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Lactic dehydrogenase isoenzyme in cerebrospinal fluid of children with febrile convulsions

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Aim: To study the lactic dehydrogenase isoenzyme values in children with simple and complex febrile convulsions. *Methods:* Cerebrospinal fluid samples were collected from 115 children, 57 with simple febrile convulsions, 27 with complex febrile convulsions and 31 with no neurological or intracranial pathology (controls). Lactic dehydrogenase activity and isoenzyme levels were measured on a Hitachi analyser. *Results:* Mean total lactic dehydrogenase activity was similar in the three groups. In the control group, lactic dehydrogenase-1 was the main fraction, followed by lactic dehydrogenase-2 and lactic dehydrogenase-3; only small percentages of lactic dehydrogenase-4 and lactic dehydrogenase-5 were detected. In the febrile convulsion group, the lactic dehydrogenase-1 fraction percentage was lower and lactic dehydrogenase-2, lactic dehydrogenase-3 percentages were higher than those in the control group; and the differences were statistically significant between the control and study groups ($p < 0.01$). Values of lactic dehydrogenase-4 and lactic dehydrogenase-5 were similar in all three groups.

Conclusion: This is the first report on the lactic dehydrogenase isoenzyme pattern in the cerebrospinal fluid of patients with simple and complex febrile convulsions. The important finding that focal and general febrile convulsions are not associated with cell damage and changes in aerobic and anaerobic metabolism as lactic dehydrogenase remained unchanged. Analysis of cerebrospinal fluid lactic dehydrogenase isoenzyme levels can assist clinicians in differentiating febrile convulsions from clinical situations that might mimic them.

Key words: *Cerebrospinal fluid, febrile convulsions, isoenzyme, lactic dehydrogenase*

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Lactic dehydrogenase (LDH) is a fermentative enzyme present in many tissues and body fluids, including the cerebrospinal fluid (CSF). In earlier studies higher levels of LDH in the CSF of patients with intracranial malignancies and bacterial meningitis compared with those of healthy individuals have been reported (1, 2). In 1964, Lending et al. (1) recommended that a CSF LDH activity of 40 U/L be considered the upper limit of normal. Later studies showed that healthy individuals are characterized by a specific LDH isoenzyme pattern, namely a preponderance of LDH-1, followed by LDH-2, LDH-3 and LDH-4 (2, 3). Different patterns are indicative of different diseases. For example, patients with bacterial meningitis show elevated levels of LDH-4 and LDH-5 (4).

Nelson et al. (5) described the LDH activity in the CSF with non-specific febrile convulsions. However, there are no published analyses of LDH isoenzymes in febrile seizures. In our study, we measured levels of total LDH and LDH isoenzymes in the CSF of children with simple and complex febrile convulsions.

The LDH activity of spinal fluid appears to bear no direct relation to initial pressure, erythrocyte count, protein or sugar (3).

Materials and methods

Patients

The study population included 115 children, of whom 57 had simple febrile convulsions (SFC) and 27 had complex febrile convulsions (CFC). Simple febrile convulsions were defined as generalized seizures, lasting less than 15 min and occurring only once a day, accompanied by fever of >38°C. Complex febrile convulsions were defined as focal seizures or lasting more than 15 min or occurring more than once a day, accompanied by fever of >38°C[6].

Table 1. Total LDH and isoenzyme distribution in the CSF of children with simple and complex febrile convulsions.

	Simple febrile convulsions ($n = 57$)	Complex febrile convulsions ($n = 27$)	Normal controls ($n = 31$)
Total LDH U/L	36.48 ± 6.02	34.46 ± 5.45	33.53 ± 5.75
LDH-1 (%)	33.68 ± 4.17	31.25 ± 3.77	42.19 ± 5.44*
LDH-2 (%)	38.045 ± 3.27	37.75 ± 8.01	30.96 ± 2.58*
LDH-3 (%)	25.9 ± 6.34	28.75 ± 6.29	20.64 ± 3.69*
LDH-4 (%)	2.45 ± 3.6	1.5 ± 3	6.20 ± 1.74
LDH-5 (%)	0.636 ± 1.43	1 ± 2	0 ± 0

LDH: Lactic dehydrogenase; CSF: cerebrospinal fluid; SFC: simple febrile convulsions; CFC: complex febrile convulsions.
* $p < 0.01$ for controls vs SFC and CFC.
All values are means ± SD.

All the children with febrile convulsions underwent lumbar puncture as part of the evaluation of a first febrile seizure or if there were signs or symptoms of meningitis. The decision to perform a lumbar puncture was made in accordance with the recommendations of the American Academy of Pediatrics (6, 7). CSF samples were taken 2–8 h after the convulsion. The CSF findings were compared with those in 31 children who had undergone lumbar puncture because of fever and suspected intracranial pathology, but with normal results. Children with meningitis or other neurological problems were excluded from the study.

The mean age of patients and controls was similar. No relationship between serum and spinal fluid activity was noted, and each varied independently of the other, presumably because of the blood-brain barrier.

Methods

CSF analysis included total and differential cell counts, glucose and bacterial cultures. None of the samples showed signs of blood. Red and white cells in the cerebrospinal fluid were excluded from the present study ($n = 10$). One cubic centimetre of CSF was stored at −20°C for later analysis of total LDH activity and LDH isoenzymes. Total LDH activity in the CSF samples was measured on a Hitachi-747 analyser (Boehringer-Mannheim) with an LDH kit (Boehringer-Mannheim) using the optimized standard method. In brief, LDH catalysed the reduction of pyruvate to lactate in the presence of NADH, and the consumption of NADH was followed at 340 nm. The reaction was performed at 37°C. Isoenzymes of LDH were separated on an REP-Helena system (Helena Laboratories Ltd., UK) with an REP-LD isoenzyme kit (Cat. No. 3077) according to the charge in the buffered agarose gel. The isoenzyme bands were then visualized by the fluorescence of the NADH formed after incubation of the gel with a buffer containing lactate and NAD. After drying the gel, the isoenzyme peaks were quantitated by densitometry and expressed as a percentage of the total LDH activity or as absolute activity when total LDH activity was entered. All analyses were performed with

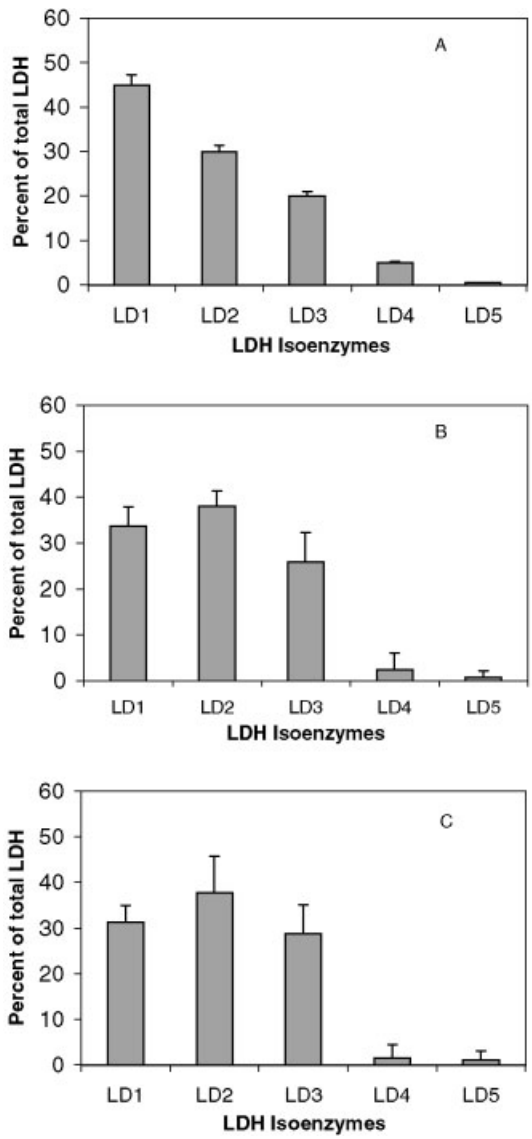

Fig. 1. A: Normal lactic dehydrogenase (LDH) isoenzyme pattern. B: Pattern in simple febrile convulsions. C: Pattern in complex febrile convulsions.

JMP Software (SAS Institute, 1995) including ANOVA and the Tukey–Kramer honestly significant difference (HSD) method for comparison of means (12). An investigator, blinded to the clinical data, carried out the LDH isoenzyme analysis.

Results

No significant differences were found among the three groups in the CSF analysis including total and differential cell counts, glucose and protein concentrations. No correlation was found between glucose protein, WBC and fever relative to LDH isoenzyme fractions among the three groups. The CSF total LDH values and the distribution of the LDH isoenzymes are presented in Table 1. Mean total LDH activity in the CSF was 33.53 ± 5.75 U/L in the control group compared with 34.46 ± 5.45 U/L in the CFC patients and 36.48 ± 6.02 U/L in the SFC patients.

The CSF LDH isoenzyme patterns in the three groups are illustrated in Fig. 1. In samples from the control group, LDH-1 was found to be the main fraction, followed by LDH-2 and then LDH-3; only small percentages of LDH-4 and trace percentages of LDH-5 were detected. By contrast, in patients with SFC and CFC, the LDH-1 fraction percentage was lower than that in the control group, whereas LDH-2 and LDH-3 were preponderant in almost all cases

The differences between LDH-1, LDH-2 and LDH-3 isoenzyme fraction levels were statistically significant between the control and the study groups ($p < 0.01$). No significant differences were found in the LDH isoenzyme between the SFC and CFC groups. LDH-4 and LDH-5 levels were similar in all three groups.

Discussion

To the best of our knowledge, this is the first report on the LDH isoenzyme pattern in the CSF of patients with simple and complex febrile convulsions. Several conditions are known to modify the normal CSF LDH isoenzyme distribution, including bacterial meningitis (increase in LDH-4 and LDH-5), viral meningitis (increase in LDH-1 and LDH-2), hydrocephalus (LDH-2 and LDH-3) intracranial tumours (LDH-5), cerebral haemorrhage (LDH-3, LDH-4 and LDH-5), leukaemic and lymphomatous infiltration (LDH-3 and LDH-4), and tuberculous meningitis (LDH-3) (2, 3). The underlying mechanism causing these CSF LDH changes in patients with pathology of the central nervous system is not understood. Some researchers have suggested a disturbance in the blood-brain barrier which enables plasma LDH to reach the CSF, or production of LDH by neoplastic tissue or by white blood cells and exogenous bacterial sources (1, 3, 9). However, neither of these theories has yet been proven. We believe the first one is unlikely because of the different patterns of LDH isoenzymes in CSF and blood.

The main finding is a discrete shift in the subfractions that are masked by analysing only the total enzyme count. Since differentiating febrile convulsions from similar clinical situations, such as chills or unusual body movements during high fever, is often difficult solely on the basis of the medical history (as given by the parents), simple CSF analyses of LDH and its enzymes could be of diagnostic importance and would ensure appropriate treatment. However, simple febrile convulsions cannot be distinguished from CFC by their total CSF LDH levels, which were found to be identical in both.

The CSF total LDH and its isoenzymes LDH-1, LDH-2 and LDH-3 apparently originate from brain tissue, which contains the same isoenzymes (2, 5, 10). All levels increase when brain tissue is damaged as a result of non-infectious neurological disorders such as hydrocephalus, brain tumours and epilepsy. Nelson et al. (5) stated that neither significant increases in CSF LDH activity nor abnormality of the isoenzyme distribution was noted in children who had had non-specific febrile convulsions. However, it is of clinical interest that in all five cases of epileptic convulsions, LDH values were greater than the presently determined upper limit of normal (5). We assumed that our patients would show CSF LDH changes similar to those found in patients with epileptic convulsions (5, 13). However, in our patients, the total CSF LDH level was found to be within the normal range. We suggest that febrile seizures, being of shorter duration than epileptic seizures, cause less damage to the brain tissue so there is less or no spillage of LDH into the CSF.

LDH levels rise moderately in viral meningitis but significantly in bacterial meningitis (10, 11). The isoenzyme patterns of these disorders are also different. In bacterial meningitis, the main isoenzymes are LDH-4 and LDH-5 and in viral meningitis, LDH-1 and LDH-2, as in other non-infectious neurologic disorders (10, 12–15). The LDH-4 and LDH-5 in bacterial meningitis originate from granulocytes crossing the blood-brain barrier while the LDH-1 and LDH-2 in viral meningitis originate from the brain tissue and possibly from monocytes (10, 11). As in most cases of febrile seizures, the changes in CSF LDH levels and its isoenzymes during systemic febrile illnesses without pleocytosis are unknown.

The important finding that focal and general febrile convulsions are not associated with cell damage and changes in aerobic and anaerobic metabolism as LDH remained unchanged. The present study shows that analysis of CSF LDH isoenzymes is not helpful in the differentiation between short- and long-lasting seizures but can help the clinician differentiate between febrile convulsions and clinical situations that might mimic them.

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