SHORT REPORT

Highly increased CSF tau protein and decreased β -amyloid (1–42) in sporadic CJD: a discrimination from Alzheimer's disease?

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Abstract

The aim was to quantify tau protein and β -amyloid (A β 42) in the CSF of patients with sporadic Creutzfeldt-Jakob disease (CJD), Alzheimer's disease (AD), and controls. Double sandwich enzyme linked immunosorbent assays (ELISAs) were used for measurements. Tau was increased 58-fold in CJD and 3.5-fold in AD compared with controls, whereas A β 42 was decreased 0.5-fold in both CJD and AD. A cut off level for tau protein at 2131 pg/ml successfully discriminated CJD from AD (100% specificity and 93% sensitivity). Tau protein concentration in CSF is probably an additional useful marker in differentiating CJD from AD.

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Sporadic Creutzfeldt-Jakob disease (CJD) is a rare and fatal human neurodegenerative disorder belonging to the transmissible spongiform encephalopathies or prion diseases. The classic triad of the clinical syndrome consists of rapidly progressive dementia, myoclonus, and a characteristic EEG. Diagnosis can only be definite at postmortem examination or brain biopsy showing the pathological isoform of the prion protein (PrP^{sc}).¹ Alzheimer's disease (AD) is the most common condition mimicking CJD, sharing some clinical and neuropathological features. Among several biomarkers, recently being evaluated for AD, the microtubule associated tau protein and the β -amyloid 1–42 (A β 42), a constituent of senile plaques, are the most reliable. Increased CSF tau protein concentrations and decreased A β 42 concentrations have been found in AD and have been proposed as candidate diagnostic markers.^{2–4} Neuronal loss with collapse of the cytoarchitecture is common in both diseases. Amyloid plaques are seen in up to 10% of patients with CJD¹ and occasionally true A β deposits have been reported.^{5,6} Due to these similarities of the two diseases we examined the above markers in patients with neuropathologically confirmed CJD, patients with well documented AD, and age matched normal controls.

Patients and methods

A total of 99 subjects were included in the study: 14 patients with CJD, 38 patients with AD, and 47 controls. The clinical diagnosis of CJD was based on progressive dementia of less than 2 years, periodic sharp wave complexes in the EEG recording, and two of the following: (1) myoclonus, (2) visual or cerebellar symptoms, (3) pyramidal or extrapyramidal tract signs, and (4) akinetic mutism. All patients had a positive test for 14–3–3 protein, a sensitive marker of the disease. Twelve out of the 14 patients were confirmed either postmortem or by biopsy as definite cases of CJD. The diagnosis of AD was based on the NINCDS-ADRDA criteria for probable AD.⁷ The control group comprised 47 elderly subjects who underwent hernia repair under spinal anaesthesia, without any history, symptoms, or signs of cognitive disorder or other neurological, malignant, or systemic disease.

Samples of CSF were obtained by lumbar puncture after consent. They were centrifuged immediately and stored in polypropylene tubes at –80°C until analysis. Tau and A β 42 concentrations in CSF were measured in duplicate by double sandwich enzyme linked immunosorbent assay (ELISA) using the Innostest htau antigen kit and the Innostest β -amyloid (1–42) kit, according to the manufacturer's (Innogenetics, Gent, Belgium) instructions. Freezing and thawing of samples were avoided. The intra-assay and interassay variabilities for tau protein were 8% and 9% respectively, and the corresponding variations for A β 42 were 7% and 10% respectively. A Kruskal-Wallis test followed by Dunn's multiple comparison test was used to compare tau protein and A β 42 concentrations among the groups. The Spearman correlation coefficient was used for correlations. Receiver operating characteristics (ROCs) curve analysis, was used to define the cut off concentrations of tau protein and A β 42 with the corresponding optimal sensitivity and specificity.

Results

Results are summarised in table 1. Age, sex, disease duration, and age of disease onset did not affect tau protein and A β 42 in any of the groups. Patients with CJD showed markedly

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Table 1 Clinical and biochemical data of the studied groups

	Controls	AD	CJD	p Value
No of patients	47	38	14	
Sex*	29/18	15/23	7/7	NS
Age (y)†	65 (10)	68 (10)	59 (4)	0.012
Disease duration (y)‡		3.6 (2.4)	0.4 (0.2)	
Tau (pg/ml)‡	137 (110–220)	490 (366–796)	7942 (3787–14750)§	<0.0001
Aβ42 (pg/ml)‡	734 (521–865)	375 (320–440)	312 (218–511)¶	<0.0001

*Men/women, comparison with χ^2 test. †Mean (SD); age compared with one way ANOVA. ‡Median (quartiles), comparison with Kruskal-Wallis test. §Minimum=1240, maximum=26570. ¶n=12.

increased tau protein ($p<0.001$) and significantly decreased Aβ42 ($p<0.001$) compared with controls. Patients with AD also had significantly increased tau protein ($p<0.001$) and significantly decreased Aβ42 ($p<0.001$) compared with controls. No difference in tau protein and Aβ42 concentrations were detected between early (duration<2 years) and late AD. Patients with CJD had significantly increased tau protein ($p<0.01$), but similar values in Aβ42 as patients with AD. For AD versus controls, the optimal cut off concentration for tau protein suggested by ROCs was 295 pg/ml (fig 1 A) and the specificity and sensitivity were 91.5% and 89.5% respectively. The optimal cut off level for Aβ42 was 445 pg/ml (fig 1 B), resulting in a specificity of 85% and a sensitivity of 76%. For CJD versus AD, the optimal cut off level for tau protein at 2131 pg/ml resulted in 100% specificity and 93% sensitivity. A significant negative correlation between tau protein and Aβ42 was found in patients with CJD (fig 2), but not in patients with AD and controls.

Discussion

The results of the present study have shown an extremely marked increase in tau protein and a 0.5-fold decrease in Aβ42 in the CSF of patients with CJD compared with controls. Patients with CJD also had significantly increased tau protein compared with patients with AD. This is the first report of combined analysis of tau protein and Aβ42 in CJD and the first comparative study versus AD and normal controls for both markers. Previous studies of combined analysis of these markers in AD have suggested that high tau protein and low Aβ42 is a characteristic profile of AD. However patients with CJD presented with a multifold increase in tau protein beyond the range seen in AD or in other dementing disorders. With the cut off point of 2131pg/ml, all except one patient were discriminated from either patients with AD or the controls. The only patient that fell in the range of the AD group was a patient with probable CJD with a relatively weaker 14–3–3 protein band, not yet having been confirmed pathologically.

Our results for tau protein are in agreement with the results of Otto *et al*⁸ who studied patients with CJD versus patients with other dementing and non-dementing neurological disorders, and also support the notion of tau protein being a sensitive marker for CJD. We specified 2131 pg/ml as the optimal cut off level for AD, the main disease in CJD differential diagnosis. A decrease in Aβ42 was in the range of the AD group. Low Aβ42 values in CSF have

been reported in CJD⁹ in one recent study and also in AD and other dementing disorders,¹⁰ suggesting that a decrease in Aβ42 is not a specific marker.

Figure 1 Concentrations of CSF tau protein (A) and Aβ42 (B) in the groups studied. Horizontal bars indicate median values and horizontal lines indicate cut off concentrations suggested by ROC analysis.

Figure 2 Significant negative correlation between CSF tau protein and A β 42 in CJD ($n=12$).

The release of both tau protein and A β 42 into CSF is considered to be a normal process, as they are detectable in normal subjects; however, the nature of these markers in CSF and their relation to neuropathology of different diseases has not been well documented. High concentrations of tau protein had at first been associated with neurofibrillary lesions, and it has been suggested that concentrations of tau protein in CSF is related to the number of tangles in patients with AD.¹¹ However, a high CSF tau protein concentration has also been reported in diseases without neurofibrillary pathology.¹² The very high concentrations of tau protein in patients with CJD are possibly correlated to the rapidly occurring neuronal loss. Our knowledge on the biochemical characteristics of CSF tau protein molecules among diseases (three versus four repeat tau protein) is also limited. The phosphorylation state is another matter under investigation as the available test cannot discriminate normal from phosphorylated tau protein.

β -Amyloid, the product of the proteolytic processing of amyloid precursors protein (APP), is constitutively secreted into the extracellular space which is in continuum with CSF.

Biochemical studies have shown that the A β 42 peptide aggregates far more rapidly into amyloid fibrils than other species, such as A β 40.¹³ The mechanism(s) underlying A β 42 reduction in the CSF is not known. Possible hypotheses include increased aggregation into insoluble fibrils (at least in AD), decreased production, implication of other unknown factors regulating cellular APP processing, and failure of detection by current methodology due to masked epitopes.⁹ In the present study, the inverse correlation between tau protein and A β 42 only in patients with CJD suggests a common mechanism affecting both substances.

In conclusion, CSF tau protein concentration seems to be an additional useful marker in differentiating CJD from AD. It should be noted that the 14–3–3 protein test is not pathognomonic, being 92%–96% specific among dementias.¹⁴ As tau protein is increased early in the course of the dementing illnesses it can be helpful in diagnosis of CJD.

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