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Abstract

Total tau protein (t-tau) levels in cerebrospinal fluid (CSF) (CSF-tau) are markedly elevated in patients with Creutzfeldt-Jakob disease (CJD). Some CSF-tau may leak into the blood. We evaluated t-tau levels in serum (serum-tau) as a possible marker for the differential diagnosis of CJD from Alzheimer's disease (AD) and other rapidly progressive dementias (RPD). Serum- and CSF-tau levels were determined in patients with sporadic CJD (n = 12), AD (n = 10) and RPD but no CJD (non-CJD-RPD; n =9) who showed RPD fulfilling the WHO criteria for possible CJD at onset and had a final diagnosis other than CJD. We also analyzed serum-tau levels in healthy volunteers as a control group (n = 10). Serum- as well as CSF-tau levels were significantly elevated in CJD group compared to those in AD, non-CJD-RPD and healthy control groups. Serum-tau would be a simple and useful marker to distinguish CJD from AD and non-CJD-RPD, requiring further large study to confirm this.

Key words

Creutzfeldt-Jakob disease, Alzheimer’s disease, tau protein, cerebrospinal fluid, serum tau protein

Introduction

Cerebrospinal fluid (CSF) markers for the diagnosis of Creutzfeldt-Jakob disease (CJD) include 14-3-3 protein, total tau protein (t-tau), neuron-specific enolase and S100B protein, and development of a new marker to detect the disease-associated form of prion protein (PrP) is ongoing. T-tau levels in CSF (CSF-tau) are markedly elevated in patients with CJD [1]. Tau is produced mainly in the central nervous system (CNS), and some CSF-tau may leak into the blood [2]. We had a hypothesis that patients with CJD may show high levels of t-tau in serum (serum-tau) in association with marked elevation of CSF-tau levels.

Previous studies have indicated that Alzheimer's disease (AD) is the most frequent alternative diagnosis for CJD [3- 7]. Although, generally, there is little difficulty in making a differential diagnosis between CJD and AD, some atypical cases of CJD may present with slowly progressive clinical course mimicking AD [8]. Other neurologic diseases with rapidly progressive dementia (RPD), such as viral encephalitis and vascular

encephalopathy, may be indistinguishable from CJD, especially during the initial phase of the disease [9].

Here we evaluated serum- as well as CSF-tau levels in patients with CJD, in comparison with those in patients with AD and patients who showed RPD mimicking CJD at the onset and later had diagnosis other than CJD (non-CJD-RPD).

Methods

We studied serum- and CSF-tau in CJD [median age ± SE (range), 73 ± 1.7 (65- 83) years] (n = 12), AD [71 ± 3.2 (53- 80) years] (n = 10), and non-CJD-RPD groups [59 ± 4.2 (43- 76) years] (n = 9).

The 12 patients with CJD were all sporadic cases, and were classified to 4 with definite (neuropathologically confirmed) CJD and 8 with probable CJD according to the World Health Organization (WHO) criteria [10]. None of these subjects had a family history of CJD or a history of receiving cadaveric dura mater grafts during neurosurgical procedures. Analysis of the PrP gene revealed no mutation, and the polymorphic codons

showed methionine homozygosity at codon 129 and glutamic acid homozygosity at codon 219 in all patients. According to Parchi's classification [11], the 4 definite cases were further classified to 3 with the MM2-cortical form characterized clinically by relatively slow progression of dementia, and 1 with the MM1 type characterized by a classic CJD phenotype showing RPD, myoclonus and positive periodic sharp-wave complexes. The 8 probable cases showed the classic CJD phenotype, probably classified to MM1 type.

Patients with AD fulfilled the NINCDS-ADRDA criteria [12] for probable AD. General physical and neurologic examinations, neuropsychological evaluations, magnetic resonance imaging (MRI) of the brain and single photon emission computed tomography (SPECT) of cerebral blood flow (CBF) were conducted to confirm the accuracy of the clinical diagnosis. The patients with AD showed medial temporal lobe atrophy on MRI and hypoperfusion in the posterior cinglate gyrus/precuneus or parieto-temporal lobe on CBF-SPECT, but no hyperintensity in the cortex or basal ganglia on diffusion-weighted MRI. The levels of cognitive impairment were defined by a Clinical Dementia Rating

(CDR) scale [13-14], and the CDR score of each patient was ≥1.0. All patients with AD were subjected to the Mini-Mental State Examination (MMSE) [22 ± 6.3 (20- 26)] [15].

The non-CJD-RPD group included patients who had fulfilled the WHO criteria for 'possible' CJD [10] at onset and had a final diagnosis other than CJD: viral encephalitis (n = 4), paraneoplastic syndrome (n = 2), hydrocephalus (n = 1), tuberculous meningitis (n = 1) and multiple cerebral infarction (n = 1).

In addition, as a control group, we analyzed serum-tau in healthy volunteers [71 ± 0.8 (66- 73) years] (n = 10). All healthy volunteers underwent medical screening, including a questionnaire regarding medical history, general and neurological examinations and neuropsychological testing with the MMSE. Those subjects with a history of head trauma, psychiatric or neurological disorders, uncontrolled major systemic diseases or an MMSE score of less than 28 were excluded. All groups were matched for age, except the non-CJD-RPD group in which ages were significantly younger than in CJD or healthy controls ($p < 0.005$ and $p < 0.05$, respectively). There was no difference in the duration of illness between the CJD group [duration (range), 3 ± 1.6 (1- 18) months] and the non-

CJD-RPD group [3.5 ± 2.1 (0.3- 18) months], but the duration of illness in the AD group [18 ± 3.8 (11- 36) months] was significantly longer comparison with the CJD and non-CJD-RPD groups ($p < 0.0001$ and $p < 0.0005$, respectively).

Written informed consent was obtained from all participants or their caregivers. The human subjects committee of our university hospital approved this study.

Serum and CSF samples were collected on the day of diagnosis. One of the patients with CJD had chronic hepatitis C and participated in a clinical trial before the onset of CJD. His blood had been stored regularly for evaluation in clinical trials for two years. We obtained permission to use the blood samples for our study.

Serum and CSF samples were centrifuged immediately after collection and stored at -80 °C until measurement in addition to a routine examination. The CSF and serum samples were analyzed for t-tau and albumin. A sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum- and CSF-tau using the manufacturer's instructions with some modification (Innotest hTAU-Ag; Innogenetics). Each ELISA plate was coated with anti-tau antibody AT120, as the capturing antibody.

After adding 75 μL of anti-tau antibody HT-7 and BT-2, as detection antibodies, 25 μL of the serum and CSF samples were applied to each well, and the plate was incubated overnight at 23ºC. After washing with phosphate buffer, the wells were incubated with 100 μL/well of horseradish peroxidase (HRP)-labeled streptavidin for 30 min at 23ºC. Bound HRP activity was assayed using tetramethyl benzidine. The HRP reaction was terminated with 2N sulfuric acid (100 μL/well), and absorbance at 450 nm was measured with a micro plate reader. All samples and standards were run in duplicate on the same day with the same lot of standards. The relative concentration estimates of serum- and CSF-tau were calculated according to the standard curve. The standard curve was composed of 7 points (1200, 600, 300, 150, 75, 37.5 and 18.8 pg/mL) and dilutions were made with phosphate buffer. The blank was phosphate buffer alone. Ten determinations of the lowest standard (18.8 pg/mL) were significantly higher than 50 determinations of the blank ($p<0.0001$) for an absorbance of 450 nm. Thus we determined the lowest detection limit to be 18.8 pg/mL. Values below the detection limit were set to zero for further statistical analyses. Quantitative determination of albumin in serum and CSF was

performed by nephelometry using standard methods. The CSF/serum albumin ratio (albumin ratio) [16], [CSF-albumin (mg/L)/serum-albumin (g/L)], was calculated as a measure of the blood-brain barrier (BBB) function. Further, the serum/CSF tau ratio (tau ratio), [serum-tau (pg/mL)/CSF-tau (pg/mL)], was calculated as a measure of leakage of t-tau from CSF to blood. The groups were compared using an analysis of variance or the Kruskal-Wallis test according to the distribution of the data, and if applicable, with a post hoc analysis. Correlations were evaluated by Spearman's rank correlation test. Values with $p < 0.05$ were regarded significant. Statistical analyses were performed using SPSS version 12.0J for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Serum-tau levels were significantly higher in the CJD group [median ± SE (range), 193 ± 72.6 (35.0- 919.0) pg/mL] compared to those in the AD ($p < 0.001$) [0 ± 3.3 (0- 33.3) pg/mL], non-CJD-RPD ($p < 0.01$) [22 ± 21.8 (0- 185.0) pg/mL] and healthy control ($p = 0.001$) groups [0 ± 9.37 (0- 89.0) pg/mL] (Fig. 1). Serum-tau levels from 3 patients

with the MM2-cortical form of sporadic CJD [50 ± 137.4 (40.0- 457.0) pg/mL] tended to be higher than those in AD group patients ($p = 0.0619$) and healthy controls ($p = 0.0767$) but similar to those in the non-CJD-RPD group. CSF-tau levels in the CJD group were significantly higher than those in the AD ($p < 0.05$) and non-CJD-RPD ($p < 0.05$) groups (Fig. 2). The albumin ratio did not differ significantly between the CJD (median ± SE, 7.6 ± 1.18), non-CJD-RPD (9.1 ± 2.83) and AD (5.7 ± 1.26) groups. There was no difference in the tau ratio between the CJD group (median ± SE, 0.019 ± 0.04), non-CJD-RPD group (0.024 ± 0.07) and AD group (0 ± 0.01). There were no significant correlations between the albumin ratio and tau ratio in total or in any of the groups.

Serum samples obtained before onset of CJD (12, 3, 2 and 1 month before onset) showed undetectable serum-tau levels.

DISCUSSION

Our results revealed that serum-tau as well as CSF-tau were significantly elevated in the CJD group compared to those in the AD, non-CJD-RPD, and control groups. When

the CJD group was limited to the MM2-cortical form of sporadic CJD characterized by relatively slow progression of dementia, serum-tau levels tended to be higher than those in the AD or control groups.

Microtubule-associated protein tau is localized in axons of the CNS neurons [17]. Previous studies have reported that the concentration of serum-tau was elevated in patients with acute neurologic disorders, such as stroke, and it was below the detection limit in neurologically healthy participants [18-19]. Little is known about the passage of tau from the brain into the blood. Disruption of BBB might be related to the leakage of tau from CSF to blood. We could not have the albumin ratio for the control group, as we had no CSF samples for it. The albumin ratio was reported to be 5.44 ± 2.16 (mean ± SD) in healthy individuals (n=231) in a previous study [19]. The albumin ratios in the CJD, AD, and non-CJD-RPD groups in this study were higher than those reported in healthy controls [19], which suggests some damage of BBB in CJD, AD, and non-CJD-RPD. Further, our results revealed no correlations between the albumin ratio and tau ratio in the CJD, AD or non-CJD-RPD groups, indicating that leakage of tau from CSF into the

blood would not be simply associated with BBB disruption represented by the albumin ratio.

Analysis of serial changes of serum-tau indicated that serum-tau levels would be elevated to be detectable after onset of CJD. Regarding serial changes of CSF-tau levels, it was reported in a patient with CJD that CSF-tau level was relatively low at 2 months before disease onset, and then rose sharply at 6 weeks after onset [20]. The CSF-tau levels before disease onset reported in the previous study with CJD [20] appear consistent with those of serum-tau levels in our study.

The serum-tau levels could be measured in all samples of the CJD group, on the other hand, they were below the detection limit in 4 of 9 samples of the non-CJD RPD group. We suggest that the possibility of CJD should be carefully examined if serum-tau was detectable in RPD. Medical procedures such as neurosurgery and ophthalmic surgery before the diagnosis of CJD may be associated with risk of secondary transmission of CJD through contaminated instruments. Our recent study revealed that 0.8% of patients with CJD underwent neurosurgery after the onset, but before the diagnosis of CJD, and

1.8% of patients with sporadic CJD ophthalmic surgery [21]. Measurement of serum-tau before neurosurgery and ophthalmic surgery in patients with dementia may be a simple and useful tool for the screening of CJD.

The limitations of this study were the small sample size. Only 4 cases with neuropathologically confirmed CJD were available. Probably due to very high proportion of MM genotype at PrP codon 129 in the Japanese population (93%) [22], all the 12 patients with sporadic CJD in this study had MM genotype at codon 129, including 3 with the MM2-cortical form; the MM2-type sporadic CJD is common among atypical CJD cases in Japan [22]. While, no patients with VV or MV genotype, rare genotypes in Japan [22], could be included in this study, although VV2-type sporadic CJD is the second commonest form in European countries and the USA [11][23]. In addition, to reveal the statistical significance in serum-tau levels for the MM2-cortical form of sporadic CJD, further study with a larger number of the patients is necessary.

In conclusion, serum-tau would be a simple and useful marker to distinguish CJD from AD and non-CJD-RPD, requiring further large study to confirm this.

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Figure Legends

Fig. 1

Serum levels of total tau protein (serum-tau) in sporadic Creutzfeldt-Jakob disease (CJD), Alzheimer's disease (AD), patients with rapidly progressive dementias but no CJD (non-CJD-RPD) and healthy control groups.

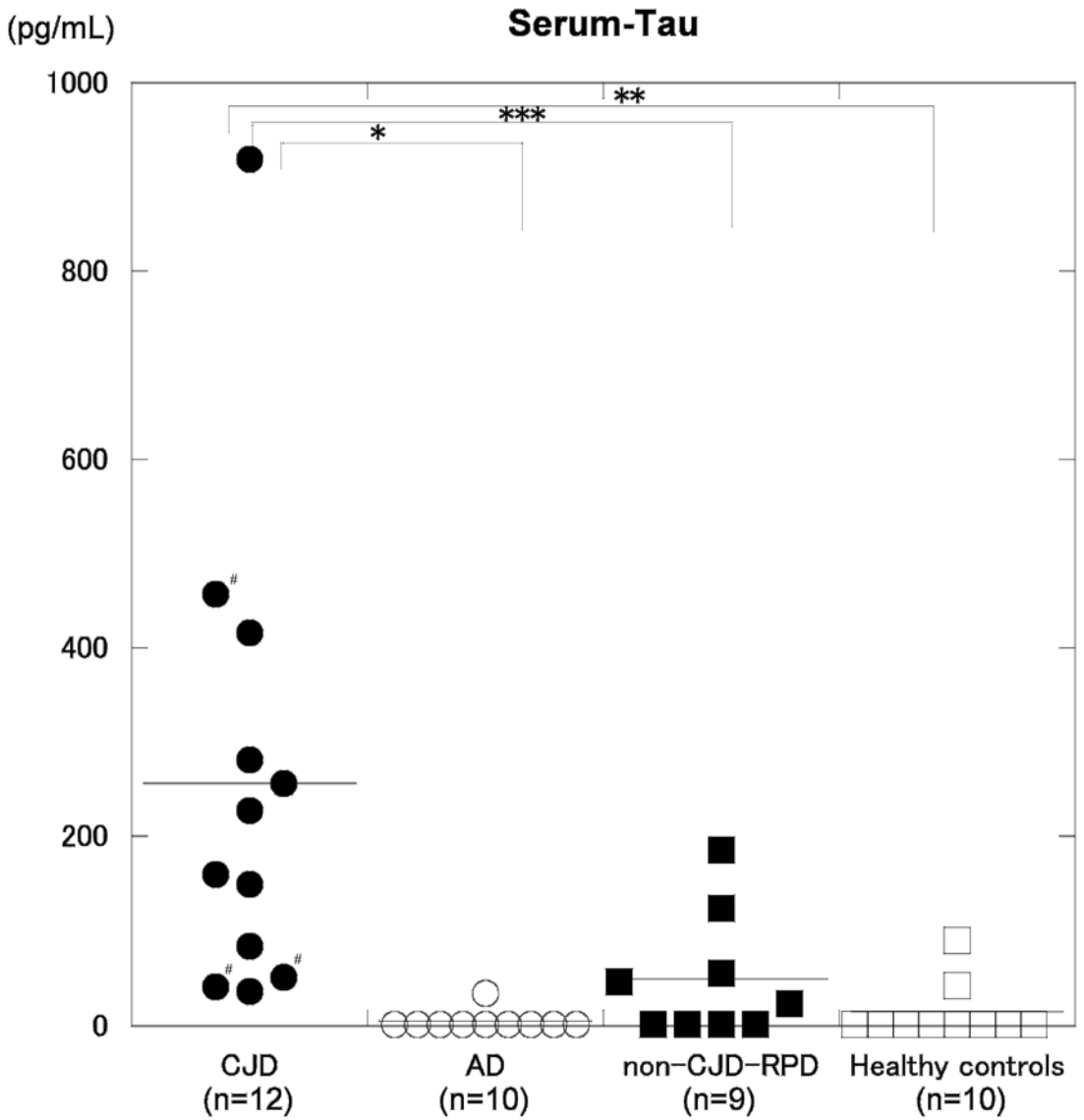
The black circles plotted with a sharp (●$^{\#}$) indicate patients with the MM2-cortical form of sporadic CJD. Values below the detection limit were set to zero. Serum-tau in CJD group patients was significantly different from that in AD group patients (*$p < 0.001$), healthy controls (**$p = 0.001$) and non-CJD-RPD group patients (***$p < 0.01$).

Fig. 2

Cerebrospinal fluid (CSF) levels of tau (CSF-tau) in sporadic CJD, AD and non-CJD-RPD groups. The black circles plotted with a sharp (●$^{\#}$) indicate patients with the MM2-cortical form of sporadic CJD. Values below the detection limit were set to zero. CSF-tau in the CJD group [median ± SE (range): 4538 ± 5599.4 (693- 69680) pg/mL] were

significantly different from those in AD [790.5 ± 174.1 (210- 1743) pg/mL; ****p < 0.05] and non-CJD-RPD groups [496 ± 250.5 (70- 2400) pg/mL; ****p < 0.05]

Fig 1.


Serum-Tau
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CJD
(n=12)
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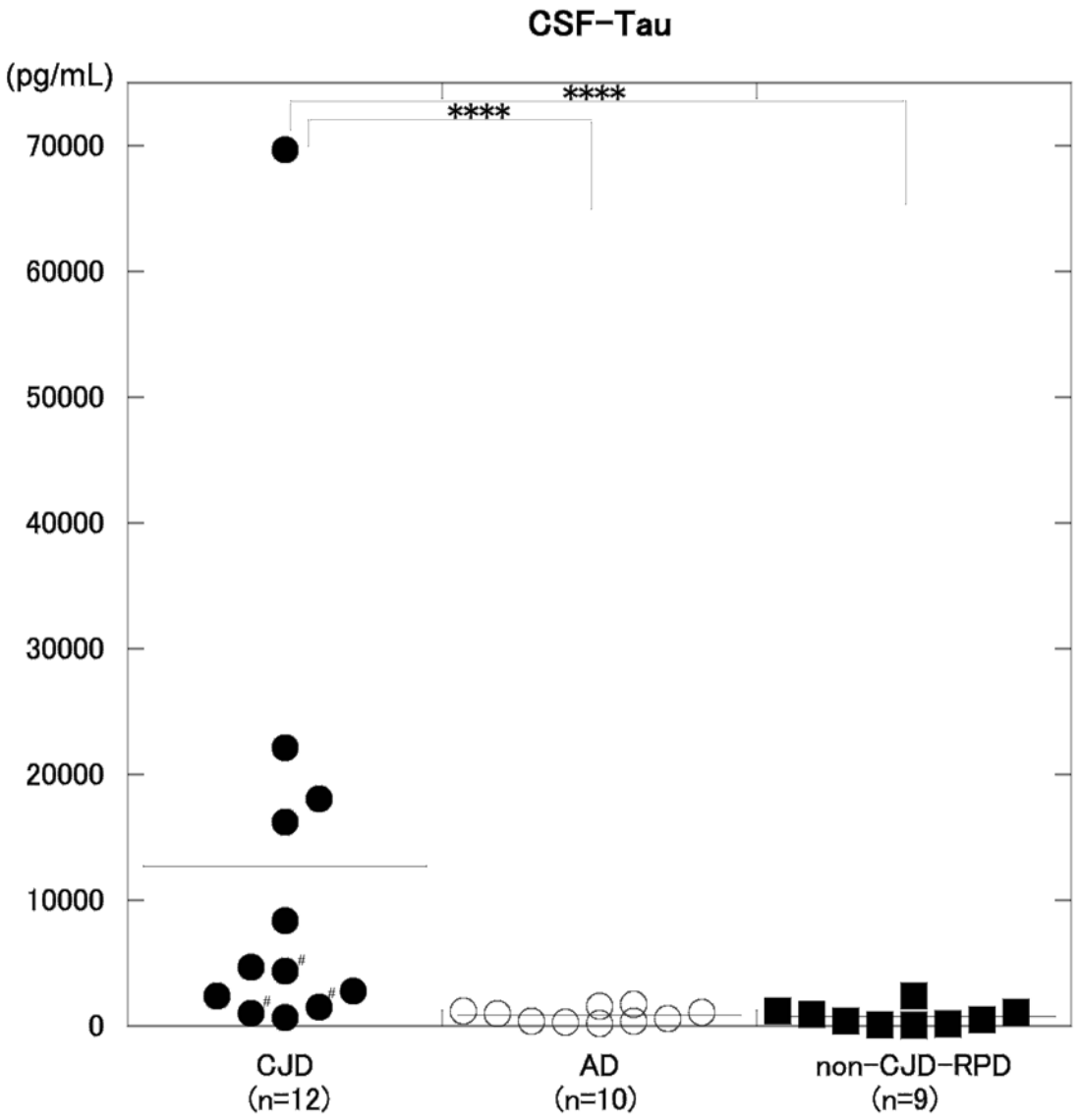
non-CJD-RPD
(n=9)
Healthy controls
(n=10)

Fig.2


CSF-Tau
(pg/mL)
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CJD
(n=12)
AD
(n=10)
non-CJD-RPD
(n=9)