Transthyretin as a potential CSF biomarker for Alzheimer’s disease and dementia with Lewy bodies: effects of treatment with cholinesterase inhibitors

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Introduction

Clinical and neuropathological criteria exist for the diagnoses of both Alzheimer’s disease (AD) and dementia with Lewy bodies (DLB), but there is an overlap of the symptoms. Biomarkers for AD and DLB are indeed essential to facilitate disease diagnosis, to monitor disease progression, and for assessment of responses to existing and future treatments. A combination of decreased levels of amyloid beta peptide (A\textsubscript{beta}), increased levels of total tau (t-tau) and phosphorylated tau (P-Tau) are increasingly used as biomarkers in CSF in AD, but no specific CSF biomarkers for DLB exist at present [1–4]. Although cognitive decline is the central characteristic of dementia, depression affects around 50% of all AD and DLB patients [5]. The underlying neurobiological mechanisms of psychiatric symptoms in DLB or AD are not fully understood. In AD, where comorbid depression has been studied to a greater extent than in DLB, it has been suggested that depression may exist as both a predisposing factor and a prodromal symptom of disease [6]. Interestingly, a meta-analysis of case–control studies found that a history of depression approximately doubles the risk of developing AD [7].

Transthyretin (TTR), a protein involved in the transport of thyroxin across the blood-brain barrier, has been found to be reduced in CSF from patients with depression as well as AD [8,9]. Also, one recent study showed that CSF TTR was further reduced in AD patients compared to DLB patients [10]. The role of TTR in the pathogenesis of AD has been hypothesized...
to be as a scaffolding protein binding to Aβ [11] and thereby possibly protecting against neurodegeneration [12] by its clearance of Aβ [13,14]. A reduction in TTR in CSF would thereby supposedly lead to increased Aβ-plaque formation, a core neuropathological characteristic of AD. In depression, the thyroid hormone transporter function of TTR has been emphasized, as reduced levels of TTR have been found in CSF from patients with major depressive disorder, which could potentially lead to lower levels of thyroid hormone in the brain [8]. The fact that CSF TTR has been found to be reduced in patients with both dementia and depression suggests that TTR may provide a potential common link between these two conditions. Furthermore, initial observations indicate that it can also be used to distinguish between AD and DLB. Therefore, the aim of this study was to evaluate whether CSF TTR indeed can be used for differentiating between AD and DLB patients with or without medication and to assess whether CSF TTR correlates to depression in these two disorders.

Methods

Subjects

In this retrospective study, clinical data and CSF samples from 85 subjects at the Memory Disorders Clinic at Rigshospitalet in Copenhagen, Denmark, the Psychogeriatric Clinic at Lund University Hospital in Lund, and the Neuropsychiatric Clinic, Sahlgrenska University Hospital, Gothenburg, Sweden were analyzed. The groups consisted of 34 women and 25 men with AD, 6 women and 7 men with DLB, and 8 women and 5 men in the control group. The 85 subjects were chosen out of a cohort originally consisting of 99 subjects to create age- and sex-matched groups. The study was approved by the medical ethics committees at the three hospitals. Patients fulfilled the consensus criteria for DLB [15] or AD (NINCDS-ADRDA) [16], respectively. Depression was diagnosed according to the ICD-10 [17], and memory function was assessed with the mini mental state examination (MMSE). Controls were recruited from senior citizen organizations and via information meetings on dementia. Inclusion criteria were that the individuals should be in good physical and mental health and not experience or exhibit any cognitive impairment. Minor vascular or other disorders in a stable phase did not lead to exclusion. All controls were thoroughly interviewed about their somatic and mental health by a research nurse before inclusion in the study.

Biochemical analyses

TTR levels in CSF were assessed using the enzyme-enhanced Mancini method [18,19]. The Mancini method is an immunochemical precipitation method that allows quantitation of antigens by single radial immunodiffusion on an agar gel labeled with the primary antibody [19]. The diffusion period of the CSF antigen was 7 days at 4°C in the present study. The enzyme enhancement was obtained with the horse radish peroxidase (HRP)-conjugated secondary antibody using carbazole as the chromogen [18]. The primary anti-TTR antibody (made in rabbit) was used at 1:800, and the secondary swine anti-rabbit antibody was used at 1:100 (both from Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Statistical analysis

The Statistical Package for the Social Sciences (spss; SPSS Inc., Chicago, IL, USA) program version 15.00 for Windows was used for all statistical analyses. For multiple comparisons, one-way Analyses of Variance (ANOVA) were used. For comparisons between two groups, Student’s t-test was used. Tests of non-parametric correlations were performed using Spearman’s rho.

Results

There were no significant differences in age between the three groups of controls, patients with AD or DLB (Table 1). MMSE scores were significantly reduced in the groups with DLB and AD compared to controls (Scheffe’s post hoc test P < 0.001; Table 1). We did not

<table>
<thead>
<tr>
<th>Table 1 Demographic characteristics</th>
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<tbody>
<tr>
<td><strong>Controls</strong></td>
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<tr>
<td>---</td>
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<tr>
<td>Gender (female/male)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>TTR (mg/l)</td>
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<tr>
<td>MMSE (points)</td>
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</table>

Data stated as mean ± standard deviation.

AD, Alzheimer’s disease; DLB, dementia with Lewy bodies; TTR, transthyretin, MMSE, mini mental state examination.
detect any significant differences in CSF TTR between AD and DLB patients or between these two disorders and controls (Fig. 1a; Table 1). Also, no significant difference in CSF TTR could be seen between women and men or between depressed and non-depressed patients, neither in the diagnostic groups nor in the cohort as a whole. There were also no significant correlations between TTR and MMSE in the different groups (AD: Spearman’s rho = 0.06, P = n.s.; DLB: Spearman’s rho = 0.23, P = n.s.; Controls: Spearman’s rho = 0.11, P = n.s.) or in the whole cohort (Spearman’s rho = 0.033, P = n.s.).

However, in the AD patient group, there was a significant reduction (14%; P < 0.001) in CSF TTR in patients medicated with cholinesterase inhibitors (n = 22) compared to those who were not (n = 37) (Fig. 1b). In the DLB group, a trend to reduction in TTR in the cholinesterase inhibitor-treated group (n = 7) compared to the non-cholinesterase inhibitor-treated group (n = 6) was found (16%; P = 0.098).

**Discussion**

In our study of clinically well-defined and age-matched AD and DLB patients, we found reductions in CSF TTR in the two patient groups with ongoing treatment with cholinesterase inhibitors. Although a protective role of TTR in Aβ-pathology has been suggested from studies performed in animal models [11,12,20], a recent study showed that TTR accelerates vascular Aβ deposition in a mouse model of AD [21], which would be in line with our finding of reduced TTR levels after treatment with cholinesterase inhibitors. We could not detect any significant differences in CSF TTR levels between AD and DLB patients, or compared to control subjects. However, conflicting results on CSF TTR in dementia exist in the literature. Although the majority of the reports have presented results of decreased levels of TTR in AD, one study has shown increased levels of CSF TTR (Table 2). Studies on CSF TTR in DLB patients have also shown different results. Significantly increased levels of CSF TTR in DLB patients in comparison with controls have been shown by Abdi et al. [22], but no significant differences in CSF TTR between DLB patients and controls have been reported by Gloeckner et al. [10] (Table 3). Taken together, CSF TTR does not appear to be a robust biomarker for differentiating AD from DLB and controls in all cohorts.

We found no differences in CSF TTR between dementia patients with or without depression. It has previously been shown that CSF TTR levels are reduced in depressed patients compared to controls [8,23,24] (Table 4). Therefore, it was postulated that the low levels of TTR resulted in reduced levels of thyroid hormones being distributed throughout the body.
Acknowledgements

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References


Table 3 Studies on CSF TTR in DLB

<table>
<thead>
<tr>
<th>Author/reference</th>
<th>Method</th>
<th>Subjects</th>
<th>TTR change</th>
<th>Change in percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schultz et al., this study</td>
<td>Enzyme-enhanced Mancini</td>
<td>13 DLB, 13 controls</td>
<td>→</td>
<td>No significant change</td>
</tr>
<tr>
<td>Gloeckner et al. [10]</td>
<td>Nephelometry using N-antiserum against human prealbumin</td>
<td>23 DLB, 19 controls</td>
<td>→</td>
<td>No significant change</td>
</tr>
<tr>
<td>Abdi et al. [22]</td>
<td>Quantitative proteomic iTRAQ</td>
<td>5 DLB, 10 controls</td>
<td>↑</td>
<td>&gt; 50%</td>
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</tbody>
</table>

Table 4 Studies on CSF TTR in depression

<table>
<thead>
<tr>
<th>Author/reference</th>
<th>Method</th>
<th>Subjects</th>
<th>TTR change</th>
<th>Change in percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schultz et al., this study</td>
<td>Enzyme-enhanced Mancini</td>
<td>59 AD, 13 DLB, 13 controls</td>
<td>→</td>
<td>No significant change</td>
</tr>
<tr>
<td>Schultz et al. [25]</td>
<td>Enzyme-enhanced Mancini</td>
<td>108 suicide attempters/15 controls</td>
<td>→</td>
<td>No significant change</td>
</tr>
<tr>
<td>Sullivan et al. [24]</td>
<td>Radio immunoassay</td>
<td>17 MDD, 15 controls</td>
<td>↓</td>
<td>10%</td>
</tr>
<tr>
<td>Sullivan et al. [23]</td>
<td>Dot-blot immunoassay</td>
<td>18 MDE /1 BPD, 24 controls</td>
<td>↓</td>
<td>39%</td>
</tr>
<tr>
<td>Hatterer et al. [6]</td>
<td>Rocket immunoelectro-phoretic procedure</td>
<td>8 MDD, 9 neurologic patients</td>
<td>↓</td>
<td>28%</td>
</tr>
</tbody>
</table>

TTR, transthyretin; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies.