Cerebrospinal Fluid and Serum Beta Secretase Activities in Alzheimer's Disease

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Summary

Objective: In this study, we aimed to investigate β secretase activities in cerebrospinal fluid (CSF) and serum of patients with Alzheimer's disease (AD) as a possible biological marker in AD.

Methods: The serum and the CSF samples were investigated in both patient and control groups. For serum analysis, thirteen patients diagnosed with ‘probable AD’ according to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM IV) and National Institute of Neurological Disorders and Stroke–Alzheimer Disease and Related Disorders (NINCDS/ADRDA) criteria and twelve controls were studied. For CSF analysis twelve patients diagnosed with ‘probable AD' according to DSM IV and NINCDS/ADRDA criteria and nine controls were studied.

Results: There was no statistical difference in CSF β secretase activity between AD patients (mean±S.E., 343.8 ± 47.9) and the controls (mean±S.E., 409.6 ± 34.9). There was also no statistical difference in serum β secretase activity between the patients (mean±S.E., 2298.9 ± 70.3) and the controls (mean±S.E., 2438.2 ± 119.4).

Conclusions: In conclusion, these results indicate that beta-secretase activity is not changed in Alzheimer's disease.

Key words: β secretase, cerebrospinal fluid, serum, Alzheimer's disease

Alzheimer Hastalığında Beyin Omurilik Sıvısı ve Serumda Beta Sekretaz Aktivitesi

Özet

Amaç: Biz bu çalışmada beta sekretaz aktivitesini, Alzheimer hastalığı (AH) için olası bir biyolojik belirteç olarak, Alzheimer hastalarının beyin omurilik sıvılarında ve-serumlarında araştırmayı amaçladık.


Bulgular: Alzheimer hastaları (Ortalama±S.H., 343.8 ± 47.9) ve kontrol grubu (Ortalama±S.H., 409.6 ± 34.9) arasında BOS beta sekretaz aktiviteleri açısından istatistiksel fark yoktu. Alzheimer hastaları (Ortalama±S.H., 2298.9 ± 70.3) ve kontrol grubu (Ortalama±S.H., 2438.2 ± 119.4 ) arasında serum beta sekretaz aktiviteleri açısından istatistiksel fark yoktu.

Sonuç: Sonuç olarak bu rakamlar beta sekretaz aktivitesinin Alzheimer hastalığındaki değişimidirini göstermektedir.

Anahtar Kelimeler: Beta sekretaz, beyin omurilik sıvısı, serum, Alzheimer hastalığı
INTRODUCTION
Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by neuronal death associated with amyloid plaques and neurofibrillary tangle deposits in the brain. By using National Institute of Neurological Disorders and Stroke–Alzheimer Disease and Related Disorders (NINCDS–ADRDA) criteria, the diagnosis of AD can be achieved clinically in 80-90%. The definite diagnosis can only be achieved by postmortem pathological examination of the brain. In the last 10 years our knowledge about the biological basis of AD has improved, but we still need biological markers that help us in the diagnosis of AD, especially in the early stage of disease. It would be greatful to understand which patients will develop AD which patients will not, by just examining blood or cerebrospinal fluid (CSF) sample.

The amyloid plaques and neurofibrillary tangles are present in different neurodegenerative disorders called taupathies, but are also very important in the diagnosis of AD. Amyloid plaques are composed of β amyloid peptides which are derived from the Amyloid Precursor Protein (APP). APP may be proteolysed between positions 671-672 and anywhere between 710-715. As a result 39-43 amino acid peptides known as Aβ are occurred. Cleavage of the Met-Asp bond at 671–672 generates the N terminus of Aβ and is catalysed by a protease activity known as β-secretase. There are two 'β-secretase' proteases known as β-site APP cleaving enzymes 1 and 2 (BACE1 and BACE2). These proteases are widely regarded as being responsible for APP cleavage at the β-secretase site in vivo. In BACE1 knockout rats β amyloid peptides were not detected and accumulation of APP C-terminal fragments C99/C89 were not observed. For that reason, we aimed to investigate BACE as a rate limiting enzyme in APP's amyloidogenic pathway as a possible biological marker in AD.

MATERIAL AND METHODS
Patients
The serum and the CSF samples were investigated in both patient and control groups. For serum analysis, thirteen patients diagnosed with 'probable AD' according to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM IV) and NINCDS/ADRDA criteria and twelve controls were studied. For CSF analysis twelve patients diagnosed with 'probable AD' according to DSM IV and NINCDS/ADRDA criteria and nine controls were studied. The patients underwent a through clinical examination, including medical history, physical, neurological, and psychiatric status, psychological tests, laboratory screening tests, electrocardiography, chest X-rays, brain CT scan, neuropsychological assessment, and a comprehensive cognitive evaluation with the use of the Functional Assessment Staging of Alzheimer's disease and the Mini-Mental State Examination (MMSE). The MMSE was also applied to the control subjects to exclude any cognitive impairment. The study was approved by the local ethics committee and informed consent for participation in this study was obtained from patients or their families and control subjects prior to inclusion.

Cerebrospinal fluid samples
CSF was collected from AD and control cases at the time of initial evaluation. CSF samples were obtained from AD patients in lateral decubitis position by lomber puncture. Control CSF samples were obtained from age-matched patients without history of any neurological disorders and were undergoing spinal anesthesia for any surgery at the Dokuz Eylül University Hospital. 2.5 cc. CSF samples were frozen at -80 °C until assay All samples were analyzed at the same time.

Serum samples
The blood samples were collected for immunological assessment in sterile tubes.
All samples were centrifuged for 10 min at 3,000 rpm, and the sera were frozen at -80°C until assay.

**β secretase assay**
The activity of BACE in CSF and serum was investigated by using a fluorogenic based kit (BACE Activity Assay Kit, Calbiochem, USA).

**Statistical analysis**
SPSS 11.0 program was used in statistical analyses. Statistical analyses results were presented as mean ± SE. Comparison between two experimental groups was based on Mann–Whitney U test. The correlation was made by Spearman rank correlation test. p<0.05 was considered to be significant.

**RESULTS**
There was no difference in age and sex between clinical groups (Table 1,2). The MMSE scores of the AD group were significantly lower than control group (p=0.001, p=0.000) (Table 1,2).

**Table 1**: Demographic data and clinical characteristics of patients with AD and controls (CSF)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=9)</th>
<th>Alzheimer’s Disease (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>8/1</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (mean ± SE)</td>
<td>68.4 ± 3.2</td>
<td>68.8 ± 3.3</td>
</tr>
<tr>
<td>MMSE (mean ± SE)</td>
<td>27.3 ± 0.5</td>
<td>18.6 ± 1.9*</td>
</tr>
</tbody>
</table>

* p= 0.001, significantly different from control subjects.

**Table 2**: Demographic data and clinical characteristics of patients with AD and controls (Serum)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=12)</th>
<th>Alzheimer’s Disease (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>9/3</td>
<td>8/5</td>
</tr>
<tr>
<td>Age (mean ± SE)</td>
<td>65.8 ± 4.1</td>
<td>71.2 ± 2.4</td>
</tr>
<tr>
<td>MMSE (mean ± SE)</td>
<td>27.2 ± 0.6</td>
<td>17.9 ± 1.9*</td>
</tr>
</tbody>
</table>

* p= 0.000, significantly different from control subjects.

There was no statistical difference in CSF BACE activity between AD patients (mean±S.E., 343.8 ± 47.9) and the controls (mean±S.E., 409.6 ± 34.9) (Figure 1). There was also no statistical difference in serum BACE activity between the patients (mean±S.E., 2298.9 ± 70.3) and the controls (mean±S.E., 2438.2 ± 119.4) (Figure 2).

We also evaluated the relation between the clinical parameter of dementia and activity of BACE. There was not any correlation between activity of BACE and clinical parameters.
DISCUSSION
In the present study, we evaluated CSF and serum β-secretase activity in patients with AD since excessive Aβ deposition occurs in AD. Although the cause of this in sporadic cases remains unknown, increased BACE1 activity may play a role.

Searching biomarkers in the CSF seems a logical starting point in AD, so that we firstly analyzed activity of BACE in CSF. We found that the activity of BACE in CSF patients with AD were not different from controls. Previous reports have shown an increase in both BACE-1 protein and activity in AD brain as compared with that of control subjects\(^\text{4,11,20}\). First time Holsinger et.al. showed increased beta-Secretase activity in cerebrospinal fluid of Alzheimer’s disease subjects\(^\text{6}\). We could not confirm their results. Number of patients is the difference between these two studies. Holsinger et al. obtained CSF postmortem and antemortem from five AD, five control cases and four AD, three control cases, respectively. Recently Wu et al. searched CSF beta-Secretase activity in patients with AD. They found that beta-secretase activity is decreased in cerebrospinal fluid of patients with AD.

Lumbar puncture is a relatively invasive procedure, on the other hand, blood is easy to obtain. Also a half liter CSF is absorbed into the blood every day, serum may offer a rich source of AD disease biomarkers. Increasing amount of evidence indicates that AD is a systemic disorder manifesting in non-neural tissues\(^\text{2,14}\). Previously reported that there is elevated platelet beta-secretase activity in subjects with Mild Cognitive Impairment (MCI) and Alzheimer’s dementia\(^\text{9,10,12,19}\). Since serum analysis is easier than platelet analysis, in our study we chose analysing of beta-secretase activity in serum. First time, we have been able to detect beta-secretase enzymatic activity in serum. But we did not find any difference in serum BACE activity between patients with AD and control. Recently reported that platelet Alpha- and Beta- Secretase activities are not significantly affected by Dementia or MCI in Swedish Patients\(^\text{6}\). Beta-secretase activity in platelets correlated with age\(^\text{17}\), but we did not find any correlation between serum BACE activity and age.

Figure 1: The activity of BACE in CSF: The results are given as the proportions of BACE values to the protein levels. There is no statistical difference between the groups. \((p>0.05)\)

Figure 2: The activity of BACE in serum: The results are given as the proportions of BACE values to the protein levels. There is no statistical difference between the groups. \((p>0.05)\)
We also evaluated the relation between the clinical parameter of dementia and activity of BACE. There was not any correlation between activity of BACE and clinical parameters. Johnston et.al. reported that there was not any correlation between platelet membrane beta-secretase activity and MMSE score in the AD group\textsuperscript{(15)}.

In conclusion, while these results indicate beta-secretase activity did not alter in AD, but the numbers in our patient groups were small and a more extensive study is needed to determine whether beta-secretase activity presents a suitable biomarker for AD.

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REFERENCES


