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Anomalously phosphorylated tau and Aβ fragments in the CSF correlates with cognitive impairment in MCI subjects

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of extracellular amyloid deposits, consisting largely of Aβ peptide and the presence of intraneuronal aggregates of neurofibillary tangles formed by tau. Development of cerebrospinal fluid (CSF) biomarkers has become a rapidly growing research field, considering the need for diagnostic tools for AD, thus allowing therapeutic compounds to have the greatest potential for being effective. We have focused on the relationships between critical biomarkers such as tau and Aβ in the CSF and the cognitive impairment of patients, as assessed by a battery of neuropsychological tests derived from CDR and CERAD, of value in the evaluation of AD patients. As part of a longitudinal study, we analyzed by ELISA and Western blots the levels and molecular patterns of hyperphosphorylated tau in the CSF of three different groups of patients: AD patients between 69- and 73-years-old, a group characterized with mild cognitive impairment (MCI) between 65- and 70-years-old, and a non-demented neurological control group of comparable ages. The levels of AT8-reactive phosphorylated tau were significantly higher ($P < 0.05$) in AD patients (0.604 ± 0.078, $n = 23$) as compared with the control group (0.457 ± 0.086, $n = 25$). No differences between the levels of AT8-reactive tau of MCI patients (0.510 ± 0.090, $n = 45$) and controls were observed. However, when the MCI group was divided on the basis of the total box score (TBS) from CDR, those subjects with a TBS < 1.5 presented tau levels (0.456 ± 0.032, $n = 31$) similar to controls, whereas those patients with TBS ≥ 1.5 displayed tau levels (0.590 ± 0.086, $n = 14$) comparable with those of AD. Western blot analyses revealed a higher AT8 reactivity in CSF samples of AD patients as compared with MCI and control samples, indicating higher levels of AD tau phosphoepitopes in the CSF. Tau heterogeneity was observed in samples of AD and MCI with higher impairment as compared with controls. As expected from previous reports, levels of Aβ (1–42) were lower (0.052 ± 0.005) than controls (0.070 ± 0.010), whereas the levels of MCI group were 0.060 ± 0.007. The MCI group with a TBS ≥ 1.5 presented Aβ levels of 0.053 ± 0.005 similar to those of AD patients, whereas the MCI group with TBS < 1.5 exhibited Aβ levels (0.066 ± 0.007) similar to controls. Studies highlight the relationships between anomalously phosphorylated tau markers in CSF with the information from TBS analysis of the different groups of patients.

Keywords: Amyloid; Tau protein; apoE Alleles; Cerebrospinal fluid; Mild cognitive disorders; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is the commonest cause of dementia and a major public health problem that may reach epidemic proportions if no cure is found within the next decade [24]. The neuropathological features of AD are a gradual and widespread neuronal loss, the extraneuronal β-amyloid deposit formation or senile plaques (SP), alterations in cerebral

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blood vessels, and the presence of neurofibrillary tangles (NFTs). SPs are mainly made up of β-amyloid, especially the Aβ (1-42) variant. The major constituent of NFTs is the microtubule-associated protein tau, which is hyperphosphorylated in AD brains [29,30].

The diagnosis of AD is based on clinical and neuropsychological examination, identification of symptoms of AD and exclusion of other known causes of dementia [18,31,41,44], as outlined by the NINCDS-ADRDA Work Group, and the *Diagnostic and Statistical Manual* of the American Psychiatric Association. AD is characterized by a progressive decline of cognitive functions, memory, language and visuospatial orientation. Neuropsychiatric symptoms such as depression and behavioral changes are common. However, diagnosis based on these instruments is unsatisfactory, indicating the need of highly sensitive and reliable approaches, selective for AD and based on biological markers [7–9]. Ideally, such markers should reflect the pathophysiological mechanisms of AD, which according to the current hypotheses, derive from the actions of two major protein aggregates: SP and NFTs [12]. There is evidence that the CSF levels of Aβ (1–42) are significantly reduced in AD patients as compared with senile controls, while increased levels of tau have been revealed [25,32,33]. The CSF levels of these proteins reflects their metabolism in the central nervous system [14]. ELISA and immunochemical methods for quantification of these markers have been used [19,42]. A number of studies suggest that CSF markers in combination with neuroimaging and neuropsychological tools, adds to the accuracy of AD diagnosis [18]. A recent study about the correlation of CSF biomarkers and the neuropathological diagnosis, confirmed an association between elevated CSF tau levels pre-mortem and the pathological hallmarks of AD, suggesting the value of anomalously high CSF tau for AD diagnosis [12]. Besides AD [17,20,22,25,26], CSF biomarkers have also been analyzed in dementia with Lewy bodies [16] and different groups with MCI [37].

The search of methods for AD detection in the pre-clinical phase could provide a hope for its prevention and treatment [11]. Early diagnosis would allow treatment with agents that delay cognitive deterioration in the initial phase of the disease, thus slowing its progression. The use of biomarkers in the pre-clinical phase operate as instruments of early diagnosis and differentiate AD from other similar pathologies [8,10]. This is applicable to patients with MCI without a differential diagnosis. In recent years a variety of syndromes have been proposed to characterize subjects with cognitive decline without dementia. Among them MCI has gained increasing attention [36,37,43], and refers to a transitional state between the cognition of normal aging and mild dementia, characterized by memory impairment, or a mild decline in some abilities [13]. The concept of MCI is one of the clinical entities proposed to characterize a heterogeneous group of individuals cognitively impaired but not demented. The causes of MCI are not yet clearly understood. No genetic link has yet been found for MCI, although that like AD, a genetic component in addition to sporadic components, might be a risk factor for people with MCI to develop AD [37,38,43]. Thus, the study of the potential markers in the cerebrospinal fluid (CSF) constitutes an important source of information in different neurological disorders. In these processes, brain pathological and structurally altered proteins among other normal components are released from the central nervous system toward the CSF, an appropriate target for the analysis of markers in AD [3,4].

We focused on the relationships between CSF biomarkers and the levels of cognitive impairment of three human subpopulations, by evaluating the relationships between the levels of hyperphosphorylated tau and the Aβ (1–42) peptide, and data from neuropsychological tools derived from CDR and CERAD, of proven diagnostic efficiency for AD. This research is part of a longitudinal study derived from the screening of over 600 patients, and in which a group of 93 patients was evaluated with the entire battery of neuropsychological tests and biological assays.

2. Methods and subjects for the study

2.1. Sampling

The subjects in the study consisted of 93 elderly patients, evaluated in the *Hospital El Salvador*, from the general population resident in the eastern metropolitan area of Santiago, Chile, who fulfilled the inclusion criteria of the study. Subjects were divided into three different groups: 23 patients with probable AD, 45 subjects with mild cognitive impairment (MCI) as defined by Petersen et al. [36,37], and 25 non-demented neurological controls. Participants were recruited through the printed media and underwent a multistage screening procedure. To be included in the study, participants needed to be more than 60 years old, to be free of significant medical illness, and to be willing to participate in the study. The study and the experimental protocols were approved by the Committee on Ethical Issues of the Faculty of Medicine, University of Chile, and all subjects provided informed consent prior to the initiation of the study. In the cases of demented subjects, in agreement with the guidelines of this Committee, the informed consent for participation in the study was obtained from their caregivers. The clinical diagnosis of AD was made according with the criteria for AD as outlined by the National Institute of Neurologic, Communicative Disorders and Stroke, AD and Related Disorders Association (NINCDS-ADRDA) Work Group [31].

2.2. Application of the semi-structured interview

The CDR ratings were obtained using a semi-structured interview specially adapted by Daly et al. [13], from the validated original version of Hughes et al. [21]. This interview was specially adapted to be used with a population with very mild impairments. It includes a standardized medical,