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CSF Lactate Dehydrogenase Activity in Patients with Creutzfeldt-Jakob Disease Exceeds That in Other Dementias

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Key Words
Lactate dehydrogenase · Creutzfeldt-Jakob disease · Dementia · 14-3-3 protein · Tau protein · Alzheimer disease

Abstract
The diagnosis of Creutzfeldt-Jakob disease (CJD) is still made by exclusion of other dementias. We now evaluated lactate dehydrogenase (LDH) in the cerebrospinal fluid (CSF) as a possible additional diagnostic tool. CSF LDH levels of patients with CJD (n = 26) were compared with those in other dementias (n = 28). LDH isoenzymes were determined in a subset (n = 9). Total LDH and isoenzyme LDH-1 were significantly higher, whereas the fractions of LDH-2 and LDH-3 were significantly lower in CJD patients. We conclude that in addition to established CSF parameters, LDH and its isoenzymes might serve as a further help to discriminate between CJD and other dementias.

Introduction

Creutzfeldt-Jakob disease (CJD) is a rare transmissible disease with rapidly progressive dementia as the leading symptom. Patients usually die within less than a year after the onset of symptoms [1, 2]. A definite diagnosis of CJD can only be made by neuropathological or immunochemical demonstration of the pathological isoform of the prion protein (PrPSc) in human brain tissue. Up to now, there are no reliable diagnostic tests for PrPSc in serum or cerebrospinal fluid (CSF). The ante-mortem diagnosis is made according to typical clinical symptoms [3, 4], supported by typical EEG findings [5], increased concentrations of neuron-specific enolase (NSE) [6, 7], tau protein [8] protein 14-3-3 [6, 9, 10] and S-100 protein [11] in the CSF. The latter is also elevated in serum of patients with CJD [12]. However, these markers only reflect neuronal destruction. LDH is another accepted marker for cellular damage. LDH is released by all types of cells after loss of membrane integrity. CSF-LDH activity is increased in central nervous system (CNS) infections [13, 14], head trauma [15, 16], vascular accidents [17], intracerebral lymphoma [18], organophosphate poisoning [19], and metastatic CNS disease [20]. However, LDH has not been investigated, yet in CJD.

Methods

Patients

We investigated CSF samples of 54 patients. All patients with suspected CJD were initially evaluated clinically [12]. Twenty-six patients were classified as having CJD by standard clinical criteria [4, 21, 22], the remaining 28 patients suffered from other dementias.

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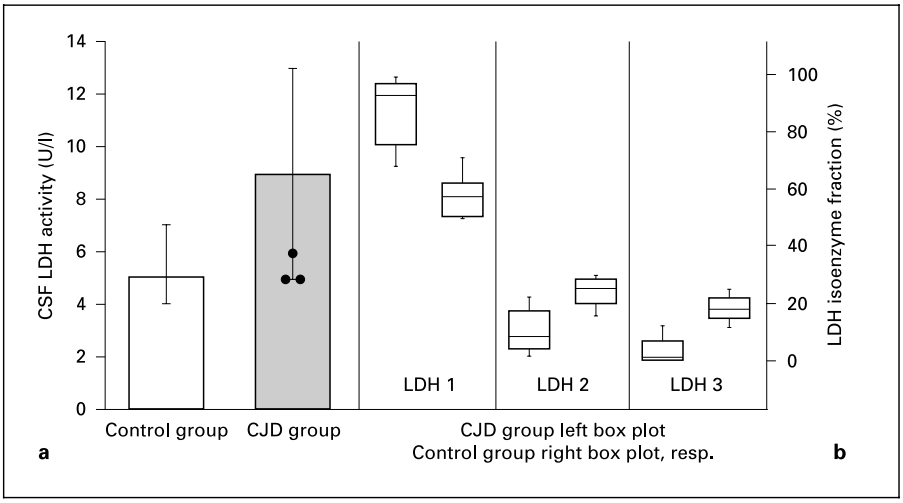

Fig. 1. a Total CSF activity of LDH in patients with CJD dementia (n = 26) and non-CJD dementia (n = 28); CSF LDH of patients negative for 14-3-3 protein (n = 3) are marked as dots. **b** LDH isoenzymes in patients with CJD dementia and non-CJD dementia. Median, 25th/75th percentile (box), 5th/95th percentile (whisker cap); n = 9 in each group.

The male-to-female ratio of patients with CJD was 4/22, their age was 65.0 ± 10.7 years (mean ± standard deviation).

Twenty-three of 26 CJD patients were positive for the 14-3-3 protein in the CSF, in 23 of 26 CJD patients the diagnosis was confirmed neuropathologically [23]. All three 14-3-3-negative patients were among the neuropathologically confirmed cases of CJD. All CJD patients had an intact blood to CSF barrier, reflected by a normal CSF to serum albumin ratio according to Reiber [24].

Other Dementias

This group comprised 28 patients (11 men, 17 women; age 62.2 ± 8.2 years, mean ± SD). The final diagnosis in these patients was made on the basis of the most recent clinical assessment according to ICD-10 or DSM-IV. The group with other dementias comprised the following diagnoses: Alzheimer's disease (n = 12), vascular dementia (n = 4), dementia due to encephalitis (n = 3), multisystem atrophy (n = 2), Huntington's disease (n = 1), paraneoplastic syndrome (n = 1), multiple sclerosis (n = 1), dementia of unknown origin (n = 4).

The study was approved by the local ethics committee in Göttingen.

LDH Measurement

The total LDH activity was measured enzymatically according to McKenzie and Henderson [25] in all patients. In 9 patients with CJD and 9 with other dementias CSF LDH isoenzymes were also measured: CSF had to be concentrated five- to tenfold by ultracentrifugation (Centrifree-System®, Millipore, Inc., USA). Afterwards, the concentrated CSF was mounted onto agarose gel and separated electrophoretically. A substrate-specific staining of the different fractions of LDH was performed by addition of lactate and NAD yielding NADH which in turn was allowed to react with *p*-nitroblue tetrazolium chloride (NBT) and phenazine methosulfate. This step results in oxidation of NADH to NAD and the formation of an NB-formazan dye which reveals isoenzyme bands. The isoenzyme bands were quantitated by scanning densitometry at a wavelength of 600 nm. The test procedure was performed with a commercial electrophoresis system (LD-Paragon®, Beckman Inc., USA).

Statistical Analysis

As the results of the CJD and non-CJD groups did not follow a Gaussian distribution, calculations of median (25/75th percentile) and significances calculated with Mann-Whitney U test are given. We obtained a cut-off value using the Youden Index [26], though the limited number of patients does not allow its clinical use.

Results

CSF LDH activity was significantly higher in CJD patients (mean 9, range 5–13 U/l) than in the non-CJD group (mean 5, range 4–7 U/l; p = 0.007). The levels of LDH in 14-3-3-protein-negative CJD patients (n = 3) ranked among the lowest in the CJD group (fig. 1).

The best cut-off value was 8 U/l, yielding a sensitivity of 69%, a specificy of 84% (Youden Index 0.53). Isoenzymes LDH-1, LDH-2 and LDH-3 could be detected, fractions of LDH-4 and LDH-5 were usually below the detection limit.

The percentage of isoenzyme fractions differed significantly: LDH-1 was higher in CJD patients (median 92%; 25th/75th percentile 75/96% vs. 57%; 50/61%; p = 0.001), while both LDH-2 (8%; 4/16% vs. 25%; 21/28%; p = 0.003) and LDH-3 (1%; 0/9% vs. 18%; 15/21%; p = 0.001) were lower as compared with the control group.

Discussion

LDH release in the CSF occurs in various conditions of acute brain cell damage. Even though CJD has a subacute clinical course, the results show that the death rate of

brain cells is still important enough to cause a higher release of LDH into the CSF than in dementia of other origins.

Since LDH-1 is the predominant brain LDH isoenzyme [20], the increased proportion of this isoenzyme and the increased total CSF LDH activity reflect the CJD-induced brain damage well.

Three of the CJD patients were negative for 14-3-3 protein. Their LDH activities were distinctly lower than the median value of all CJD patients. Hence, the absence of the scaffolding protein 14-3-3 in CSF might be a sign of comparatively conserved cellular integrity in 14-3-3-negative CJD patients.

Our calculated cut-off value doubtless needs to be interpreted with caution because of the small number of investigated patients. However, it is already evident that its differential diagnostic test is as good as that of other markers, such as 14-3-3, tau protein or S-100B.

We conclude that CSF analysis of total LDH activity and its subfractions can help in the differential diagnosis of CJD in a cost-effective manner. Patients lacking 14-3-3 in the CSF might have attenuated brain cell damage as compared with those CJD patients who are positive for this protein.

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