Plasma and Cerebrospinal Fluid Levels of Amyloid β Proteins 1-40 and 1-42 in Alzheimer Disease

Pankaj D. Mehta, PhD; Tuula Pirttilä, MD, PhD; Sangita P. Mehta, MS; Eugene A. Sersen, PhD; Paul S. Aisen, MD; Henryk M. Wisniewski, MD, PhD

Background: In brains with AD, Aβ is a major component of diffuse plaques. Previous reports showed that CSF Aβ42 levels were lower in patients with AD than in controls. Although studies showed higher plasma Aβ42 levels in familial AD, a recent report has indicated that plasma Aβ42 levels were similar in a sporadic AD group and controls. However, no information is published on plasma Aβ40 and Aβ42 levels in relation to Apo E genotype or severity of dementia in sporadic AD.

Objective: To examine plasma and cerebrospinal fluid (CSF) levels of amyloid β protein 1-40 (Aβ40) and 1-42 (Aβ42) levels in patients with probable Alzheimer disease (AD) and elderly nondemented control subjects in relation to the apolipoprotein E (Apo E) genotype and dementia severity.

Setting: Two university medical centers.

Patients and Methods: Levels of Aβ40 and Aβ42 were measured in plasma from 78 patients with AD and 61 controls and in CSF from 36 patients with AD and 29 controls by means of a sandwich enzyme-linked immunosorbent assay.

Results: Mean plasma Aβ40 levels were higher in the AD group than in controls (P = .005), but there was substantial overlap; Aβ42 levels were similar between the groups. Levels of Aβ40 and Aβ42 showed no association with sex or Mini-Mental State Examination scores. There was a significant relationship between age and Aβ40 level in controls but not in the AD group. Levels of Aβ40 were higher in patients with AD than in controls (P<.01). Cerebrospinal fluid Aβ40 levels were similar in the AD group and controls. However, Aβ42 levels were lower in the AD group than in controls (P<.001). The levels showed no association with severity of dementia.

Conclusions: Although mean plasma Aβ40 levels are elevated in sporadic AD and influenced by Apo E genotype, measurement of plasma Aβ40 levels is not useful to support the clinical diagnosis of AD. Lower levels of CSF Aβ42 in the AD group are consistent with previous studies.

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SUBJECTS AND METHODS

SUBJECTS

The study included 42 patients with sporadic AD and 46 elderly, nondemented controls from the Alzheimer Disease Research Center, Mount Sinai Medical Center, New York, NY, and 36 patients with probable AD and 29 controls from the Department of Neurology, Tampere University Medical Center, Tampere, Finland. Informed consent was obtained from all participants or their guardians. The diagnosis of probable AD was made according to the criteria of the National Institute of Neurological Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association.13 Age, sex, Apo E genotype, and MMSE scores of the AD group and controls are given in Table 1. The median duration of disease in the AD group was 3 years (range, 0.5-10 years).

PLASMA AND CSF COLLECTION

Blood was collected from both centers between 10 AM and 2 PM in tubes containing sodium ethylenediaminetetraacetic acid. After 15 minutes, plasma samples were centrifuged at 3000 rpm at 4°C. Supernatants were collected, divided into aliquots, and frozen at −80°C. Plasma samples were collected from 78 patients with AD and 61 controls. Cerebrospinal fluid samples were collected from all subjects recruited from the Tampere center. The controls included patients with headache (n = 16) and mild depression (n = 13). Samples were obtained at the time of diagnostic lumbar puncture, divided into aliquots, and stored frozen at −80°C until further study. All CSF samples were tested for cell counts and levels of glucose and total protein. Samples contaminated with blood were excluded.

APOLipoprotein E genotypes of blood samples from the AD and control groups were determined using polymerase chain reaction methods as previously described.20 The polymerase chain reaction products were digested with HhaI subjected to polyacrylamide gel electrophoresis, and separated DNA fragments were visualized using ethidium bromide staining.

ANTISERUM SAMPLES

We conjugated Aβ32-40 and Aβ33-42 peptides synthesized commercially (Ana Spec, San Jose, Calif) to keyhole-limpet hemocyanin in phosphate-buffered saline solution (PBS) with 0.3% glutaraldehyde. Rabbits were immunized with 1 ng/mL of Aβ42 was examined using a sandwich enzyme-linked immunosorbent assay (ELISA). There was a strong response of R162 with 1 ng/mL of Aβ40 but no detectable response with 10 ng/mL of Aβ42. Similarly, R164 was found to be specific to Aβ42 but showed no reactivity to Aβ40. Western blot analysis also showed that R162 was specific to Aβ40 and that R164 was specific to Aβ42 as described previously.21

Aβ40 AND Aβ42 ELISA

Levels of Aβ were measured using monoclonal antibody 6E10 (specific to an epitope present on 1-16 amino acid residues of Aβ), rabbit antiserum R162 (specific to a peptide corresponding to Aβ40), and rabbit antiserum 164 (specific to a peptide corresponding to Aβ42) in a double-antibody sandwich ELISA as described previously.22 Briefly, 100 μL of monoclonal antibody 6E10 (2.5 μg/mL) diluted in carbonate-bicarbonate buffer (pH 9.6) was coated in wells of microtiter plates and incubated at 4°C overnight. After washing the plates with PBS containing 0.05% polyoxymethylene sorbitan monolaurate (Tween 20; Sigma-Aldrich Corp, St Louis, Mo) (PBST), wells were blocked for an hour with 200 μL of 10% normal sheep serum in PBS to avoid nonspecific binding. Plates were washed again, and 100 μL of standards (Aβ40 and Aβ42; Bachem, Torrance, Calif) diluted in PBST with 0.5% bovine serum albumin or plasma (undiluted) were applied and incubated 2 hours at room temperature and 4°C overnight. After washing, the plates were incubated with biotinylated rabbit antiserum samples 162 or 164 diluted in PBST with 0.5% bovine serum albumin at room temperature for 1 hour 15 minutes. After washing, NeutrAvidin-horseradish peroxidase conjugated (Pierce, Rockford, Ill) diluted in PBST was added into the wells, and plates were incubated 1 hour at room temperature. Plates were washed again, and 100 μL of o-phenylenediamine dihydrochloride (Sigma-Aldrich Corp) in 50-mmol/L citric acid and 100-mmol/L sodium phosphate buffer (pH 5.0) was added in each well. The reaction was stopped by adding 100 μL of 1N sulfuric acid. The optical density (OD) was measured at 490 nm in a micro-ELISA reader. The relationship between OD and the Aβ concentrations was determined using a 4-feature logistic logarithm function. Nonlinear curve fitting was performed with a commercially available program (KinetiCalc; Biotek Instruments, Inc, Winooski, Vt) to convert OD of plasma to estimated concentrations. All samples were coded; investigators were unaware of group assignment (AD vs control group) until levels measured and recorded.

ASSAY SENSITIVITY AND PRECISION

Detection limit of the assay was 20 pg/mL for Aβ40 and 40 pg/mL for Aβ42. The percentage coefficients of variation ranged from 8% to 14% (interassay) and 10% to 18% (intraassay). When Aβ40 and Aβ42 levels in CSF and plasma from 20 patients with AD were quantitated using a second set of antisera samples specific for Aβ40 (R163) and Aβ42 (R165) and compared with the levels quantitated using R162 and R164, there was a significant correlation for Aβ40 (r = 0.83; P < .001) and for Aβ42 (r = 0.71; P = .005).

STATISTICAL ANALYSIS

The groups were compared for each constituent, using the Kruskal-Wallis 1-way analysis of variance with a Bonferroni correction for multiple comparisons. Pearson correlation with Bonferroni correction was used to analyze the relationship between the variables. Pairs of groups were compared using the Mann-Whitney test. The level of P < .01 was considered significant.

AD, this assay is not used widely because it requires lumbar puncture.

Recently, 2 studies measured plasma Aβ40 and Aβ42 levels in patients with AD.13,14 One study13 showed that plasma Aβ40 and Aβ42 levels were 2- to 3-fold higher in patients with familial AD and with AβPP and presenilin 1 and 2 mutations compared with patients with sporadic AD and controls. The second
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Herein, we report the quantitation of plasma and CSF Aβ40 and Aβ42 levels in patients with probable AD and elderly nondemented controls and analyze the relationships with age, sex, MMSE score, and Apo E genotype.

Table 1 shows the demographic characteristics of plasma samples from the AD and control groups. The groups did not differ significantly (P = .79) by age or sex. The Apo E ε4 allele frequencies were 52 (67%) of 78 in the AD group and 13 (21%) of 61 in the control group. The higher frequency of Apo E ε4 allele reported in the AD group was consistent with previous findings in Finland. The frequency of Apo E ε4 allele in controls was similar to that reported previously.

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**PLASMA LEVELS**

Levels of Aβ40 were higher in the AD group than in controls (P = .003) (Figure 1). The levels were higher in patients with AD and the Apo E ε4 allele than those without the allele, but with the borderline significance (P = .03) (Table 2). However, the levels were higher than those in controls with and without the Apo E ε4 allele (P = .01). Levels of Aβ42 were similar between patients with AD and controls; they were also similar in both groups with and without Apo E ε4 allele.

There was a significant association between Aβ40 and Aβ42 levels for patients with AD (r = 0.38; P = .008).
However, a similar relationship reached borderline significance in controls ($r = 0.31; P = .03$). There was a significant relationship between age and Aβ40 levels in controls ($r = 0.37; P = .008$), but not in patients with AD ($r = 0.27; P = .07$). However, there was no significant relationship between age and Aβ42 level in patients with AD or controls. There were no significant differences in Aβ40 and Aβ42 levels in men compared with women. There was no significant association between MMSE scores and levels of Aβ40 ($r = 0.27; P = .07$). However, there was no significant relationship between MMSE scores and levels of Aβ42 ($r = .03; P = .73$). There was no significant association with age or sex in either group. There was also no significant overlap between both groups, measurement of plasma Aβ40 levels is not useful as a diagnostic tool to distinguish patients with sporadic AD from elderly, non-demented controls. The finding that plasma Aβ42 levels are similar between patients with AD and controls is consistent with those recently reported. Discrepancies between both groups, measurement of plasma necessitate a sensitive and reliable laboratory quantitation assay. Also, Aβ binds to carrier proteins such as Apo E and Apo J that are present in plasma. Antibody epitopes of Aβ may be masked by such binding and interfere with detection of true Aβ values in body fluids using sandwich ELISA. Investigators have also reported cross-reactivity between Aβ and several plasma proteins, including immunoglobulin G and fibrinogen. However, our immunoblotting studies did not show staining of additional bands with specific antibodies to Aβ40 and Aβ42. The monoclonal antibody 6E10 recognizes AβPP and Aβ in CSF and plasma. However, AβPP did not cause any interference in our assay as confirmed by the recovery data of Aβ40. The significance of plasma Aβ levels in relation to Aβ accumulation in the brain is unclear. If plasma Aβ originates from tissues other than brain, there may not be an association between plasma Aβ levels and Aβ deposited in the brain. However, investigators have shown that Aβ–Apo E and Aβ–Apo J complexes cross the blood–brain barrier; thus, Aβ present in plasma may contribute to the development of Aβ deposits in the brain. Previous studies showed that Aβ levels varied highly in brains of patients with AD, and those with massive amyloid deposition contained predominantly Aβ40. In some patients with sporadic AD, high plasma levels of Aβ40 may

### CSF LEVELS

Levels of Aβ40 in CSF were similar in patients with AD and controls. However, Aβ42 levels were lower in patients with AD than in controls ($P<.001$) (Figure 2). The levels were lower in patients with AD with the Apo E e4 allele than in controls without the allele ($P = .004$) (Table 3). There was no significant difference in Aβ42 levels between patients with AD with the Apo E e4 allele and controls with the allele. The levels showed no association with age or sex in either group. There was also no relation between the levels and MMSE scores in patients with AD and controls.

### COMMENT

Unlike earlier studies, our results showed that mean plasma Aβ40 levels were elevated in patients with AD, compared with controls. Because there was a considerable overlap between both groups, measurement of plasma Aβ40 levels is not useful as a diagnostic tool to

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**Table 3. Cerebrospinal Fluid Aβ40 and Aβ42 Levels in Patients with AD and Controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Specimens</th>
<th>Aβ40, ng/mL (Median, Range)</th>
<th>Aβ42, pg/mL (Median, Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with sporadic AD</td>
<td>36</td>
<td>11.5 (4.7-23.4)</td>
<td>36 (25-325)†</td>
</tr>
<tr>
<td>With Apo E e4</td>
<td>28</td>
<td>11.9 (4.7-23.4)</td>
<td>38 (25-325)†</td>
</tr>
<tr>
<td>Without Apo E e4</td>
<td>8</td>
<td>11.0 (6.9-21.0)</td>
<td>25 (25-255)</td>
</tr>
<tr>
<td>Elderly non-demented controls</td>
<td>29</td>
<td>9.8 (5.3-23.1)</td>
<td>111 (25-1060)†</td>
</tr>
<tr>
<td>With Apo E e4</td>
<td>10</td>
<td>8.1 (5.3-11.8)</td>
<td>56 (25-250)‡</td>
</tr>
<tr>
<td>Without Apo E e4</td>
<td>19</td>
<td>10.7 (5.8-23.1)</td>
<td>135 (35-1060)</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnote to Table 2. P<.004, analysis of variance with Bonferroni correction.

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contribute to the accumulation of Aß40 in preexisting plaques.

Age and Apo E genotype are major risk factors for sporadic AD. We found a significant relationship between age and plasma Aß40 levels in controls and between Aß40 and Apo E genotype in patients with AD. These results are interesting, since investigators have suggested that higher plasma Aß40 or Aß42 levels may play a part in 10% to 20% of sporadic AD before the onset of clinical symptoms. 13 Although our findings showed increased plasma Aß40 levels in AD with the Apo E e4 allele, we do not know whether the levels were present several years before the onset of probable AD. Further longitudinal measurements are needed in individuals with mild cognitive symptoms or asymptomatic first-degree relatives of patients with AD in diagnosing early AD.

Although we found no relationship between severity of dementia and Aß levels in the cross-sectional sample, longitudinal studies will be necessary to determine conclusively whether there is a relationship between plasma Aß and progression of AD. Such studies are particularly important to determine whether modulation of plasma Aß may be a useful measure of disease-modifying therapies.

Our CSF results showing lower mean Aß42 levels in AD are consistent with those of previous reports. 9-12 The absence of correlation with age or sex is in agreement with earlier reports. Our studies showed no significant relationship between these levels and MMSE scores, consistent with the data of Motter et al 9 and Tamaoka et al 10; others showed weak 12 or strong 11 correlation between the levels and dementia severity. The reason for the discrepancy may be that in our study, the sample size was small, and most patients with AD were mildly demented. However, our results are consistent with the histopathological data, which showed no correlation between number of plaques and Aß deposition in the brain. 12-14 Further studies with a greater number of samples are essential to clarify these controversial findings.

Our findings that CSF Aß42 levels were lower in patients with AD and the Apo E e4 allele than in patients without the allele agree with those of a number of published reports. However, our studies showed no significant differences in CSF Aß42 levels between patients with AD with the Apo E e4 allele and controls with the allele. The findings are different from those reported by Galasko et al 12 and Hulstaert et al, 35 who reported significant differences. The reason for this discrepancy may be that in our studies, the number of controls with the Apo E e4 allele is smaller than in the latter studies. 12, 35

The values of CSF Aß40 and Aß42 differed between research laboratories. For example, we found mean CSF Aß42 levels in AD to be 35 pg/mL, in contrast to the range seen from 125 fmol/mL to 833 pg/mL in previous studies. 9-12 Conflicting findings may result from differences in the affinity of specific Aß antiseraum samples and the purity and solubilization of peptides used as standards. In addition to the differences in the ELISA methods, conflicting data could result from differences in sample collection and storage conditions. It has been reported that Aß values decreased over time, even if the CSF samples were frozen. 36 Repeated freezing and thawing of CSF could also result in the loss of Aß. Several studies have shown that Aß levels are lower in CSF collected in glass or polystyrene tubes than in polypropylene tubes. Although our samples were divided into aliquots and well frozen in polypropylene tubes, storing samples frozen over a long time and other unknown factors might have influenced the true value of Aß in CSF.

In summary, although blood is easy to obtain, it is still unclear if there are systemic changes specific for AD, and to what extent changes in blood composition reflect pathologic changes seen in the brain. Cerebrospinal fluid may better represent brain abnormalities than blood, but drawing of CSF is an invasive procedure. Further measurements of Aß40 and Aß42 levels in matched plasma, CSF, and autopsy brain tissues in correlation with dementia severity and Apo E genotype could increase our understanding of the significance of plasma and CSF measurements.

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Reprints: Pankaj D. Mehta, PhD, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd, Staten Island, NY 10314 (e-mail: pdmehta@worldnet.att.net).

REFERENCES


