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

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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 and the Apo E ε4 allele 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.

Arch Neurol. 2000;57:100-105
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. 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 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.

APO E GENOTYPING

Apolipoprotein E genotypes of blood samples from the AD and control groups were determined using polymerase chain reaction methods as previously described. 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 the peptides, and the specificity of antiserum samples (PBS) with 0.5% glutaraldehyde. Rabbits were immunized with 1 ng/mL of Aβ was examined using a sandwich enzyme-linked immunosorbent assay (ELISA). 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 the peptides, and the specificity of antiserum samples (PBS) with 0.5% glutaraldehyde. Rabbits were immunized with 1 ng/mL of Aβ was examined using a sandwich enzyme-linked immunosorbent assay (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. 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% polyoxyethylene 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, NeutraAvidin-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 Aβ40 and Aβ42 concentrations was determined using a 4-feature logistic logithm function. Nonlinear curve fitting was performed with a commercially available program (KinetiCalc; Bioteck 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 were 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 antiserum 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. One study 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
Apolipoprotein E (Apo E) is a 34-kd polymorphic protein that is involved in the transportation and redistribution of lipids among various tissues. The Apo E $\varepsilon4$ allele is a significant risk factor for the development of sporadic and familial late-onset AD.15,16 Although studies17,18 have shown an association of Apo E $\varepsilon4$ alleles with increased amyloid deposition in brains with AD, none examined the relationship between plasma $\beta_40$ and $\beta_42$ levels and the Apo E genotype.

Herein, we report the quantitation of plasma and CSF $\beta_40$ and $\beta_42$ 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 $\varepsilon4$ 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 $\varepsilon4$ allele reported in the AD group was consistent with previous findings in Finland.23,24 The frequency of Apo E $\varepsilon4$ allele in controls was similar to that reported previously.15,16

Table 1. Demographic Characteristics of Patients With AD and Controls*

<table>
<thead>
<tr>
<th>Specimen, Group</th>
<th>Mean Age, (Range), y</th>
<th>Sex</th>
<th>Median MMSE Score (Range)</th>
<th>Apo E $\varepsilon4$ Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with AD (n = 78)</td>
<td>74 (55-99)</td>
<td>39</td>
<td>39</td>
<td>15.5 (1-28)†</td>
</tr>
<tr>
<td>Controls (n = 61)</td>
<td>67 (44-92)</td>
<td>27</td>
<td>34</td>
<td>30 (27-30)</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with AD (n = 36)</td>
<td>71 (55-85)</td>
<td>7</td>
<td>29</td>
<td>22 (1-29)‡</td>
</tr>
<tr>
<td>Controls (n = 29)</td>
<td>62 (54-74)</td>
<td>11</td>
<td>18</td>
<td>30 (28-30)</td>
</tr>
</tbody>
</table>

*AD indicates Alzheimer disease; MMSE, Mini-Mental State Examination; Apo E, apolipoprotein E; and CSF, cerebrospinal fluid. Unless indicated otherwise, data are given as number of patients.†Fifty-three patients had scores of greater than 12; 6, of 12 or less.‡Thirty patients had scores of greater than 12; 6, of 12 or less.

Table 2. Plasma $\beta_40$ and $\beta_42$ Levels in Patients With AD and Controls*

<table>
<thead>
<tr>
<th>Specimens</th>
<th>No. of</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\beta_40$, pg/mL</td>
</tr>
<tr>
<td>Patients with sporadic AD</td>
<td>78</td>
<td>272 (100-770)†</td>
</tr>
<tr>
<td>With Apo E $\varepsilon4$</td>
<td>52</td>
<td>290 (101-770)‡</td>
</tr>
<tr>
<td>Without Apo E $\varepsilon4$</td>
<td>26</td>
<td>228 (100-620)</td>
</tr>
<tr>
<td>Elderly nondemented controls</td>
<td>61</td>
<td>219 (35-490) †</td>
</tr>
<tr>
<td>With Apo E $\varepsilon4$</td>
<td>13</td>
<td>224 (35-301)‡</td>
</tr>
<tr>
<td>Without Apo E $\varepsilon4$</td>
<td>48</td>
<td>213 (35-490) ‡</td>
</tr>
</tbody>
</table>

*AD indicates Alzheimer disease; $\beta_40$, amyloid $\beta$ protein ending at residue 40; $\beta_42$, amyloid $\beta$ ending at residue 42; and Apo E, apolipoprotein E. †P<.005, analysis of variance with Bonferroni correction.‡P<.01, analysis of variance with Bonferroni correction.

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 $\varepsilon4$ 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 $\varepsilon4$ allele reported in the AD group was consistent with previous findings in Finland.23,24 The frequency of Apo E $\varepsilon4$ allele in controls was similar to that reported previously.15,16

PLASMA LEVELS

Levels of $\beta_40$ were higher in the AD group than in controls ($P = .005$) (Figure 1). The levels were higher in patients with AD and the Apo E $\varepsilon4$ allele than in 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 $\varepsilon4$ allele ($P = .01$). Levels of $\beta_42$ were similar between patients with AD and controls; they were also similar in both groups with and without Apo E $\varepsilon4$ allele.

There was a significant association between $\beta_40$ and $\beta_42$ levels for patients with AD ($r = 0.38; P = .008$).

Figure 1. Box plots of plasma levels of amyloid $\beta$ protein ending at residues 40 ($\beta_40$) (top) and 42 ($\beta_42$) (bottom) in patients with Alzheimer disease (AD) and elderly, nondemented control subjects. Boxes indicate 25th to 75th percentiles; small squares, median values; and upper and lower bars, range from 0 to 100th percentile.
However, a similar relationship reached borderline significance in controls ($r = 0.31; P = .03$). There was a significant relationship between age and $A\beta 40$ 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 $A\beta 42$ level in patients with AD or controls. There were no significant differences in $A\beta 40$ and $A\beta 42$ levels in men compared with women. There was no significant association between MMSE scores and levels of $A\beta$ in patients with AD or controls. The MMSE scores did not differ significantly between patients with AD and the Apo E $e4$ allele and those without the allele.

## CSF LEVELS

Levels of $A\beta 40$ in CSF were similar in patients with AD and controls. However, $A\beta 42$ 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 $A\beta 42$ 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,13,14 our results showed that mean plasma $A\beta 40$ levels were elevated in patients with AD, compared with controls. Because there was a considerable overlap between both groups, measurement of plasma $A\beta 40$ levels is not useful as a diagnostic tool to distinguish patients with sporadic AD from elderly, nondemented controls. The finding that plasma $A\beta 42$ levels are similar between patients with AD and controls is consistent with those recently reported.13,14 Discrepancies between our results and those reported by others13,14 may result from differences in patient populations. Our data suggest that Apo E genotype influences $A\beta 40$ levels; the frequency of Apo E $e4$ allele was not reported in the earlier studies. Also, different ELISA methods and specificity of antibodies likely influence the data. However, these factors are not likely responsible for the differences seen in plasma $A\beta 40$ levels between patients with AD and controls, as our data on CSF $A\beta 40$ and $A\beta 42$ levels are consistent with a number of published data.9-12

There are various difficulties in the measurement of $A\beta$ levels in body fluids. Low concentrations of $A\beta$ in plasma necessitate a sensitive and reliable laboratory quantitation assay. Also, $A\beta$ binds to carrier proteins such as Apo E and Apo J that are present in plasma.25,26 Antibody epitopes of $A\beta$ may be masked by such binding and interfere with detection of true $A\beta$ values in body fluids using sandwich ELISA. Investigators have also reported cross-reactivities between $A\beta$ and several plasma proteins, including immunoglobulin G27 and fibrinogen.28 However, our immunoblotting studies did not show staining of additional bands with specific antibodies to $A\beta 40$ and $A\beta 42$.29 The monoclonal antibody 6E10 recognizes $A\beta$ in the brain but may not recognize $A\beta$ in plasma.29 Hence, investigators have not investigated the development of $A\beta$ deposits in the brain. Previous studies showed that $A\beta$ levels varied highly in brains of patients with AD, and those with massive amyloid deposition contained predominantly $A\beta 40$.30 In some patients with sporadic AD, high plasma levels of $A\beta 40$ may

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**Figure 2.** Box plots of cerebrospinal fluid levels of amyloid $\beta$ protein ending at residues 40 ($A\beta 40$) (top) and 42 ($A\beta 42$) (bottom) in patients with Alzheimer disease (AD) and control subjects. Symbols are explained in the legend to Figure 1.

**Table 3.** Cerebrospinal Fluid $A\beta 40$ and $A\beta 42$ Levels in Patients With AD and Controls*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Specimens</th>
<th>$A\beta 40$, ng/mL</th>
<th>$A\beta 42$, pg/mL</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 nondemented 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.
†$P < .001$, analysis of variance with Bonferroni correction.
‡$P < .004$, analysis of variance with Bonferroni correction.
contribute to the accumulation of Aβ40 in preexisting plaques.

Age and Apo E genotype are major risk factors for sporadic AD. We found a significant relationship between age and plasma Aβ40 levels in controls and between Aβ40 and Apo E genotype in patients with AD. These results are interesting, since investigators have suggested that higher plasma Aβ40 or Aβ42 levels may play a part in 10% to 20% of sporadic AD before the onset of clinical symptoms. Although our findings showed increased plasma Aβ40 levels in AD with the Apo E ε4 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 Aβ levels in the cross-sectional sample, longitudinal studies will be necessary to determine conclusively whether there is a relationship between plasma Aβ and progression of AD. Such studies are particularly important to determine whether modulation of plasma Aβ may be a useful measure of disease-modifying therapies.

Our CSF results showing lower mean Aβ42 levels in AD are consistent with those of previous reports. 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 and Tamaoka et al, others showed weak or strong 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 the number of plaques and Aβ deposition in the brain. Further studies with a greater number of samples are essential to clarify these controversial findings.

Our findings that CSF Aβ42 levels were lower in patients with AD and the Apo E ε4 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 Aβ42 levels between patients with AD and the Apo E ε4 allele and controls with the allele. The findings are different from those reported by Galasko et al and Hulstaert et al, who reported significant differences. The reason for this discrepancy may be that in our studies, the number of controls with the Apo E ε4 allele is smaller than in the latter studies.

The values of CSF Aβ40 and Aβ42 differed between research laboratories. For example, we found mean CSF Aβ42 levels in AD were 35 pg/mL, in contrast to the range seen from 125 fmol/mL to 833 pg/mL in previous studies. Conflicting findings may result from differences in the affinity of specific Aβ antisera 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 Aβ values decreased over time, even if the CSF samples were frozen. Repeated freezing and thawing of CSF could also result in the loss of Aβ. Several studies have shown that Aβ levels are lower in CSF collected in glass or polystyrene tubes than 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 Aβ 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 Aβ40 and Aβ42 levels in matched plasma, CSF, and autopsy brain tissues and correlation with dementia severity and Apo E genotype are needed to increase our understanding of the significance of plasma and CSF measurements.

Accepted for publication June 2, 1999.

The work was supported by funds provided by New York State through its Office of Mental Retardation and Developmental Disabilities, Albany.

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REFERENCES


