Total and phospho-tau (Thr 181) CSF levels in patients with mild cognitive impairment and Alzheimer’s disease

Poziom Tau całkowitego i tau fosfo (Thr 181) w płynie mózgowo-rdzeniowym u pacjentów z zespołem lekkich zaburzeń poznawczych oraz z otępieniem typu Alzheimera

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Summary

Background: Recent studies have found significantly increased CSF tau levels in patients with Alzheimer’s disease (AD), which suggests that this protein could be a molecular marker of AD. Since early diagnosis of AD can improve therapeutic interventions, both total tau and phosphorylated tau (phospho-tau) should be investigated in patients with mild cognitive impairment (MCI) and AD.

Material and methods: CSF total tau and tau protein phosphorylated at threonine 181 concentrations were determined by ELISA in 132 AD patients, 29 patients with MCI, and 24 controls. Total tau and phospho-tau levels were compared between mild, moderate, and severe AD, MCI, and controls, and were correlated with age and severity of dementia.

Results: CSF total tau levels were significantly higher in patients with severe and moderate AD than in the remaining study groups. In mild AD and MCI, total tau levels differed significantly from both severe-to-moderate AD and controls. CSF phospho-tau levels were significantly higher in severe AD than in the other groups. Phospho-tau levels were significantly higher in moderate and mild AD compared to controls, but not in MCI. In AD, total tau and phospho-tau levels correlated significantly with severity of dementia, but not with age.

Conclusions: Both CSF total and phospho-tau protein concentrations are important in the diagnosis of AD and MCI. However, there is a large overlap between patients and controls, which warrants
Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder associated with neurofibrillary tangle formation as one major neuropathological hallmark. The microtubuli associated tau protein is released into the cerebrospinal fluid (CSF) during neurofibrillary tangle aggregation. With an apparent molecular mass of 46kDa it is measurable by ELISA in the CSF. Several studies found significantly increased CSF tau levels in patients with AD in comparison to controls indicating the potential relevance of this protein as a molecular marker of AD (for review see [1]). In most but not all studies, this finding also applied for the comparison with patients with vascular dementia (VaD) [2,3,4,5,6,7,1].

Since early diagnosis of AD is warranted to enable early therapeutic interventions CSF tau protein levels were investigated in preclinical conditions of AD such as mild cognitive impairment (MCI). Compared to controls, CSF total tau protein levels were significantly increased in patients with MCI or incipient AD (for review see [8]).

Most recently, phosphorylated tau protein (phospho-tau) was discovered as potential biomarker of AD. CSF phospho-tau levels refer to the phosphorylation state of the protein which is supposed to be decisive for neurofibrillary tangle formation in AD. Thus, CSF phospho-tau levels might represent a direct indicator of ongoing AD pathology. This hypothesis was emphasized by the finding of almost no change of CSF phospho-tau concentration following acute stroke [9] or in Creutzfeldt-Jakob disease [10] whereas total tau shows a marked increase in these conditions. In recent studies, CSF phospho-tau levels were found to be significantly increased in patients with AD compared to controls [2,11,12,13,14,15,16,17,18,19,8]. Concerning CSF phospho-tau levels in MCI patients only few studies were conducted revealing significantly increased CSF concentrations compared to controls [20,21,22,23,24]. However, in these studies only a subset of tau protein species phosphorylated at different epitopes (e.g. threonine 231, serine 235) has been investigated.

Studies investigating both CSF total and phospho-tau, and including AD patients with different dementia severity as well as patients with MCI and healthy controls are rare although they may provide further inside in the clinical relevance of these biomarkers. Therefore, in the present study we investigated both CSF total and phospho-tau protein levels in mild, moderate, and severe AD compared to patients with MCI and controls.

Methods

One hundred thirty two (88 females and 44 males) patients with AD (NINCDS-ADRDA criteria [25]) and twenty nine (15 females and 14 males) patients with MCI according to Petersen et al. [26] were included in the study. According to the Mini Mental State Examination score (MMSE [27]) AD patients were classified as mild AD (MMSE 21 to 25), moderate AD (MMSE 13 to 20), or severe AD (MMSE ≤12). Participants were consecutively admitted patients of the Section for Geriatric Psychiatry, University of Heidelberg. Patients were excluded if they had evidence of stroke or other neurological disorders, severe uncontrolled diabetes or uncontrolled hypertension. The Hachinski ischemic score modified by Loeb and Gandolfo [28] of the AD and the MCI patients was less than three. Clinical diagnosis was based on all relevant information including history, clinical examination, blood parameters (full blood count, blood chemistry, erythrocyte sedimentation rate, thyroid function tests), neuropsychological, and neuroradiological findings. The control group consisted of 24 individuals (13 females and 11 males) without cognitive impairment or psychiatric diseases from whom CSF samples were obtained during spinal anaesthesia at the Department of Anaesthesia, University Hospital Heidelberg.

In all patients, lumbar puncture was performed at a fixed time of the day, between 10 and 12 am, as part of the routine diagnostic procedure to exclude inflammatory disease. The resultant CSF samples were immediately aliquoted into non-adsorbent tubes, frozen at –80°C, and stored in polypropylene...
tubes until examination. Total tau and tau phosphorylated at threonine 181 levels were determined using the Innogenetics INNOTEST-hTau-Ag-kit [29] and the INNOTEST Phospho-Tau Thr181 kit [30], respectively. Investigators were blinded to diagnosis prior to the measurement of total tau and phospho-tau concentration.

For data analysis, Pearson correlation coefficients, and analyses of variance with Duncan’s test were calculated. The study was approved by the ethics committee of the University of Heidelberg.

Results

Means and standard deviations of the clinical variables are presented in table 1. According to the results of a Duncan’s test at the 5% level, the AD patients were significantly older than the controls. AD patients and MCI patients did not differ significantly with respect to age. Similarly, there was no significant age difference between mild, moderate, and severe AD patients. Between MCI patients and controls no significant age difference arose. As expected, MMSE scores were significantly lower in AD and MCI patients than in controls and differed significantly between all diagnostic groups (mild AD, moderate AD, severe AD, MCI, controls).

Table 1: Means ± standard deviations of clinical characteristics in patients and controls with the results of a Duncan’s test at the 5% level

<table>
<thead>
<tr>
<th></th>
<th>Mild AD</th>
<th>Moderate AD</th>
<th>Severe AD</th>
<th>MCI</th>
<th>Controls</th>
<th>Duncan-Test (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>52</td>
<td>54</td>
<td>26</td>
<td>29</td>
<td>24</td>
<td>g2,g3,g1&gt;g5</td>
</tr>
<tr>
<td>age (years)</td>
<td>72.9 ±9.7</td>
<td>74.8 ±10.3</td>
<td>73.8 ±8.3</td>
<td>70.0 ±8.2</td>
<td>65.9 ±7.6</td>
<td>g2,g3,g1&gt;g5</td>
</tr>
<tr>
<td>MMSE score</td>
<td>23.2 ±1.3</td>
<td>16.1 ±2.4</td>
<td>8.6 ±2.9</td>
<td>27.1 ±1.2</td>
<td>29.3 ±0.8</td>
<td>g5&gt;g4&gt;g1&gt;g2&gt;g3</td>
</tr>
<tr>
<td>Total tau (pg/ml)*</td>
<td>506.9 ±257.3</td>
<td>569.3 ±292.0</td>
<td>624.4 ±309.0</td>
<td>412.6 ±217.4</td>
<td>240.0 ±100.0</td>
<td>g3,g2&gt;g1,g4&gt;g5</td>
</tr>
<tr>
<td>Phospho tau (pg/ml)</td>
<td>73.8 ±27.1</td>
<td>77.8 ±31.0</td>
<td>106.0 ±32.9</td>
<td>64.2 ±23.6</td>
<td>49.5 ±10.4</td>
<td>g3&gt;g2,g1,g4,g5</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>g2,g1&gt;g5</td>
</tr>
</tbody>
</table>

*g1: n=21  g2: n=32  g3: n=9  g4: n=17  g5: n=19
AD=Alzheimer’s disease, MCI=mild cognitive impairment, MMSE=MiniMental State Examination

CSF total tau protein concentrations were highest in the severe AD patients and differed significantly from those obtained in controls, MCI, and mild AD but not moderate AD. In mild AD and MCI, total tau levels were significantly higher than in the controls but significantly lower than in moderate and severe AD (Fig. 1).

CSF phospho-tau levels were highest in severe AD and differed significantly from the other diagnostic groups. Phospho-tau levels were higher in moderate AD than in mild AD but this difference did not reach statistical significance. Both groups (moderate and severe AD) differed significantly from controls with respect to phospho-tau levels. Phospho-tau levels in MCI patients were in between moderate and severe AD, and controls although these differences were not significant (Fig. 2).

In the AD patients, no significant correlations between total-tau or phospho-tau levels, and age arose. Severity of dementia (MMSE scores) was significantly correlated with total tau (r=-0.3 p<0.0005), and phospho-tau (r=-0.4 p<0.0005) levels. In the AD patients, the correlation between CSF total tau and phospho-tau levels was highly significant (r=0.9 p<0.0005). Age, severity of dementia, CSF total tau, and phospho-tau levels showed only minor, non-significant differences between patients with probable and possible AD.
**Fig. 1:** Tau levels (pg/ml) in patients with mild, moderate, and severe AD, MCI, and controls. Results of a Duncan’s test at the 5% level: severe AD, moderate AD > mild AD, MCI > controls

**Fig. 2:** Phospho-tau levels (pg/ml) in patients with mild, moderate, and severe AD, MCI, and controls. Results of a Duncan’s test at the 5% level: severe AD > mild AD, moderate AD, MCI, controls; mild AD, moderate AD > controls

**AD**-Alzheimer’s disease, **MCI**-mild cognitive impairment
Discussion

Findings of our study demonstrate: (1) CSF total tau levels are significantly elevated in patients with severe and moderate AD, differing significantly from patients with mild AD, patients with MCI, and controls. In mild AD and MCI, total tau levels are between severe and moderate AD, and controls, respectively, differing significantly from both. (2) CSF phospho-tau levels are significantly elevated in severe AD compared to moderate and mild AD, MCI, and controls. Phospho-tau levels are significantly higher in moderate and mild AD compared to controls but not between MCI and these groups. (3) In AD, total tau and phospho-tau levels are significantly correlated with severity of dementia but not with age.

Our results confirm previous studies demonstrating increased total tau levels in AD compared to controls without cognitive deficits (for review see [31]). Moreover, we could demonstrate a continuing increase of total tau levels from MCI versus mild and moderate AD to severe AD indicating an increasing release of total tau into the CSF with disease progression. From a clinical point of view, it seems to be important to emphasize the similarity of CSF total tau levels in mild AD and MCI which were significantly increased compared to controls but still significantly lower than in moderate and severe AD. This finding reflects and confirms the clinical judgement of an incipient neurodegenerative disorder in a considerable portion of MCI patients.

However, MCI includes a variety of conditions which might not join a common pathological pathway. Corresponding to this, we found a different distribution of the CSF phospho-tau levels among the diagnostic groups. Again, the highest phospho-tau levels were found in severe AD which differed significantly from the other patients and the controls. However, phospho-tau levels in MCI did not differ significantly neither from controls nor from mild and moderate AD. This finding could be explained by the assumption that MCI indeed comprises neuropathologically heterogeneous conditions whereas CSF phospho-tau levels rather indicate an AD specific neurodegenerative process.

Hitherto, only a few studies investigated CSF phospho-tau levels in MCI patients. The majorities of these studies found significantly increased CSF levels of tau protein phosphorylated at threonine 231 compared to controls. In our study, similar results were obtained for CSF levels of tau protein phosphorylated at threonine 181 indicating the usefulness of phospho-tau levels irrespective of phosphorylation site in the differentiation of MCI from healthy controls.

In addition, tau levels in the AD group showed a high variability: In a recent study, we could demonstrate that patients with tau levels below the 25%-percentile of the distribution, who were characterized by a high percentage of severely demented patients, showed tau levels similar to those measured in controls [1]. This result can not be explained by the limited accuracy of clinical diagnosis alone. Heterogeneity of AD may provide an alternative explanation. Indeed, the group of AD patients without elevated tau levels was characterized by a significantly higher percentage of patients with presenile onset.

In the AD group, a significant correlation between both total tau levels and phospho-tau levels, and severity of disease arose. In contrast, in a 6-year follow-up study of AD, CSF tau protein levels phosphorylated at threonine 231 declined during the course of AD [32]. In future studies, the baseline levels of CSF tau protein concentration should to be taken into account to clarify this discrepancy since the mechanisms of tau protein release and sequestration remain uncertain. The findings of our study apply at least for a subgroup of AD patients which might be characterized by total and phospho-tau levels which are increasing with disease severity.

Although all studies on CSF tau protein levels in AD agree with increased tau levels in AD compared to healthy controls, potential confounding factors have to be discussed. Since neuropsychiatric symptoms, such as depressive symptoms and agititation, are frequent in AD psychotropic medication is often described in these patients. Therefore, one has to take into account potential psychotropic medication effects on tau protein release. In the past, medication effects were mainly investigated...
for beta amyloid 1-40 and 1-42 levels [33]. However, in a recent study we could exclude a correlation of CSF total tau levels, and dosage and type of psychotropic medication in patients with AD [1].

While the present study focused on the differentiation between normal ageing and different stages of AD it seems also pertinent to ask for the feasibility to separate AD from other dementias. In a recent study, we therefore investigated the relevance of CSF tau levels for the differential diagnosis of AD and VaD. In accordance with some (for review see [5]) but not all previous studies ([34,35,36]) we found significantly elevated tau levels in AD compared to VaD [1]. Discrepancies between these studies may be explained by elevated tau levels in some of the investigated VaD patients following an acute damage to cerebral tissue induced by an ischemic event. The temporal relationship between an ischemic episode and the CSF examination could thus explain part of the variance of tau levels observed in VaD patients. This assumption is in line with the finding by Arai et al. [5] who observed tau levels in patients with acute cerebral infarction to be increased 2-3 weeks after the event, but to normalize several months later. Hesse et al. [9] reported a marked increase of tau levels in patients with acute ischemic stroke, peaking at three weeks and returning to normal after three months. Furthermore, the divergent results of studies comparing tau levels between AD and VaD may refer to the presence of a concomitant degenerative pathology rather than pure vascular changes in a considerable portion of patients diagnosed with VaD since several neuropathological studies have shown that a high proportion of clinically diagnosed VaD patients have notable concomitant AD pathology [37,38].

Furthermore, other putative markers of AD, such as atrophic cerebral changes, might parallel CSF total and phospho-tau protein concentration. Recently, CSF tau protein levels were expected to correlate with measures of hippocampal atrophy which is generally considered as a surrogate marker for AD related brain pathology [39,40,41]. In contrast to the finding of a significant correlation between β-amyloid 1-42 CSF levels and cerebral volume changes as revealed by magnetic resonance imaging [33] we did not find any correlations between measures of cerebral atrophy and CSF tau protein levels in a recent study [1].

In conclusion, our results underline the importance of CSF total and phospho-tau protein concentrations in the diagnosis of AD and MCI. However, a large overlap between patients and controls demonstrates the potential heterogeneity of the diagnostic entities and warrants further investigations. From a clinical point of view, one may conclude that increased tau levels confirm the clinical diagnosis of AD while normal values do not exclude this disease.

References


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