

The Correlation Study between Plasma A β Proteins and Cerebrospinal Fluid Alzheimer's Disease Biomarkers

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Background and Purpose Alzheimer's disease (AD) is characterized by the accumulation of amyloid- β proteins (A β). In this study we explored the correlation of plasma A β 40 and A β 42 concentrations with A β 42, total tau (tTau) and phosphorylated tau 181 (pTau181) levels in cerebrospinal fluid (CSF) in AD and control subjects to further understand the characteristics of plasma A β proteins levels.

Methods The consecutive subjects (44 AD and 47 controls) in this study were recruited. The plasma levels of A β 40 and A β 42 were measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits. And the corresponding CSFs were analyzed in terms of A β 42, tTau and pTau181 concentrations using INNOTEST ELISA kits. Further, the albumin levels were measured both in serum and CSF and albumin ratio was obtained to check the integrity of blood-brain barrier.

Results CSF A β 42 concentrations were significantly decreased while tTau and pTau181 levels were significantly increased in AD subjects. The plasma levels of A β 42 were significantly lower ($p=0.007$), while the A β 40/A β 42 ratio was significantly higher ($p<0.001$) in AD patients than in controls. The overall plasma A β 42 levels showed a positive correlation with those of CSF A β 42 ($p=0.001$), but not with the others in CSF. In subgroup analysis, the CSF A β 42 demonstrated positive correlation with not only plasma A β 42 but also A β 40 levels in controls. However, no significant relationship was noted between plasma and CSF A β proteins in AD group.

Conclusions The plasma A β 42 and A β 40 concentrations were shown to have a close relationship with CSF A β 42 levels in controls, but not in AD subjects. Our results suggest that the correlation between plasma A β 40 and CSF A β 42 levels is perturbed in AD.

Key Words Alzheimer's disease, amyloid β proteins, biomarker, blood, cerebrospinal fluid.

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INTRODUCTION

Alzheimer disease (AD), the most common degenerative brain disease, is characterized by the accumulation of amyloid β (A β) and phosphorylated tau proteins in cerebral cortex¹ which accompanying clinical symptoms of cognitive dysfunctions.² Pathophysiologic change of deposition of A β fibrils can be detected by positron emission tomography (PET) using A β -specific ligand. And the increased brain burden of A β 42 and tau proteins are suggested to be reflected by the decre-

ment of A β 42 and increased tau protein levels in cerebrospinal fluid (CSF).³ These measurements based on brain pathology have been recognized as an effective early diagnostic tool and recently incorporated into the diagnostic criteria of AD to increase the premortem accuracy of AD diagnosis.⁴ However, high cost of PET and inconvenience of lumbar puncture make it difficult for these to be widely used in clinical practice. Thus, to develop blood-based diagnostic biomarkers has been one of major concern in AD research field.⁵ As the increase of A β protein concentrations in brain and the decrease of A β 42 in CSF are the characteristic in AD, its alterations in plasma had been expected to be useful in diagnosis of AD. However, many trials failed to demonstrate its significance due to controversial outcomes.⁶ The plasma amount of A β proteins is affected by numerous factors,^{7,8} such as age, sex, renal function, platelet count,

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plasma protein level,⁹ obesity,¹⁰ cerebral ischemia,¹¹ and race.¹² Further, plasma A β proteins are easily bound to other proteins and degraded in blood.¹³ Therefore, not correcting these variables in prior literatures would contribute to diminish the reliability of plasma A β concentration as diagnostic biomarker of AD to some extent. Recently we identified the significant rise of plasma A β 40/A β 42 ratio in young AD patients with clinical onset of AD less than 65 year-old¹⁴ after the correction of these variables. At that time we tried to compare the levels of CSF AD biomarkers with plasma A β proteins concentrations to understand the characteristics of plasma A β protein concentrations. However, it was done in small population, 32 of AD patients vs. 25 of controls. In current study, we aimed to explore the correlation repeatedly between the plasma and CSF AD biomarkers including more samples. Further, the subgroup analysis was newly performed within individual group to check if the correlation differs depending on diagnosis of AD.

METHODS

Subjects

For this study, 47 healthy control group who had no evidence of cognitive dysfunction or brain disease and 44 AD patients were enrolled from 2012 March to 2016 February from our institute. All subjects gave an agreement to participate in this study which was approved by institutional review board. When the clinical characteristics of patients were compatible with probable AD based on recent National Institute on Aging-Alzheimer's Association criteria, we diagnosed AD.¹⁵ All subjects underwent Korean version of Mini-Mental State Examination (K-MMSE),¹⁶ clinical dementia rating (CDR), CDR-sum of box (CDR-SOB), Seoul Neuropsychological Battery¹⁷ and brain magnetic resonance imaging (MRI). Brain MRI included T1 axial, T2 axial, fluid-attenuated inversion recovery imaging axial, gradient echo axial, and thin-slice (0.5–1 mm) T1 coronal series. All patients with brain structural lesions which were not compatible with AD were excluded. Control groups included patients who underwent lumbar puncture for various clinical purpose and showed normal findings in K-MMSE, CDR, CDR-SOB and neuroimaging (computed tomography in 27 and MRI in 20).

Plasma analysis

Blood sample was conducted after minimum fasting of 8 hours. All samples were collected with EDTA tube and centrifuged for 15 minutes at 2000 \times g at 4°C. Supernatants of centrifuged samples were dispensed at polypropylene frozen tube with screw cap (Sarstedt PP, 500 μ L cat# 72.730.006; Sarstedt,

Nümbrecht, Germany), and stored at -80°C. Before measuring the concentration of plasma biomarkers, samples were thawed at room temperature. Using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Wako Chemicals, Richmond, VA, USA), plasma A β 40, A β 42 proteins concentration were measured. Each sample was measured in duplicate and the mean level was used as final level.

CSF analysis

Spinal tapping was conducted at L3–4 or L4–5 between 8 AM and 10 AM. First 2 mL of CSF were used for routine analysis, cell count, and protein and glucose levels. Following 10–12 mL of CSF were collected with polypropylene collecting tube (BD Falcon, #352096; BD Biosciences, Bedford, MA, USA), and subsequently centrifuged for 10 minutes at 4000 \times g. 400 μ L of supernatants were dispensed at polypropylene frozen tube with screw cap (Sarstedt PP, 500 μ L cat# 72.730.006), and stored at -80°C. Samples were thawed just before the measurements of CSF biomarker level. Using commercially available INNOTEST ELISA kit (Fujirebio Diagnostics, Ghent, Belgium), A β 42, tTau, and pTau181 levels were checked. All the process for collection and storage of CSF followed the agreed protocol.¹⁸ CSFA β 42, tTau, and pTau181 levels were checked in duplicate. Additionally, CSF albumin level was measured with Bromocresol Green albumin assay kit (Sigma-Aldrich, St. Louis, MO, USA) and used for calculating CSF albumin (mg/dL)/plasma albumin (g/dL) ratio (albumin ratio).

Statistical analysis

All data were analyzed using SPSS statistic package, version 19.0 (SPSS Inc., Chicago, IL, USA). Because none of CSF AD biomarkers demonstrated normal distribution the logarithmic transformations was performed before the statistical analysis.¹⁹ The plasma A β protein levels showed normal distribution and these were used as raw data in statistics. *t*-test was used for group comparison of continuous variables, and chi-square test for categorical variables. Pearson's correlation coefficient was used to check the relationship between plasma and CSF parameters. Nonparametric Mann-Whitney test was used for analyzing albumin ratio since it showed skewed deviation even after logarithmic transformation. A *p*-value<0.05 was considered significant.

RESULTS

Demographic analysis

The demographic and laboratory characteristics according to diagnosis group are presented in Table 1. MMSE scores were

significantly low (18.1 ± 6.6 vs. 28.3 ± 1.7 in AD vs. controls, $p < 0.001$), while CDR (1.1 ± 0.6 vs. 0.0 ± 0.0 , $p < 0.001$) and CDR-SOB (5.5 ± 4.0 vs. 0.0 ± 0.0 , $p < 0.001$) scores were high in AD patients. Apolipoprotein E $\epsilon 4$ allele was more common in AD group [18 (41%) in AD vs. 10 (20%) in controls, $p = 0.040$]. The age, gender distribution, the year of education, current history of diabetes mellitus and hypertension and familial history of dementia were comparable between the groups (in all, $p \geq 0.05$). The platelet count, and plasma protein and creatinine concentrations did not significantly differ (in all, $p \geq 0.05$).

Plasma A β protein level

There were no statistically significant differences in A $\beta 40$ protein levels between AD patients and controls ($p = 0.17$). However, plasma A $\beta 42$ level was noticeably low in AD patients compared to controls (16.1 ± 10.0 PM vs. 22.3 ± 10.8 PM, $p = 0.007$) (Table 2). And the A $\beta 40$ /A $\beta 42$ ratio was much higher

in AD patients than in controls (5.4 ± 2.5 vs. 3.4 ± 1.9 , $p < 0.001$). The CSF albumin concentrations did not differ between the groups ($p = 0.387$), while its plasma levels were lower in controls (4.0 ± 0.4 vs. 3.8 ± 0.4 , $p = 0.032$). However, the albumin ratio (CSF albumin/plasma albumin) which reveals the integrity of blood-brain barrier²⁰ rather tended to be higher in AD group ($p = 0.085$).

Correlations between CSF AD biomarkers and plasma A β proteins

CSF A $\beta 42$, tTau and pTau181 level are known useful as a biomarker of early diagnosis of AD.²¹ In consistent with the prior literature,²¹⁻²³ A $\beta 42$ levels were markedly decreased (320.6 ± 92.4 vs. 689.0 ± 170.1 pg/mL, AD vs. controls, $p < 0.001$), while tTau (520.9 ± 209.7 vs. 204.4 ± 62.4 pg/mL, $p < 0.001$) and pTau181 levels (91.7 ± 129.2 vs. 42.8 ± 12.3 pg/mL, $p < 0.001$) were much higher in AD subjects (Table 3).

The correlations between plasma A β proteins and individ-

Table 1. Clinical and blood sample information of participants

	AD (n=44)	CON (n=47)	p
M:F	16:28	13:34	0.500
Age (year-old)	62.2 \pm 7.2	60.6 \pm 6.2	0.256
Education (year)	10.5 \pm 4.4	9.3 \pm 3.9	0.190
Diabetes	5	8	0.556
Hypertension	16	16	0.827
MMSE	18.1 \pm 6.6	28.3 \pm 1.7	<0.001*
CDR	1.1 \pm 0.6	0.0 \pm 0.0	<0.001*
CDR-SOB	5.5 \pm 4.0	0.0 \pm 0.0	<0.001*
APOE $\epsilon 4$ carrier	18 (41%)	10 (20%)	0.040*
One allele	12	9	
Two allele	6	1	
Platelets (/mL ⁶)	238.9 \pm 56.4	224.5 \pm 74.9	0.341
Plasma proteins (g/dL)	6.7 \pm 0.5	6.5 \pm 0.6	0.107
Plasma creatinine (mg/dL)	0.9 \pm 0.0	0.9 \pm 0.2	0.128

Mean \pm standard deviation.

* $p < 0.05$ is significant on *t*-test or chi-square test.

AD: Alzheimer's disease, APOE: apolipoprotein E, CDR: clinical dementia rating, CDR-SOB: CDR-sum of box, CON: control, F: female, M: male, MMSE: Mini-Mental State Examination.

Table 2. Plasma beta amyloid protein level, albumin level and its ratio

	AD (n=44)	CON (n=47)	p
Plasma A $\beta 40$ (PM)	70.8 \pm 22.0	64.2 \pm 23.5	0.170
Plasma A $\beta 42$ (PM)	16.1 \pm 10.0	22.3 \pm 10.8	0.007*
Plasma A $\beta 40$ /A $\beta 42$	5.4 \pm 2.5	3.4 \pm 1.9	<0.001*
Plasma albumin (g/dL)	4.0 \pm 0.4	3.8 \pm 0.4	0.032*
CSF albumin (mg/dL)	21.9 \pm 11.1	18.6 \pm 6.6	0.387
Albumin ratio [†]	5.6 \pm 2.8	4.3 \pm 1.6	0.085

Mean \pm standard deviation. *t*-test was used for group comparison.

* $p < 0.05$, [†]CSF (mg/dL)/plasma albumin levels (g/dL).

AD: Alzheimer's disease, CON: control, CSF: cerebrospinal fluid.

Table 3. Level of CSF biomarkers

	AD (n=44)	CON (n=47)	p
CSF A β 42 (pg/mL)	320.6 \pm 92.4	689.0 \pm 170.1	<0.001*
CSF tTau (pg/mL)	520.9 \pm 209.7	204.4 \pm 62.4	<0.001*
CSF pTau (pg/mL)	91.7 \pm 129.2	42.8 \pm 12.3	<0.001*
CSF albumin (mg/dL)	21.9 \pm 11.1	18.6 \pm 6.6	0.387

Mean \pm standard deviation. Log-transformed values of CSF AD biomarkers were used for statistical analysis, but the raw values were presented for easy understanding. CSF albumin was compared by non-parametric analysis. For the others *t*-test was used.

**p*<0.05.

AD: Alzheimer's disease, CON: control, CSF: cerebrospinal fluid.

Table 4. Analyses of correlation between CSF biomarker and plasma beta amyloid

	CSF A β 42 (pg/mL)	CSF tTau (pg/mL)	CSF pTau181 (pg/mL)
Both			
A β 40 (PM)	<i>r</i> =0.029, <i>p</i> =0.781	<i>r</i> =0.100, <i>p</i> =0.353	<i>r</i> =-0.031, <i>p</i> =0.773
A β 42 (PM)	<i>r</i> =0.364, <i>p</i> =0.001*	<i>r</i> =-0.160, <i>p</i> =0.144	<i>r</i> =-0.114, <i>p</i> =0.297
CON			
A β 40 (PM)	<i>r</i> =-0.391, <i>p</i> =0.006*	<i>r</i> =-0.153, <i>p</i> =0.305	<i>r</i> =-0.121, <i>p</i> =0.424
A β 42 (PM)	<i>r</i> =0.339, <i>p</i> =0.023*	<i>r</i> =0.029, <i>p</i> =0.851	<i>r</i> =-0.087, <i>p</i> =0.576
AD			
A β 40 (PM)	<i>r</i> =0.135, <i>p</i> =0.381	<i>r</i> =0.141, <i>p</i> =0.374	<i>r</i> =-0.133, <i>p</i> =0.388
A β 42 (PM)	<i>r</i> =0.141, <i>p</i> =0.374	<i>r</i> =0.271, <i>p</i> =0.091	<i>r</i> =0.154, <i>p</i> =0.331

r: Pearson's correlation between plasma levels of A β and log-transformed CSF levels of AD biomarkers.

**p*<0.05 in significant on Pearson's correlation analysis.

AD: Alzheimer's disease, CON: control, CSF: cerebrospinal fluid.

ual CSF AD biomarkers were computed in all participants (Table 4). The plasma A β 42 levels revealed a significant positive correlation with those of CSF A β 42 protein (*r*=0.364, *p*=0.001) (Fig. 1). However, there was no correlation in the other pair of plasma A β protein and CSF AD biomarkers. However, on subgroup analysis the control group demonstrated intimate relationship not only between plasma A β 42 and CSF A β 42 protein levels (*r*=0.339, *p*=0.023), but also between plasma A β 40 and CSF A β 42 protein concentrations (*r*=0.391, *p*=0.006). In contrast, within AD group, CSF A β 42 levels did not reveal significant correlation with any of plasma A β protein levels (*p*=0.381 for A β 40 and *p*=0.374 for A β 42).

DISCUSSION

There have been several reports considering plasma A β 42 levels as a possible diagnostic tool in AD,²⁴⁻²⁶ however it remains controversial.^{5,27} In our prior investigation, the rise of plasma A β 40/A β 42 ratio was found useful for diagnosis of early-onset AD.¹⁹ Consistently, we confirmed it again in the this study which increased sample size, although the part of the current samples (20 in AD and 18 in controls) were the same ones which were used for the prior study.¹⁴ There have been controversy in the direction of change plasma A β protein levels in the presence of AD.^{5,27} However, in relation to the rise

of plasma A β 40/A β 42 ratio recent literatures support it as consistent a finding in AD.^{8,25}

Technical limitations of measuring level of A β protein are more conspicuous in plasma than CSF. It is attributed to that lower quantity of A β protein in plasma and its high tendency to bind with other protein.²⁸ In this study, plasma protein and creatinine level showed no differences between AD group and controlled group. However, plasma albumin level was slightly higher in AD group. Because plasma albumin is the binding target for A β protein, the possibility that increased level of albumin affected the lower plasma A β 42 level in AD patients cannot be excluded. However, it might be less likely based on the following. The result of this study corresponds to previous studies¹⁴ and the extent of decrease of plasma A β 42 was more remarkable (*p*=0.007) compared to the slight increase of plasma albumin level (*p*=0.032). Core pathophysiology of AD is rise of A β protein burden in brain.²⁹ Thus, it is possible that the alteration in plasma A β protein level in AD patient is related to the increase of A β proteins in AD brain. In current study the correlation between plasma A β 42 and CSF A β 42 was identified in overall subjects. It is in agreement with our previous study, in which we checked the correlation not dividing the subjects according to the diagnosis. However when we did subgroup analysis, the positive correlation was persistent only in controls. Further, the additional correlation between

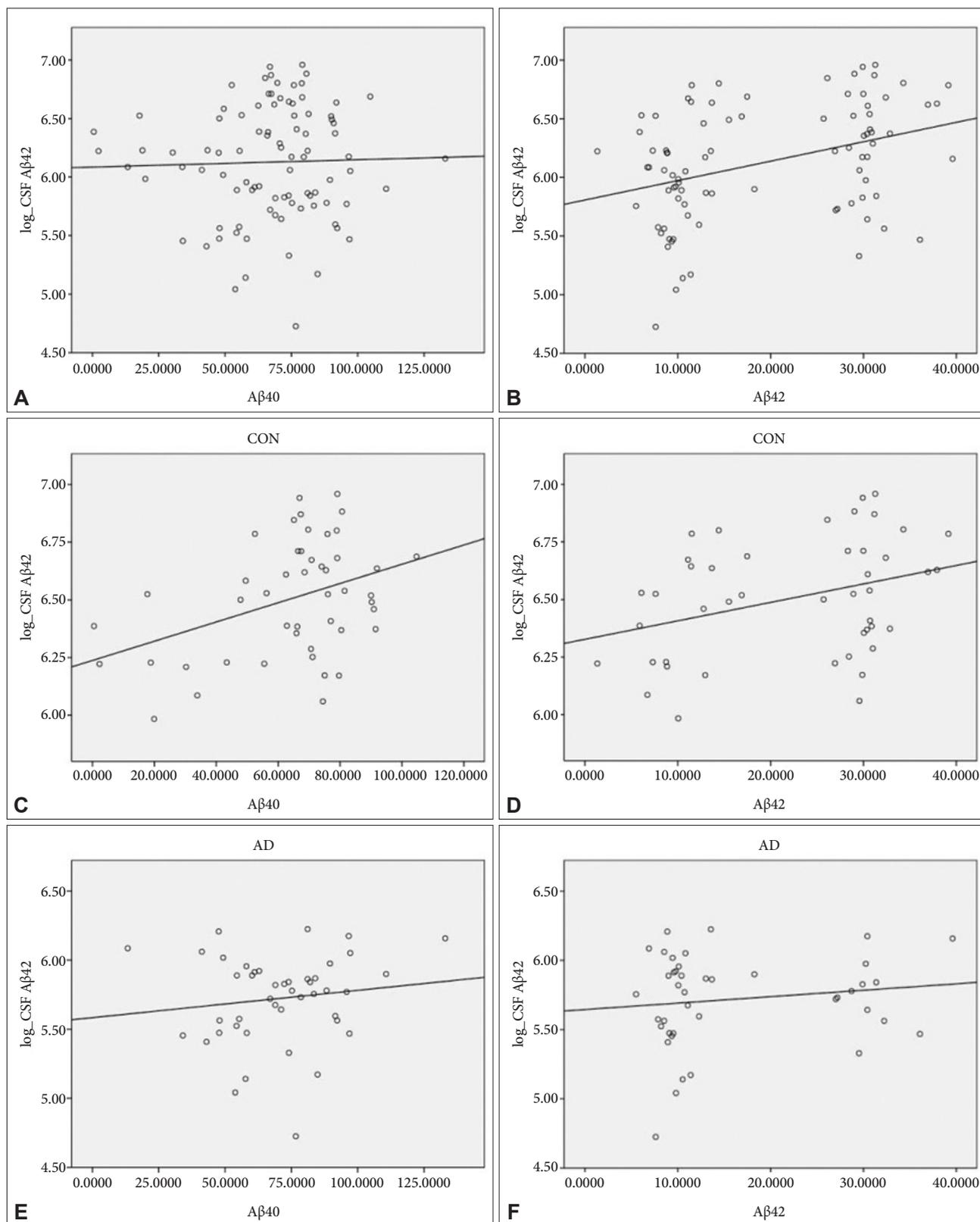


Fig. 1. Correlation between CSF A β 42 and plasma A β 40, A β 42. A and B: Correlation between CSF A β 42 and plasma beta amyloid in all participants (A-A β 40, B-A β 42). C and D: Correlation between CSF A β 42 and plasma beta amyloid (C-A β 40, D-A β 42). E and F: Correlation between CSFA β 42 and plasma beta amyloid (E-A β 40, F-A β 42). AD: Alzheimer's disease, CON: control, CSF: cerebrospinal fluid.

plasma A β 40 proteins and CSF A β 42 concentrations was identified significant in control group. It is contrast to AD group, where there was no correlation between plasma A β protein and CSF A β 42 protein. It is consistent with the previous reports which described minor or no correlation between plasma and CSF A β proteins levels in AD patients.^{11,30-32} Therefore, it is suggested that the loss of correlation between CSF and plasma A β protein concentrations might be related to the development of AD. Although it did not reach significant level ($p=0.085$), the diminished blood-brain integrity, increased albumin ratio, was suspected AD group. It might be possible mechanism underlying loss of correlation between plasma A β 40 (or 42) and CSF A β 42 protein concentrations. However, we are very cautious about our result because the current study is based on the small population. The following study is necessary to confirm our result and to elucidate underlying mechanism.

In conclusion, this study demonstrated the decrease in plasma A β 42 level and the increase in A β 40/A β 42 ratio in AD patients. Furthermore, the apparent correlation of plasma A β level and CSF A β 42 only presented in control subjects, not in AD group, which suggests that decrease of correlation of CSF-plasma A β proteins may be related to the pathology of AD.

Conflicts of Interest

The authors have no financial conflicts of interest.

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