

Original Research Article

Brain-Derived Neurotrophic Factor Serum Levels and Hippocampal Volume in Mild Cognitive Impairment and Dementia due to Alzheimer Disease

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Keywords

Alzheimer disease · Blood biomarker · Brain atrophy · Hippocampus · Mild cognitive impairment

Abstract

Background/Aims: Hippocampal atrophy is a recognized biomarker of Alzheimer disease (AD) pathology. Serum brain-derived neurotrophic factor (BDNF) reduction has been associated with neurodegeneration. We aimed to evaluate BDNF serum levels and hippocampal volume in clinical AD (dementia and mild cognitive impairment [MCI]). **Methods:** Participants were 10 patients with MCI and 13 with dementia due to AD as well as 10 healthy controls. BDNF serum levels were determined by ELISA and volumetric measures with NeuroQuant[®]. **Results:** MCI and dementia patients presented lower BDNF serum levels than healthy participants; dementia patients presented a smaller hippocampal volume than MCI patients and healthy participants. **Discussion:** The findings support that the decrease in BDNF might start before the establishment of neuronal injury expressed by the hippocampal reduction.

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Introduction

Alzheimer disease (AD) is associated with hippocampal atrophy and memory decline [1, 2]. Memory impairment is one of the most common symptoms in patients in the early stages of AD; it is characterized by explicit memory deficit, particularly episodic and working

memory functions [3]. These deficits are reflective of the involvement of the hippocampal formation [4]. Impaired verbal and episodic memory were correlated with reduced volume of the hippocampal formation and entorhinal cortex, which may be reduced by 25% using volumetric scanning [5]. Atrophy was most marked in the entorhinal cortex and superior temporal gyrus and extended to the anterior cingulate [5].

Recently, there has been a conceptual shift towards the proposal that AD exists before the onset of dementia [6]. Therefore, a clinical phenotype of memory/cognitive impairment without significant functional impairment, called mild cognitive impairment (MCI), was included in the diagnostic criteria of the International Working Group [7] and the National Institute of Aging-Alzheimer's Association (NIA-AA) [6]. The NIA-AA criteria also proposed several biomarkers to support this prodementia or prodromal AD diagnosis. Among these biomarkers, structural neuroimaging with atrophy of the medial temporal region as positive diagnostic information was included. Combinations of distinct biomarkers, including hippocampal volumetry, present a good predictive value to differentiate AD from normal aging in patients with MCI [6].

Brain-derived neurotrophic factor (BDNF), a neurotrophin highly expressed in the hippocampus [8], is a vital component of synaptic plasticity and memory formation [9, 10]. BDNF has been related to hippocampal plasticity [9, 11] and its action plays a central role in the mechanism of long-term potentiation – one of the most accepted models for memory formation – resulting in structural synaptic changes [9, 10]. However, despite all the available information, there is no consensus about the serum BDNF profile in neurodegenerative diseases, particularly AD. While some studies have reported lower BDNF serum levels in AD patients in comparison with healthy subjects [12–14], other studies demonstrated higher BDNF serum levels in AD individuals [15] or no difference between AD and controls [16].

Likewise, studies on cerebrospinal fluid (CSF) BDNF levels in AD and other dementias report conflicting results. Forlenza et al. [17] demonstrated decreased CSF BDNF concentration associated with progression to AD in amnesic MCI patients. Blasko et al. [18] did not find a significant difference in BDNF between AD and controls, and Laske et al. [19] showed lower CSF BDNF concentrations among AD patients in comparison to healthy controls and non-AD dementia patients. Furthermore, a study with healthy older adults showed an association of lower CSF BDNF levels with poorer memory performance and faster cognitive decline [20].

The effect of the BDNF genetic variability or circulating levels of BDNF on memory and hippocampal volume in healthy older adults and in individuals with MCI or early dementia has also been evaluated showing discrepant results. Among individuals with amnesic MCI and high amyloid- β accumulation, BDNF Val66Met carriers showed a significant and larger decline in episodic memory and hippocampal volume [21]. A significant correlation between lower circulating BDNF and memory deficit was demonstrated in a cohort with MCI participants [14]. BDNF serum levels and hippocampal volume were found to positively correlate in a study with healthy older adults [22], but this relation was not further observed [23]. Additionally, no significant BDNF effect, in terms of genotype or plasma, in both hippocampal volume and memory was found in 2 large independent middle- and old-age cohorts of cognitively normal and early-stage dementia participants [24]. Finally, aging itself is associated with reduced hippocampal volume, lower levels of serum BDNF, and poorer memory performance [22].

Therefore, the objective of the study was the evaluation of BDNF serum levels and hippocampal volume in individuals with MCI and dementia due to AD, as well as in healthy elderly participants. The relationship between BDNF serum levels and hippocampal volume was also evaluated in the whole sample.

Methods

Participants

The sample was composed of 10 healthy elderly individuals, 10 participants with MCI, and 13 with dementia due to AD. Healthy elderly individuals were community-dwelling residents who were accompanying Internal Medicine patients, were functionally independent, and presented a Mini-Mental State Examination (MMSE) score >26, cognitive performance above the cutoffs for this population, and a Clinical Dementia Rating Scale score of 0. The NIA-AA core clinical criteria for MCI due to AD were used to identify the MCI group: (1) cognitive concern reflecting a change in cognition reported by the patient or informant or clinician (i.e., historical or observed evidence of decline over time); (2) objective evidence of impairment in 1 or more cognitive domains, typically including memory (i.e., formal or bedside testing to establish level of cognitive function in multiple domains); (3) preservation of independence in functional abilities; and (4) not demented. In addition, vascular, traumatic, medical causes of cognitive decline and the autosomal dominant form of AD were ruled out by clinical history [6]. All dementia patients met the NIA-AA criteria for probable dementia due to AD [25]. Patients (MCI and dementia) were selected from the Dementia Outpatient Clinic of the Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

All subjects underwent a clinical history and physical examination, the MMSE [26, 27], the Clinical Dementia Rating Scale [28–30], the Activities of Daily Living and Instrumental Activities of Daily Living functional scales [31] and a multidomain cognitive evaluation. This cognitive battery assessed episodic memory, executive function, attentional control, visuospatial skills and language by the Rey Auditory Verbal Learning Test [32], the figure copying and the CERAD word list test [33], the clock-drawing test [34], the Trail Making Test [35], the digit span forward [36], and the letter and category fluency [37]. The available age and educational norms were considered to evaluate the scores of each test.

All participants and/or a proxy gave written informed consent and all procedures were approved by the Hospital de Clínicas de Porto Alegre Research Ethics Committee (# 110275).

BDNF Measurement

Blood nonfasting samples were collected in serum-separating tubes during clinical evaluations, centrifuged, aliquoted, and stored at -80°C .

BDNF levels in serum samples were determined by sandwich ELISA using monoclonal antibodies specific for BDNF (R&D Systems, Minneapolis, MN, USA). Briefly, microtiter plates were coated overnight at room temperature with the monoclonal anti-BDNF antibody at $4\ \mu\text{g}/\text{mL}$ in PBS. Then, plates were washed 3 times with wash buffer and blocked for 1 h at room temperature with PBS containing 5% nonfat milk powder. After washing, plates were coated overnight at 4°C with the samples diluted 1:200 in sample diluent (PBS with 1% BSA) and the standard curve ranged from 7.8 to $500\ \text{pg}/\text{mL}$ of BDNF. Plates were washed again and biotinylated anti-BDNF antibody at $0.2\ \mu\text{g}/\text{mL}$ in PBS was added, which was incubated for 2 h at room temperature. After washing, incubation with streptavidin-peroxidase conjugate (diluted 1:1,000 in sample diluent) for 1 h at room temperature was performed. Plates were washed and incubated with the substrate for 20 min at room temperature. Finally, the stop solution was added and the amount of BDNF was determined by absorbance at 450 nm. The standard curve demonstrated a direct relationship between optical density and BDNF concentration.

Magnetic Resonance Imaging Acquisition

Magnetic resonance imaging (MRI) data were obtained in a Philips Achieva 1.5-Tesla scanner (Amsterdam, the Netherlands). T1 high-resolution sagittal 3D magnetization-prepared rapid acquisition gradient echo was acquired with NEX = 1, image matrix = $256 \times$

Table 1. Demographic and clinical data of the studied groups

Variables	Healthy participants (n = 10)	MCI (n = 10)	Dementia (n = 13)	p value
Age, years ^a	68.60±4.62	69.20±7.88	76.08±6.08*	0.012
Female, n (%) ^b	6 (60%)	6 (60%)	10 (76.9%)	0.602
Education, years ^a	12.20±6.37	9.60±6.07	6.92±6.17	0.145
CDR-SB ^a	0.24±1.14	1.39±1.13	7.43±1.06**	0.001
MMSE score ^a	28.85±1.14**	24.88±1.12**	16.63±1.05**	0.001

CDR-SB, Clinical Dementia Rating Scale Sum of Boxes; MMSE, Mini-Mental State Examination. ^a One-way ANOVA with Bonferroni post hoc test. ^b χ^2 test with Fisher exact test. * p < 0.05; ** p < 0.001.

232, flip angle = 8°, TE = 4 ms, TR = 8.63 ms, and voxel size = 1 × 1 × 1 mm³ yielding 160 slices. Axial fluid-attenuated inversion recovery was acquired with TR = 11,000 ms, TE = 140 ms, TI = 2,800 ms, turbo factor = 55, EPI factor = 1, NEX = 3, slice thickness = 5 mm, and matrix = 186 × 512 providing pixel measurements of 1 × 0.86 mm given the 220 × 220 mm field of view. Study time for this version of fluid-attenuated inversion recovery was 2 min and 56 s.

Volumetric Analysis

We obtained volumetric measurements from the high-resolution anatomic images using NeuroQuant[®] (CorTechs Labs Inc., San Diego, CA, USA), which is a fully automated program that has been validated against other segmentation procedures and found sensitivity to volumetric changes in mild AD [38] and MCI [39]. This program provides volumes for 2 medial temporal lobe regions (hippocampus and inferior lateral ventricles). NeuroQuant (automated) analyzes 3D MRI scans offering accurate, consistent, and reproducible measures and segmentations of brain subcortical structures comparing them with standardized values according to age, sex and cranial volume. The NeuroQuant computer-automated analysis routinely provides volume data on 9 brain regions, left and right sides, for a total of 18 volume measurements (<http://www.cortechs.net/products/neuroquant.php>). For the present study, we only used the hippocampal and the forebrain parenchyma volumes. The volume of the hippocampus was analyzed as the sum of the right and left hippocampus (total volume), as well as the forebrain parenchyma (total volume).

Statistical Analysis

Statistical analyses were performed using PASW Statistics 18.0 for Windows (SPSS, Chicago, IL, USA). Descriptive statistics (mean, SD and frequency) were calculated for demographic, cognitive and MRI data. The χ^2 test with the Fisher exact test were used for categorical variables, and the Spearman nonparametric correlation was also performed. ANCOVA was used for the analyses controlling for age as covariate. The Bonferroni test was used for the post hoc comparisons.

Results

Demographic and clinical data are presented in Table 1. Dementia patients were significantly older than MCI and healthy subjects. Moreover, they presented lower MMSE and higher Clinical Dementia Rating Scale Sum of Boxes scores.

Table 2. Age-controlled comparison of BDNF serum levels and MRI volumetric data among studied groups (ANCOVA with Bonferroni post hoc test)

Variables	Healthy participants (n = 10)	MCI (n = 10)	Dementia (n = 13)	F	p
BDNF level, pg/mL	26.25 ± 2.90*	20.57 ± 2.93	14.59 ± 2.75	3.72	0.036
Forebrain parenchyma volume, cm ³	949.80 ± 27.22	926.40 ± 26.86	860.55 ± 25.21	2.73	0.082
Total hippocampal volume, cm ³	6.48 ± 0.23	6.29 ± 0.26	4.91 ± 0.24**	9.74	0.001

BDNF, brain-derived neurotrophic factor; MCI, mild cognitive impairment. * $p < 0.05$; ** $p < 0.001$.

Table 2 shows the comparison of BDNF serum levels, hippocampal and forebrain parenchyma volumes among the 3 groups. Healthy participants had higher BDNF levels ($p < 0.001$) than dementia and MCI patients. The dementia group presented a significantly lower total hippocampal volume than MCI and healthy participants ($p < 0.001$), while the 2 latter groups did not differ. No significant difference in the total forebrain parenchyma volume (cm³) among the groups was observed. All analyses were age-adjusted.

To strengthen the results, the correlation analysis was carried out in the whole sample, allowing higher variability of each variable and a larger sample size. However, no significant correlation of serum BDNF with hippocampus volume was observed (Spearman rho = -0.04; $p = 0.87$).

Discussion

The present study was carried out to evaluate BDNF serum levels and hippocampal volume in healthy elderly participants and in patients with MCI and dementia due to AD according to the NIA-AA criteria [6, 25]. We also aimed to analyze the correlation of serum BDNF and hippocampal volume in the whole sample. We have hypothesized a decreasing gradient of BDNF serum levels and hippocampal volume from healthy participants toward MCI and dementia due to AD, based on the following assumptions: (a) BDNF plays a central role in long-term memory potentiation mechanism [9, 10]; (b) memory impairment is an early and typically clinical phenotype of AD pathology [6, 7]; (c) episodic memory impairment and hippocampal volume reduction are associated [1–3, 40]; and (d) hippocampal volumetry was included in the set of biomarkers proposed to differentiate AD from normal aging in patients with MCI. We also hypothesized a relation of BDNF levels with hippocampus volume, taking into account the high expression of BDNF in the hippocampus [8] and its relation with hippocampal plasticity [9, 11]. Our main findings are: (1) healthy participants present significantly higher BDNF serum levels than patients with MCI and dementia due to AD; (2) dementia patients present a significantly smaller hippocampal volume than MCI and healthy participants; and (3) BDNF serum levels do not correlate with hippocampal volume. Therefore, our prior hypotheses were partially confirmed.

Healthy participants presented higher BDNF serum levels than MCI and dementia patients. Patients with MCI and those with dementia due to AD presented similar levels. Our findings are similar to the group of studies reporting higher BDNF levels in healthy elderly individuals and reduced levels in MCI and AD patients [12–14, 24, 41]. However, there is no consensus on the pattern of serum BDNF in these conditions, because another set of studies reported higher [15, 42] or similar BDNF levels [16, 43, 44] in patients with AD. Nonetheless,

our findings support the reduction of serum BDNF in neurodegenerative disease, such as MCI and dementia due to AD. In the study of Forlenza et al. [17], reduced CSF BDNF concentrations predicted progression of MCI to AD, suggesting the involvement of BDNF in the pathophysiology of neurodegenerative changes in AD.

Analyzing brain volumes, we found a higher hippocampal than forebrain reduction among dementia patients, but not among MCI and healthy participants, emphasizing the disproportional atrophy of these regions in mild stages of dementia due to AD (according to the Clinical Dementia Rating Scale Sum of Boxes distribution). The specific hippocampal atrophy in AD has already been encompassed by the NIA-AA diagnostic criteria recommendation as a structural biomarker of early AD pathology and a predictor of progression [6, 25, 45]. Looking particularly at the MCI findings, no significant difference in hippocampal volume was found as compared to healthy participants. This could be explained by the use of the NIA-AA MCI core clinical criteria in the present investigation, a syndrome defined by clinical, cognitive, and functional criteria. The NIA-AA group reported that, similar to dementia due to AD, other etiologies in addition to the AD pathophysiological process may coexist in an individual who meets the criteria for MCI due to AD [6]. Such heterogeneity could have impacted the progression of neuronal injury, measured by the volume of the hippocampus, in our MCI patients. Although MCI clinical criteria are recommended to be applied in clinical settings, they are different from MCI research criteria which include a set of neuroimaging and CSF biomarkers [6]. Among these biomarkers, hippocampal volumetry is one of them. Then, starting with the clinical identification of cases we cannot expect full biomarker positivity in all MCI patients. Levels of certainty according to the presence and nature of the biomarker findings were also proposed for the final set of criteria for MCI due to AD [6]. Finally, although the automated program used as segmentation procedure for the volumetric measures is valid and sensitive [38, 39], automated hippocampal segmentation algorithms need to be validated against the manual segmentation gold standard [46].

It is accepted that reduced BDNF serum levels may correspond to the lack of neurotrophic support due to increased amyloid- β accumulation contributing for the progressive degeneration of specific brain regions affected by Alzheimer pathology [19]. Taking our results together (lower BDNF levels in MCI and dementia due to AD and smaller hippocampal volume in dementia due to AD), we can hypothesize that the decrease in BDNF may start before the establishment of neuronal injury expressed by the hippocampal reduction.

The BDNF can cross the blood-brain barrier in both directions and BDNF in the blood and in the cerebral cortex showed a positive correlation in animal studies [47, 48]. The hippocampus is one of the main regions of BDNF expression in the cerebral cortex, supporting the a priori hypothesis of the present study. However, our findings refute this hypothesis since no relationship of BDNF serum levels with hippocampal volume was found. Therefore, serum BDNF did not seem a consistent marker for hippocampal atrophy.

In agreement with our result, another investigation showed BDNF serum levels reflecting some aspects of neuronal integrity, although no significant relationship between BDNF serum concentrations and any hippocampal brain metabolites were observed [49]. Furthermore, we did not analyze the effect of the variation of the BDNF gene (Val66Met substitution) on the hippocampus volume as already explored. However, these studies are not conclusive since some showed an association [50] or no relation of this genetic variability or of the BDNF-circulating levels with the hippocampus volume in healthy and early dementia subjects [24]. An early effect of the reduction of BDNF on hippocampal volume, i.e. neuronal injury, would be another possibility to explain this finding.

The current study has limitations such as the small sample size and the cross-sectional design preventing another data approach as well as the longitudinal assessment of these patients. However, some methodological strengths of the study should be emphasized. The

comparison of BDNF levels and hippocampal volume in the 3 groups was age-adjusted, since age alone can affect the hippocampal volume, serum BDNF, and memory performance [22]. In addition, the use of neuropsychological tests such as the Rey Auditory Verbal Learning test to measure episodic memory impairment (i.e., the ability to acquire and retain new information), which have age and gender norms for the Brazilian population, provided higher reliability in establishing the clinical AD phenotype.

Finally, this is an exploratory investigation that supports the role of reduced BDNF serum levels (easily measurable) in amnesic phenotypes, as well as the established neuronal injury detected by the hippocampal loss in dementia due to AD. The relation of circulating BDNF with hippocampal volume was not observed, but this issue deserves further investigation with larger samples, a longitudinal design and BDNF cerebrospinal measures.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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