA per tube and this was continued till the albumin-stained marker had moved to the lower end of the separator gel. Gels were removed and stained for LDH.[9] The staining solution contained lithium lactate (substrate), nicotinamide adenine dinucleotide (coenzyme), phenazine methosulphate and nitro blue tetrazolium. Gels were incubated in the staining solution at 37°C in the dark for 1 hour. Purple-coloured bands of formazan indicated the site of LDH isoenzymes. Patterns were scrutinized visually and an arbitrary judgement arrived at in terms of normal, absent, increased or reduced, and the Ef values calculated by use of the conventional formula:

$$\text{Ef of LDH isoenzyme} = \frac{\text{Distance travelled by LDH isoenzyme band}}{\text{Distance travelled by albumin marker}}.$$

RESULTS

Figure 1.

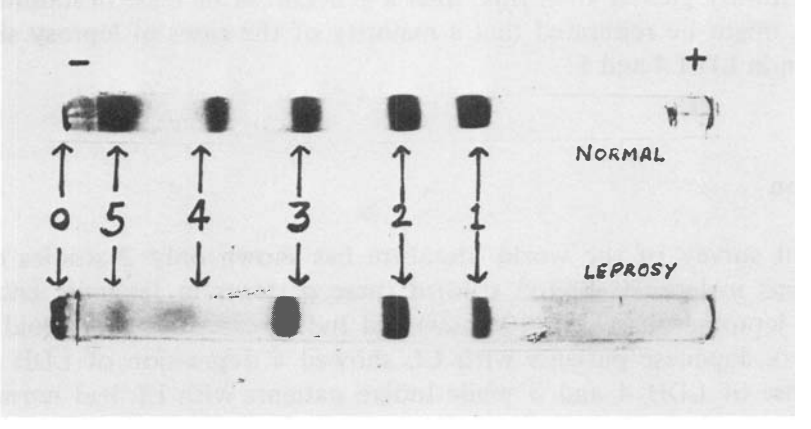

Figure 1. Lactate dehydrogenase isoenzyme separators of sera on polyacrylamide gel disc electrophoresis. Two separations – normal and a case of leprosy – are illustrated. The 5 bands 1–5 in decreasing order of electrophoretic mobility are marked. 0 = origin. Note the diminution in LDH 4 and 5 in the serum from a case of leprosy; this was the common pattern.

CONTROLS

A normal pattern of LDH isoenzymes was obtained in 22 samples. The LDH isoenzyme bands 1–5 in decreasing order of electrophoretic mobilities had Ef values of 0.66, 0.5, 0.37, 0.22 and 0.08 with a variation of ± 0.04. As described in the literature[2] the relative intensity of the bands in decreasing order was $2 > 1 > 3 > 4$ and 5. The remaining three samples showed four bands the missing band always being LDH 5.

CASES

Sera from the patients with leprosy exhibited a diversity of LDH zymograms. Only 11 cases showed the normal pattern. There was no correlation between the clinical type of disease and any particular LDH isoenzyme pattern. However, the dominant feature of all the cases as a whole was a deficiency of the two cathodal LDH isoenzymes 4 and 5 in as many as 28 cases (39%); in 7 cases these bands were absent and in 21 they were faint (Figure 1). In 13 cases faint LDH 4 and 5 bands were associated with prominent bands 1 and 2. Table 1 summarizes the results. The miscellaneous group of patterns seen in 22 cases consisted of: 18 cases with an isolated reduction or absence of either LDH 4 or 5 the other being normal; one case with reduction of both LDH 1 and 2; one case with an elevation of LDH 1 and 2; and 2 cases showing only a single band LDH 1. Eleven cases showed an isomorphic pattern characterized by an increased staining density of all five LDH isoenzymes, though this result could be the result from an inadvertant increase in the sample size, because it is difficult to pipette 5 μl with absolute precision. If the volume on occasion is inadvertantly greater than this, then a generalized increase in staining would occur. It might be reiterated that a majority of the cases of leprosy showed a reduction in LDH 4 and 5.

Discussion

A careful survey of the world literature has shown only 2 studies on LDH zymograms in leprosy. Saito[3] studied these patterns in Japanese and Indian cases of lepromatous (LL) (10 cases) and Indian cases of tuberculoid leprosy (13 cases). Japanese patients with LL showed a depression of LDH 1 and 2 with a rise of LDH 4 and 5 while Indian patients with LL had normal LDH 1, 2 and 3 and an elevation of LDH 4 and 5. In contrast the Indian cases of TT showed a fall or an absence of LDH isoenzymes 4 and 5. The changes were ascribed to damage of the skin, muscle or liver or on account of faulty clearance of LDH from the blood stream to a deranged reticuloendothelial system. The other study[4] noted a normal LDH zymogram in 18 cases of leprosy.

The present study indicates marked differences in the LDH zymograms between patients with leprosy and normal individuals. The commonest observation was a deficiency of the two slow-moving (cathodal) LDH isoenzyme bands 4 and 5 with or without a corresponding rise in the fast-moving (anodal) 1 and 2 bands. Leprosy granulomas have been shown to have a depressed anaerobic glycolysis.[10] It is therefore possible that the deficiency of LDH 4 and 5, noted in the present study, indicates a general shift in the metabolic pattern in leprosy towards aerobic preponderance. This might be reflected in an elevated

Table 1. Serum LDH isoenzyme patterns in 72 cases of leprosy

Type of leprosy	Total no. of cases	Results of serum LDH isoenzyme analysis						
		Normal LDH pattern	Increase of all bands (isomorphic)	Depressed or absent LDH 4 and 5				Other pattern* (miscellaneous)
				Normal LDH 1 and 2		Prominent LDH 1 and 2	Total	
				Absent 4 and 5	Diminished 4 and 5			
ENL	3	1	1	0	0	1	1 (33)	0
LL (active)	15	1	2	1	1	4	6 (40)	6
LL (regressing)	23	5	4	2	2	5	9 (39)	5
BL	5	2	1	0	1	0	1 (20)	1
BT	7	0	3	0	1	0	1 (14)	3
TT	18	2	0	4	3	3	10 (56)	6
ID	1	0	0	0	0	0	0	1
Total	72	11 (15)	11 (15)				28 (39)	22 (31)

*Details of these patterns are given in the text.
Figures in brackets are percentages.

LDH 1 and 2 which was observed in some of the cases. The isomorphic (general elevation) pattern seen in 11 cases, if it is not a technical artefact, could be due to damage to the cleaving or degradation mechanisms of LDH catabolism.

Tissue LDH have also been studied in leprosy. Zuravieva[10] analysed leprosy granulomas in 69 cases by histochemical methods and noted a loss of LDH activity in the lesions; this was ascribed to a depression of anaerobic glycolysis. Saito[11] studied homogenates of tissues from 18 cases of leprosy by agar gel electrophoresis. This author found LDH 3 was increased in ENL leprosy and both 3 and 4 in LL. We described anomalous (additional) LDH isoenzymes in tissue homogenates of cases of leprosy. These correlated with presence of viable *Mycobacterium leprae* in the tissues and were thought to originate from the parasite.[12]

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