Decreased butyrylcholinesterase and oxytocin levels versus increased dopamine levels in advanced Alzheimer’s patients

Durmus Ayan, Esma Ozmen, Serpil Ersan, Ismail Sari, Etem Erdal Ersan, Sibel Soylemez

Omer Halisdemir University, Research and Training Hospital, Department of Medical Biochemistry, Nigde, Türkiye
Omer Halisdemir University, Faculty of Medicine, Department of Medical Biochemistry, Nigde, Türkiye
Omer Halisdemir University, Faculty of Medicine, Department of Psychiatry, Nigde, Türkiye
Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

ARTICLE INFO

Keywords:
Alzheimer
Dopamine
Oxytocin
Butyrylcholinesterase

Received: Mar 02, 2022
Accepted: May 23, 2022
Available Online: 26.08.2022

DOI:
10.5455/annalsmedres.2022.03.081

Abstract

Aim: In this study, it was aimed to examine the serum levels of dopamine, oxytocin, and butyrylcholinesterase (BchE) enzyme activity in advanced Alzheimer’s disease (AD).

Materials and Methods: For our preliminary study, a total of 40 participants were included in the study. 20 of the participants consisted of patients and the other 20 made up the control group. In the serum samples of the patient and control group, the levels of dopamine and oxytocin were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA) method, while the BchE activity was measured by the spectrophotometric method.

Results: We found that serum BchE and oxytocin levels in the AD group were statistically lower than the controls (p<0.01, p=0.027), whereas serum dopamine levels in the AD group were statistically higher than the control group (p=0.02). Furthermore, we analyzed that there was no significant correlation between the measured parameters (p>0.05).

Conclusion: Our results indicate that the decrease in BchE activity and oxytocin levels and the increase in dopamine levels may have a relationship with the progression of the disease in the AD group. However, we believe that studies are needed with larger numbers of patients with different levels of AD.

Introduction

Alzheimer’s Disease (AD), which is the most common reason for dementia and affects a wide population across the world, is a disease characterized by memory and learning ability loss in the elderly [1]. AD manifests itself with a progressive neurodegenerative disorder, deficiencies in the cholinergic system, beta-amyloid (Aβ) accumulation as neurofibrillary tangles and amyloid plaques [2,3]. It is also known that AD pathology is characterized by chronic brain inflammation that subsidizes disease pathogenesis [4]. The butyrylcholinesterase enzyme (BchE) (EC 3.1.1.8) is a non-specific enzyme that is also known as "pseudo" cholinesterase and that can hydrolyze the different types of choline esters. BchE activity is present in many tissues in the organism, but it also has important functions in the liver, pancreas, central nervous system, and serum [5]. AD is histopathologically characterized by the formation of senile amyloid plaque, and BchE has been reported to have played an incentive role for Aβ aggregation in the early stages of synonym plaque formation [6]. BchE shows structural homology with Aβ and this is the underlying reason for the additive effect in the Aβ aggregation of BchE. These structural similarities make it easy to create the BchEβ- Aβ complexes and form much more neurotoxic structures [7]. Namely, BchE can interact directly with Aβ and increase Aβ accumulation in unsolved plaques. This process plays an important role in the pathology of AD [8]. The lack of spatial orientation in advanced Alzheimer’s patients of low plasma cholinesterase activity is associated with the reduction in the ability to perform basic activities of everyday life and the life expectancy [9]. Approximately 80 of 200 peptides, known to exist in the body, functions as neurotransmitters, and approximately 20 of them are associated with AD [10]. Dopamine and oxytocin (OXT) are also among these AD-related neuro-
transmitters. OXT is produced in neurons of the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei and in much smaller cells of the suprachiasmatic (SCN) nucleus [11]. OXT is not only a hormone that plays a role in reproduction but a hypothalamic neuropeptide that regulates facial recognition and social interaction in the brain [12]. The progress of AD pathogenesis is thought to be negatively contributing to cell loss in the PVN and SCN areas that produce OXT [13]. A study conducted on animal models shows that the increase in Aβ in the hippocampus, the main learning and memory center of the brain, causes a decrease in the signal transmission potential of neurons here. It has been reported that this degeneration has affected a particular characteristic of neurons called "synaptic plasticity", which is the ability of synapses to adapt to an increase or decrease in signal activity over time. In the study, an increase in the signal transmission potential of neurons of animals injected with OXT into the hippocampus region was detected. As a result, OXT is thought to be able to improve its adaptability in synaptic plasticity which has a role in the pathogenesis of AD [13].

It is known that oxytocin increases the dopaminergic activity in the mesocorticolimbic dopamine system, which is very important for the expression of affiliation representation, even if it is not fully clear with which neuronal mechanisms do oxytocin affect learning, memory and behavioral situations [14]. As it is known that the decrease in brain dopamine levels is generally associated with behavioral and psychiatric disorders that occur during the pathogenesis of the disease. It has been determined that, along with the advancement of AD and the damage of other neuronal pathways, brain dopamine levels have decreased over time. Therefore, the dysfunctions that occur in the dopaminergic system are a significant part of AD pathology [15].

Dopamine is not only released from the nerve endings as a neurotransmitter but also produced by the adrenal gland as catecholamine. In normal conditions, serum dopamine levels are very low while plasma dopamine is largely produced by sympathetic noradrenergic nerves [16]. Furthermore, the cholinergic system in the hippocampus plays an important role in organizing the catecholamine metabolism in the periphery. In AD, the degeneration of the cholinergic system also affects the levels of catecholamines (dopamine, noradrenaline, and norepinephrine) [17]. In light of these data, in our study, we examined the plasma dopamine, oxytocin levels, and serum BchE enzyme activity in patients with AD to better understand the possible changes in the advanced stage of the disease.

Materials and Methods
This study was approved by the Local Ethics Committee of Niğde Ömer Halisdemir University Faculty of Medicine (date: January 13, 2022; approval number: 2021-123) and conducted in accordance with the Helsinki Declaration. Our study is a prospective preliminary study, including a total of 40 participants, including 20 advanced Alzheimer’s patients who applied to Niğde Training and Research Hospital Community Mental Health Center, and 20 healthy volunteers with simple random sampling method.

Inclusion criteria
It is formed of individuals diagnosed with AD after the clinical examination that was conducted using the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM), published by the American Psychiatric Association (APA). The Mini-Mental Test (MMT) was applied to evaluate the patients’ cognitive states after the evaluation of their physical examination findings. The patients whose scores were below the threshold value of 17 of the MMT, which has an interval of score range between 0-30, are considered to be advanced Alzheimer’s patients.

Exclusion criteria
Those with an MMT value of 17 and above, those who use cholinesterase inhibitors, have liver failure, and with neurodegenerative diseases other than Alzheimer’s disease, have been excluded from the study.

Blood samples collected from patient and control groups were centrifuged at 4000 rpm for 10 minutes and were preserved until the study day at -20 degrees. On the study day, serum samples obtained from the patient and control group were first held at +4 degrees, and then at room temperature for a period to be dissolved.

In the serum samples of the patient and control group, the levels of dopamine and oxytocin were measured by the Enzyme-Linked ImmunoSorbent Assay (ELISA) method, while the BchE activity was measured by the spectrophotometric method in Roche Cobas c500 biochemistry autoanalyzer. Sera dopamine (catalog no: CEB851Ge) and oxytocin (catalog no: CEB052Ge) levels of the study group were determined by using an ELISA kit (Cloud-Clone Corp. CCC, USA) according to the instruction of the manufacturer. The minimum detectable dose of oxytocin is typically less than 4.99 pg/mL. Intra-Assay CV is <10% and Inter-Assay CV is 12% for oxytocin. The minimum detectable dose of dopamine is typically less than 4.71 pg/mL. Intra-Assay CV is <10% and Inter-Assay CV is 12% for dopamine. The lowest measurable analyte level for butyrylcholinesterase is 100 U/L. For the intra-study accuracy values, CV values at two different levels were determined as 0.5%, while the inter-study accuracy CV value was determined as 2.6% and 1.1% for two different levels, respectively. Serum indexes such as hemolysis, icterus, and lipemia were measured for each sample. If the indexes measured were above the limits set in the kit insert determined by the manufacturer firm, the sample was excluded from the study.

Statistical analysis
The SPSS software version 15.0 (SPSS Inc. Chicago, IL) statistical package software was used. G power 3.1.6 for Windows (HeinrichHeine-Universität Düsseldorf, Düsseldorf, Germany) was used for the power analysis. The minimum required size of the study population was calculated to be 40 subjects in for a large effect size with at the 95% confidence interval for α = 0.05.

Whether the data show normal distribution or not was determined by Kolmogorov Smirnov, Shapiro-Wilk, Skewness, Kurtosis values, and Histogram graphics. Since the numerical variables did not met the normal distribution
Table 1. The age and gender distributions of the patient and control groups.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Alzheimer’s Patients (n=20)</th>
<th>Control Group (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± standard deviation</td>
<td>mean ± standard deviation</td>
<td></td>
</tr>
<tr>
<td>80 ±8.97</td>
<td>75.8 ±9.42</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer’s Patients</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>7 (35%)</td>
<td>13 (65%)</td>
<td>8 (40%)</td>
<td>12 (60%)</td>
</tr>
</tbody>
</table>

Table 2. The median and interquartile range of Alzheimer’s patients and control group for dopamine, oxytocin and BchE.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Alzheimer’s Patients Median (IQR)</th>
<th>Control Group Median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine pg/mL</td>
<td>2.64 (2.53-2.94)</td>
<td>2.29 (2.03-2.62)</td>
<td>0.002</td>
</tr>
<tr>
<td>Oxytocin pg/mL</td>
<td>1.71 (1.10-2.33)</td>
<td>2.45 (1.68-2.65)</td>
<td>0.027</td>
</tr>
<tr>
<td>BchE IU/L</td>
<td>5220 (397-6650)</td>
<td>7700 (7100-8620)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

IQR: interquartile range.

Results

The age and gender distributions of the patient and control groups in our study group are shown in Table 1.

When our results were analyzed, the serum dopamine levels of the group with advanced Alzheimer’s disease were statistically higher compared to those of the healthy control group. On the other hand, serum oxytocin levels and BchE activity were statistically lower in the group with Alzheimer’s disease compared to the control group. (Table 2) When the results of the correlation analysis were examined, there was a statistically significant correlation between Dopamine and oxytocin and BchE levels in the group with Alzheimer’s disease [(r=0.09, p=0.677), (r=0.072, p=0.762), respectively]. When the results of the control group correlation analysis were analyzed, there was a statistically negative correlation between dopamine and oxytocin (r=−0.553, p=0.011), while there was no statistical correlation between dopamine and BchE (r=−0.307, p=0.188).

Discussion

In this study, levels of serum dopamine oxytocin, and BchE activity were examined in patients with advanced AD. When the data was analyzed, our first finding was that serum dopamine levels were higher in Alzheimer’s patients than those in the healthy control group. Another noteworthy finding was that the levels of serum oxytocin and BchE enzyme activity were lower in the patients with advanced AD than the healthy controls despite among these patients nobody using any cholinesterase inhibitor. Since most of the studies aimed to study brain dopamine, oxytocin, and BchE levels and the determination of serum levels of these parameters in a very limited number of studies increases the importance of our study.

It has been confirmed by previous studies that brain dopamine levels are low in advanced Alzheimer’s patients [18,19]. One of the main centers of dopamine production in the brain is the Ventral Tegmental Area (VTA), and it is believed that VTA undergoes atrophy over time, and dopamine production is also reduced with the progression of the disease in Alzheimer’s patients. Studies on VTA’s size and memory, along with the pathologies that lead to mild cognitive disorder and dementia, have argued that decreased dopaminergic VTA activity may be important in determining early Alzheimer’s disease [18]. Results of a large meta-analysis, in which the relationship between AD and brain dopamine levels also reported that the decreased brain dopamine levels are associated with the pathophysiology of the disease [19]. While the data on the brain dopamine levels are as such, it was reported that the free dopamine levels found in the plasma were largely produced from sympathetic noradrenergic nerves [20]. When the neuronal sources of plasma dopamine levels were researched, the data found in a study confirmed that extreme stimulus, especially in sympathetic neuronal pathways, even other than the neurodegenerative diseases, have increased the plasma dopamine levels while reducing the plasma noradrenaline levels [17]. Sympathetic nervous system activity increases in AD, which is a neurodegenerative disease. In this context, cognitive decline arising in the early stages of AD may be related to sympathetic nervous system disorders [21]. Dopamine is also synthesized from the adrenal medulla as catecholamine. In a study comparing the levels of plasma catecholamine in women patients with Alzheimer’s-type dementia, vascular dementia, and patients with no dementia, plasma dopamine levels in non-dementia patients were observed to be slightly higher than patients with Alzheimer’s type dementia and vascular dementia, but this value was found not statistically significant [22]. When the urine-discarded metabolites of catecholamines produced by the adrenal gland were examined in individuals with AD, it was reported that the levels of urine catecholamine were lower than those in the healthy control group [16]. In addition to this data, studies in neurodegenerative diseases other than AD showed that serum dopamine levels are also reduced, especially with the increased motor dysfunction[dysfunction [23-25]. Contrary to these studies, in our study, it was found that the serum dopamine levels of the group with advanced AD were statistically higher compared to those in the healthy control group. In these contradictory findings, the differ-
ences in the number of individuals in the study group and methodology between studies, and our patient group consisted of all advanced AD may have an effect. Therefore, there is a need for extensive and additional studies on this subject. Although OXT has been reported with studies in the regulation of learning and logical memory performance [26,27] the number of studies that have been conducted so far on OXT’s impact on Aβ-induced cognitive distortion is very few [28]. The low levels of OXT in the cerebral spinal fluid (CSF) of Alzheimer’s patients compared to healthy individuals, supports the hypothesis that central vasopressinergic activity decreases in Alzheimer’s patients [29]. On the other hand, it has been reported that there is no significant difference in the levels of serum OXT between healthy voluntary and patients with Huntington’s disease, which is a neurodegenerative disease that has many common points with AD pathophysiology [30]. While it has been observed that the intranasal OXT application in patients with frontotemporal dementia, which has similar aspects to dementia developed during Alzheimer’s disease, may have caused some signal increases in the limbic and frontal regions of patients, further investigation into the issue is recommended to improve symptoms of frontotemporal dementia [31]. As it is known, OXT is a neuropeptide with both central and peripheral effects [32]. The lack of evidence to use the serum OXT levels in the early stages of Alzheimer’s disease for early diagnosis has created the basis for the examination of the serum OXT levels in the advanced stages of Alzheimer’s disease [32]. Because there are no studies that examine the serum OXT levels in advanced Alzheimer patients, we examined the serum OXT levels in advanced Alzheimer’s patients in our study and observed that OXT levels were low compared to the levels of the healthy participants.

Some clinical studies have reported that activities of both BchE and acetylcholinesterase (AChE) enzymes have increased in AD [33,34]. In addition to increased serum BchE activity levels in Alzheimer’s patients, low BchE activity levels have been reported in some studies [9,35,36]. It was suggested that a reduced level of BchE enzyme activity could be associated with the level of the cognitive defect and may be used as a prognostic marker [34]. Interestingly in a study of the course and mortality of the disease, three out of four patients with reduced BchE enzyme levels along with the progression of AD were reported to have lost their lives within a year [9]. In another study examining the relationship between BchE enzyme activity and life longevity in AD, its low serum BchE activity levels in 45% of the patients with AD who have lost their lives supports the previous study data [35]. Serum BchE enzyme activity level has been also examined in patients with dementia associated with Parkinson’s disease, which has another neurodegenerative disease, and low enzyme activity has been detected in the patient group. In an old study, it was reported that there was no significant change in the serum BchE levels between Parkinson’s patients and the healthy control group [37]. Our finding that BchE enzyme activity levels were significantly lower in advanced AD patients compared to the control group supports the idea that the activity of this enzyme decreases in parallel with the worsening of AD prognosis, and suggests that BchE enzyme activity may be an important marker for the prognosis of the disease.

**Conclusion**

Contrary to previous studies, further studies are needed to clarify the underlying causes of serum dopamine levels in advanced Alzheimer’s patients compared to the control group and to illuminate mechanisms that lead to high dopamine levels. In addition, the number of studies in which the serum OXT level, which we thought maybe important in the pathology of Alzheimer’s disease, is very few and insufficient. In our study, we examined the serum OXT levels in advanced Alzheimer’s patients. In this regard, additional studies are required in different stages of Alzheimer’s disease to better understand the relationship of serum OXT levels with the prognosis. Finally, BchE enzyme activity, which we determined to be significantly lower in advanced AD patients, maybe a useful marker for the prognosis of the disease. Since our study is a preliminary study, we believe that it will be a guide for researchers to plan new studies.

**Study limitations**

While our study had a good plan, there were some limitations. Firstly, we only conducted our study in advanced Alzheimer’s patients. After groups are formed according to the stages of the disease, changes in serum dopamine, OXT, and BchE enzyme levels and the relationship between the prognosis of the disease that these markers can be evaluated. In addition, the relationship of these markers with the life expectancy of patients can be examined. Secondly, the number of patient and control groups can be increased. Since dopamine levels are questionable in the serum, studies for the source of serum dopamine levels in these patients can be planned.

**Ethics approval**

This study was approved by the Local Ethics Committee of Niğde Ömer Halisdemir University Faculty of Medicine (date: January 13, 2022; approval number: 2021-123) and conducted in accordance with the Helsinki Declaration.

**References**


