Journal of Clinical Neuroscience xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical commentary

Cerebrospinal fluid biomarkers of Alzheimer's disease are associated with carotid plaque score and hemodynamics in intra- and extra-cranial arteries on ultrasonography

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ARTICLE INFO

Article history:
Received 21 June 2017
Accepted 4 December 2017
Available online xxxx

Keywords:
Alzheimer's disease
Amyloid β
Phosphorylated tau
Ultrasonography
Plaque score
Mean flow velocity

ABSTRACT

Carotid plaque score (PS) and hemodynamic abnormalities in intra- and extra-cranial arteries are related to Alzheimer's disease (AD) progression. As these parameters are measured conveniently and non-invasively by ultrasonography, we examined their association with cerebral spinal fluid (CSF) AD biomarkers amyloid β (Aβ) and phosphorylated tau (p-tau). Carotid PS, mean flow velocity (MFV) in multiple intra- and extra-cranial arteries, CSF Aβ42 and p-tau, neurocognitive function (assessed by the Mini-Mental State Examination and Alzheimer's Disease Assessment Scale-cognitive subscale, Japanese version), and blood lipids (total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride) were measured in AD patients (n = 42), mild cognitive impairment patients (n = 20), and cognitively normal controls (n = 18). The results were also compared among groups defined by PS range. After adjusting for blood lipids as covariates, Aβ42 was higher in the PS = 1.1−2.0 mm group than in the higher PS groups (2.1−3.0, 3.1−5.0, 5.1−7.0, and >7.0 mm). However, subjects with very low PS (<1.1 mm) also had a low mean CSF Aβ42. Alternatively, CSF p-tau181 did not differ between PS groups. In multiple regression analysis, Aβ42 was not associated with MFVs; however, CSF p-tau181 showed a significant association with the MFV of the internal carotid and basilar arteries. Findings suggest that carotid plaque formation may accelerate Aβ42 deposition, although it is not necessary for deposition. Hemodynamics abnormalities may cause increased CSF p-tau181. Ultrasonographic evaluation of PS and arterial hemodynamics may be a useful noninvasive method for estimating AD pathology.

1. Introduction

The two defining pathologies of Alzheimer's disease (AD) are the senile plaques formed by extracellular accumulation of amyloid β (Aβ) protein and the intracellular neurofibrillary tangles formed by the deposition of phosphorylated tau (p-tau) [1]. A decreased Aβ42 and an elevated p-tau in the cerebrospinal fluid (CSF) are used as biological markers from AD with high diagnostic reliability [2].

AD progression is associated with abnormalities in cerebral hemodynamics. These intracranial hemodynamics changes are generally measured by brain perfusion single-photon emission computed tomography (SPECT), positron emission tomography (PET), or perfusion MRI. In addition, transcranial Doppler (TCD) ultrasound is useful as a noninvasive and less expensive method for the evaluation of cerebral blood flow. Decreased middle cerebral artery (MCA) and basilar artery (BA) mean flow velocities (MFVs) as well as reduced cerebrovascular reactivity (CVR) have been reported in AD [3–5]. Furthermore, it was reported that pathological Breath-Holding Index, an indicator of CVR, is associated with an increased risk of conversion from mild cognitive impairment (MCI) to AD [6].

One of the main causes of cerebral hemodynamic anomalies in AD is circle of Willis atherosclerosis. AD patients showed more severe stenosis of circle of Willis arteries at autopsy than the nondemented control subjects [7]. Moreover, the frequency of circle of Willis atherosclerosis was related to the severity of AD pathology, such as senile plaques and neurofibrillary tangles [8]. Also, carotid atherosclerosis was associated with an increased risk for dementia and mortality [9]. Hence, evaluation of atherosclerosis and cerebral hemodynamics is important for the prediction of AD progression.

While there is strong evidence linking abnormal cerebral blood flow and atherosclerosis with AD pathology, it is unclear how

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https://doi.org/10.1016/j.jocn.2017.12.006
0967-5868/

ultrasonographic hemodynamic measures are related to levels of the AD pathology-related proteins Aβ and p-tau in CSF. The aim of this study was to investigate the association of carotid plaques and aberrant intra- and extra-cranial artery hemodynamics as measured by ultrasonography with CSF Aβ and p-tau. Such associations may allow for the estimation of AD pathophysiology noninvasively and conveniently by ultrasound.

2. Methods

2.1. Subjects

This study included patients with AD (n = 42) and MCI (n = 20) as well as cognitively normal control (NC) subjects (n = 18). AD diagnosis was performed according to criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [10], the National Institute of Neurological and Communicative Disorders and Stroke, and the AD and Related Disorders Association [11]. Mild cognitive impairment was diagnosed according to Petersen's criteria [12]. All patients with MCI in this study had the amnestic form.

Subjects were excluded from the study if they showed large territorial infarcts or multiple lacunar infarcts, were younger than 65 years of age, or had a history of diseases (other than AD or MCI) that may cause cognitive disorders. The study design was approved by the ethics committee of Tottori University.

2.2. Cognitive function tests

Cognitive function was assessed using the Mini-Mental State Examination (MMSE) [13] and the Alzheimer's Disease Assessment Scale-cognitive subscale, Japanese version (ADAS-J cog) [14]. The MMSE is a general screening test for cognitive impairment, with lower scores (out of 30) indicative of cognitive impairment. The ADAS-J cog is used to evaluate the degree of AD progression, with higher scores indicative of more severe symptoms.

2.3. CSF and blood tests

Cerebrospinal fluid samples were collected by lumbar puncture and immediately stored in polypropylene containers at −80 °C until analysis. The Aβ42 and p-tau181 levels in CSF were measured by sandwich enzyme-linked immunosorbent assays (Human Amyloid β 1–42 Assay kit; Immuno-Biological, Gunma, Japan and INNOTEST PHOSPHO-TAU (181p); Innogenetics, Ghent, Belgium). Fasting venous blood samples were collected and serum was obtained by centrifugation for the measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides.

2.4. Ultrasonography

Carotid plaques and MFVs in intra- and extra-cranial arteries were evaluated using an ultrasonic diagnostic system (UF-760AG; Fukuda Denshi Co., Ltd., Tokyo, Japan). Carotid plaques were measured in B-mode using a 7.5-MHz transducer. Plaques were defined by intima-media thickness (IMT) greater than 1.1 mm. The IMT measurements were performed from the near and far walls along four segments of the bilateral carotid arteries, common carotid artery (CCA) proximal to starting point, CCA proximal to carotid bifurcation, carotid bifurcation and internal carotid artery (ICA) [15]. The plaque score (PS) was calculated by summing maximum plaque thicknesses for the eight segments.

MFVs in the CCA, ICA, and vertebral artery (VA) were measured using a 7.5-MHz transducer, while MCA and BA MFVs were measured with a 2-MHz transducter. The transtemporal window was used to obtain MFV measures from bilateral MCAs and the transforaminal window was used to obtain MFV measures from the BA. For analysis, bilateral average MFVs were calculated for the CCA, ICA, VA, and MCA. There were also patients for whom we could not obtain adequate TCD signals from the MCAs and BA.

2.5. Statistical analysis

Subject age, MMSE score, ADAS-J cog score, CSF Aβ42 concentration, CSF p-tau181 concentration, serum lipid levels, and MFV values were compared among AD, MCI, and NC groups by one-way analysis of variance with Tukey post hoc tests for multiple comparisons. Sex ratios were compared among groups by chi-squared test. Both CSF biomarkers were compared among groups defined according to PS (PS = <1.1, 1.1–2.0, 2.1–3.0, 3.1–5.0, 5.1–7.0, >7.0 groups) by analysis of covariance, with total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride as covariates. Age, MMSE score, ADAS-J cog score, and blood lipid values were compared among groups classified according to PS by one-way analysis of variance and post hoc Tukey tests for multiple comparisons. Sex ratio was compared among PS groups by chi-squared test. Multiple regression analyses were conducted with Aβ42 and p-tau181 as dependent variables and MFVs of CCA, ICA, VA, BA, and MCA as independent variables.

A $P < .05$ was considered statistically significant, and all tests were 2-tailed. All data were analyzed using IBM SPSS Statistics 23 (IBM Japan, Tokyo, Japan) and GraphPad Prism7 (MDF Inc., Tokyo, Japan).

3. Results

3.1. Clinicodemographic characteristics of study participants

The clinical and demographic characteristics are summarized in Table 1. We obtained BA measures from 93.8% (39 AD patients, 20 MCI patients, and 16 NC subjects) and MCA measurements from 67.5% (32 AD patients, 15 MCI patients, and 7 NC subjects) of the participants. Age was slightly, but significantly, higher in the AD group than in the NC group ($p = .035$). As expected, MMSE and CSF Aβ42 were significantly lower in the AD group than in the NC group ($p < .0001$, $p < .0001$, respectively), while ADAS-J cog score was significantly higher in the AD group than in either the MCI group ($p = .037$) or the NC group ($p < .0001$), confirming greater symptom severity in the AD group. There were no significant differences in sex ratio, CSF p-tau181 levels, blood lipid concentrations, and arterial MFVs among AD, MCI, and NC subjects.

3.2. Comparison of CSF biomarker and total plaque score

Clinicodemographic values based on PS range (<1.1, 1.1–2.0, 2.1–3.0, 3.1–5.0, 5.1–7.0, and >7.0 mm) are shown in Table 2, and the comparison of Aβ42 and p-tau181 concentrations among these PS-defined groups is shown in Fig. 1. After adjusting for blood lipids as covariates, Aβ42 was higher in the PS = 1.1–2.0 group than in the four higher PS groups (2.1–3.0, 3.1–5.0, 5.1–7.0, >7.0), while the PS < 1.1 group showed no significant difference in CSF Aβ42 level compared to the other groups. The p-tau181 concentration did not differ among groups classified by PS. MMSE score was significantly higher in the group with PS = 1.1–2.0 compared to the other groups, while age, sex ratio, ADAS-J cog score, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides did not differ significantly among the six PS groups.

Please cite this article in press as: Kouzuki M et al. Cerebrospinal fluid biomarkers of Alzheimer's disease are associated with carotid plaque score and hemodynamics in intra- and extra-cranial arteries on ultrasonography. J Clin Neurosci (2017), https://doi.org/10.1016/j.jocn.2017.12.006