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Clinical Study

Glial fibrillary acidic protein as a marker of astrocytic activation in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis

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ABSTRACT

Glial fibrillary acidic protein (GFAP) has been shown to be increased in the cerebrospinal fluid (CSF) of patients suffering from neurological diseases involving the activation of astrocytes, but has not been studied in amyotrophic lateral sclerosis (ALS) patients to our knowledge. CSF samples of patients with definite ALS and of those with other neurological diseases were evaluated for their GFAP concentrations. CSF-GFAP concentrations of patients with ALS were significantly elevated by 53% compared to patients with other neurologic diseases. GFAP might serve as a biomarker in ALS. Our findings support the concept that astrocytes play a role in ALS pathogenesis.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease [1,2] characterized by selective, rapid degeneration of motor neurons [3,4]. Time to diagnosis is an average of 16–18 months from symptom onset. Since the diagnosis is purely clinical and no biomarkers exist, patients should undergo a thorough workup to exclude other diseases that may mimic ALS. At the time of writing, cerebrospinal fluid (CSF) analysis of ALS patients has not revealed a specific pattern helpful in diagnosis. Thus diagnosis can be firmly established only at an advanced stage. The pathophysiology of the sporadic disease remains elusive [5–9]. Though the major abnormality in ALS is the progressive and selective death of motor neurons, in recent years glial cells have been shown to have a pathogenic role [10,11] and *post mortem* studies of ALS patients have revealed astrogliotic changes in the spinal cord and motor cortex [12–14]. However, astrocytic markers have not been investigated in the CSF of ALS patients to our knowledge. As astrocytes may play a role in the pathophysiology of disease, and their activation involves the upregulation of glial fibrillary acidic protein (GFAP), CSF GFAP may serve as a marker for astrocytic injury in neurologic disease [15]. In experimental, as well as *post mortem* studies of ALS patients, increased tissue levels of GFAP have been detected [16,17]. Studies on the clinical usefulness of a CSF biomarker of astrocytic damage and its relation to motor neuron loss in ALS are lacking [18,19]. We therefore examined, in a prospective manner, the CSF concentration of GFAP in ALS.

2. Patients and methods

Patients were recruited from the Department of Neurology, Rabin Medical Center, a tertiary medical facility in Israel, over a period of 3 years (June 2010 to September 2014). The El Escorial diagnostic criteria [20,21] were used to classify patients with ALS and we included only patients with definite ALS. The institutional Ethics Committee approved the study, and all patients gave informed consent to participate. All patients suspected of suffering from ALS underwent a full diagnostic workup including peripheral electrodiagnostic and electromyographic evaluation, exclusion of underlying malignant neoplastic diseases, brain and cervical spinal cord imaging, and an analysis of the CSF. We identified 14 patients with definite ALS (mean age 64.6 ± standard deviation [SD] 8.0 years, range 51–76 years; 12 males, two females) and 14 randomly chosen, non-matched patients (mean age 42.2 ± SD 18.6 years, range 19–73 years; 10 males, four females; Table 1) undergoing lumbar puncture (LP) for other reasons than ALS, including pseudotumor cerebri, multiple sclerosis, acute confusional state, headache, peripheral neuropathy, and seizures.

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Table 1
Demographics and results of the CSF analysis in ALS and control patients

	ALS	Control
Patients, n	14	14
Age, years	64.6 ± 8.0 [51–76]	42.2 ± 18.6 [19–73]
Male/female	12/2	10/4
CSF		
Leukocytes (n/dl)	1.9 ± 4.4 [0–17]	6.6 ± 9.6 [0–32]
Protein (mg/dl)	44 .0 ± 18.4 [22.3–83.0]	46.4 ± 25.2 [17.5–96.0]
CPK (mg/dl)	275.2 ± 166.6 [95–532]	127 ± 95 [50–364]
Symptoms prior to LP (months)	9.3 ± 5.1 [2–18]	–
Reason for LP in control:	–	Demyelination (3) Confusional state (2) Headache (2) Neuropathy (3) Pseudotumor cerebri (2) Seizure (2)

Data are presented as mean ± standard deviation [range] unless otherwise indicated.
ALS = amyotrophic lateral sclerosis, CPK = creatine phosphokinase, CSF = cerebrospinal fluid, LP = lumbar puncture.

2.1. Serum, CSF examination, and enzyme-linked immunosorbent assay testing for GFAP

Serum samples were obtained from all patients and evaluated for complete blood count, electrolytes, creatine kinase and liver enzymes. CSF samples were sent for routine testing (cell count and differentiation, protein, glucose and chloride evaluation, gram-stain, bacterial culture, and microscopic cytology). Two sample vials of CSF (3 ml) were immediately frozen and stored at −80°C for later testing. All CSF samples were quantified for GFAP concentration using a commercially available sandwich enzyme-linked immunosorbent assay (GFAP, #A05188, Bertin Pharm, Montigny le Bretonneux, France), and assays were performed according to the manufacturer's protocols. In short, CSF samples (100 µL) were applied to anti-GFAP antibody-precoated 96-well plates and incubated for 2 hours. After washing three times with phosphate buffer saline, biotin-labeled monoclonal antihuman GFAP antibody was added and incubated for 1 hour. After washing, streptavidin-horseradish peroxidase tracer was applied for 1 hour and then washed three times. Finally, hydrogen peroxide/tetramethylbenzidine substrate was added, and after 10 minutes, the reaction was stopped with sulfuric acid solution. We measured the absorbance at wavelength 450 nm. The detection limit of GFAP in this test is 0.04 ng/mL. All CSF samples were tested twice independently with separate test kits from the same manufacturer.

2.2. Statistical analysis

Findings were evaluated using analysis of variance to determine pairwise comparisons amongst multiple data sets with a significance level of 0.05. Statistical analysis was carried out using GraphPad Prism-5 software (GraphPad, La Jolla, CA, USA). Either Student's t-test to compare two groups or the Mann–Whitney U test was applied.

3. Results

3.1. Concentrations of GFAP in the CSF are elevated in ALS patients

Comparing the mean GFAP concentration in ALS patients with that of patients with other neurological diseases, a 53% elevation in GFAP was detected. Mean GFAP levels were 1.43 ± SD 0.89 ng/ml in ALS patients and 0.94 ± 0.62 ng/ml in the control group (n = 14 each; p = 0.049; Fig. 1A). Distribution of GFAP concentrations across the ALS and control patients was wide and did not enable a clear cut-off value with high enough specificity to identify ALS solely by the CSF GFAP concentration (Fig. 1B). No differences in CSF GFAP concentrations between patients with bulbar signs, with monoparetic disease, paraparesis or quadriparesis were detected. Likewise, on comparison of CSF GFAP concentrations in patients with fasciculations with patients with mainly spastic upper motor neuron signs, no significant differences were detected.

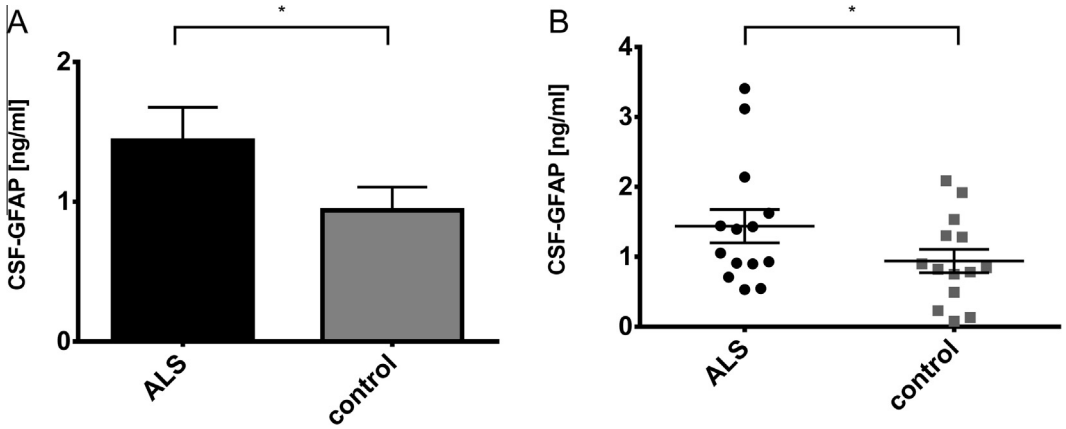

Fig. 1. Cerebrospinal fluid glial fibrillary acidic protein (CSF-GFAP) concentrations in patients with amyotrophic lateral sclerosis (ALS) and other neurological diseases. (A) Mean GFAP levels (ng/ml) in ALS and control individuals (asterisk indicating statistical significance; p = 0.049). (B) Individual patient and control subject CSF-GFAP concentrations.