Cerebrospinal Fluid Biomarkers in Alzheimer's Disease and Geriatric Depression: Preliminary Findings from Brazil

Taylor Reis,¹ Carlos Otavio Brandão,¹ Evandro Silva Freire Coutinho,² Elias Engelhardt³ & Jerson Laks¹

¹ Institute of Psychiatry of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

² National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

³ Institute of Neurology of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Keywords

Alzheimer's disease; β -amyloid; CSF; Dementia; Major Depression; P-tau; T-tau; Tau.

Correspondence

Jerson Laks, Av. Copacabana 749/802,
22050-001 Rio de Janeiro, RJ, Brazil.

Tel.: +55 21-2255-3454;

Fax: +55 21-2548-4510;

E-mail: jlaks@centroin.com.br

Received 7 October 2011; revision 4 January

2012; accepted 12 January 2012

doi: 10.1111/j.1755-5949.2012.00311.x

SUMMARY

Aims: Depression is a highly prevalent disorder in the elderly and one of the risk factors for developing dementia. The present study involves patients with Alzheimer's disease (AD), geriatric major depressive disorder (MDD) and cognitively healthy controls aiming to compare baseline cerebrospinal fluid (CSF) biomarkers. **Methods:** The study included 52 patients with more than 60 years of age with a diagnosis of MDD, AD, and healthy controls. All individuals underwent a medical history, physical, and neurologic examination, laboratory tests and neuropsychological assessment to rule out any clinical diseases or disorders. Measurement of CSF P-tau₁₈₁, T-tau, and A β 42 was performed using commercial assays (ELISA). **Results:** CSF A β 42 levels of depressed patients and normal controls were significantly higher than in AD. There was not any significant difference in measures of P-tau among the groups. T-tau, however, showed to be significantly different among the groups, with higher measures in AD group. Higher levels of P-tau were observed in four MDD patients compared with controls. **Conclusion:** CSF A β 42, T-tau, and P-tau levels may differentiate between AD and depression in a Brazilian sample.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative dementia [1], affecting nearly 10% of the population over 65 years of age [2, 3]. The characteristic findings at the microscopic level are degeneration of the neurons and their synapses together with extensive amounts of senile plaques and neurofibrillary tangles [4]. Neuronal degeneration, plaques, and tangles deposits start early in the preclinical phase and increase their amount until they reach a certain threshold to yield the first symptoms [5]. Depression, in turn, is not only a common and highly prevalent disorder in the elderly [6] but is one of the risk factors for developing dementia as well. According to epidemiological studies, depressive elderly present a 4-fold increased risk for dementia [7,8]. There are also consistent data to confirm that depression is one of the clinical presentations of a prodromal phase of a preclinical dementia [9,10]. Consequently, the clinical differential diagnosis between AD and depression is often a difficult task on clinical grounds [11].

Several studies have shown the clinical usefulness of biological markers in the cerebral spinal fluid (CSF) for the differential diagnosis between degenerative dementia and other neurological and psychiatric disorders [including depression, mild cognitive impairment, and normal aging; Refs. 12–15]. Amyloid beta (A β), total tau (T-tau) and phosphorylated tau (P-tau) are the most important

markers with regards to neurodegenerative diseases [16]. Overall, a moderate to marked decrease of CSF A β 42 levels has been found in patients with AD compared with controls [13].

CSF level of P-tau probably reflects the phosphorylation state of tau [16]. These data suggest that P-tau in CSF is not simply a marker for neuronal degeneration or damage but it specifically reflects the phosphorylation state of tau and, thus, possibly the formation of tangles in AD brain [16]. The specificity of CSF P-tau to differentiate AD from other dementias seems to be higher than for T-tau and A β 42 given the fact that increased P-tau has only been found in AD, whereas T-tau and A β 42 have been found in a number of other degenerative diseases [13,14].

The assessment of changes in the concentrations of CSF A β 42, T-tau, and P-tau in nondemented patients with depression with and without cognitive manifestations can provide important information on the clinical course and outcome of these syndromes. It might also contribute to understanding the relation between depression and dementia. Similarly, taking mild cognitive impairment (MCI) as an example of a disorder which may be already viewed as incipient AD, or may evolve to AD [10], late-onset depression is also a good focus of attention. Some depressed patients may already present dementia or be at risk for such outcome. The present study is a preliminary report of a cross-sectional

study involving patients with AD, geriatric major depressive disorder (MDD), and cognitively healthy controls to document baseline CSF biomarkers. It aims to compare the results of CSF markers in elderly patients with AD or depression. We hypothesized that A β 42, T-tau, and P-tau would present a gradient of concentrations between healthy elderly controls, depressed, and AD patients.

Methods

Participants and Study Design

The study included 52 patients (40 females) between March 2008 and October 2010. Subjects with more than 60 years of age constituted a consecutive series of patients presenting with symptoms leading to a diagnosis of MDD according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [17], AD according to DSM-IV [17] and National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association [NINCDS-ADRDA; Refs.18, 19] criteria together with healthy controls. Only first episode MDD patients were included in the study. Similarly, patients were included in the study provided that they were still drug-free at the time of the examination and lumbar puncture. All individuals underwent an evaluation that consisted of full medical history, physical, and neurologic examination, neuropsychological assessment, laboratory tests (complete blood count, serum electrolytes, glucose, creatinine, amylase, lipase, gamma-glutamyl transpeptidase, total protein, albumin, globulins, triglyceride, HDL cholesterol, SGPT, SGOT, bilirubins, uric acid, urine sediments, PSA, vitamin B12, and thyroid stimulating hormone). All laboratory tests had to be within the normal range in order for the patients to be included in the study. Exclusion criteria for all groups included heavy cigarette smoking (more than 10 packs/year) and alcohol use other than social.

All clinical and cognitive evaluations were carried out before CSF exams, and the data were kept blinded from the clinicians as well as from the physician who collected the CSF samplings.

Instruments

The Mini-Mental State Examination [MMSE; 20] was used to assess the cognitive state of the groups. The MMSE is a brief screening test for cognitive capabilities which scores range from 0 to 30. In this study, we used a Brazilian version of the MMSE [21]. The clinical dementia rating [CDR; 22] was performed to assess the presence of dementia and to stage its severity under a Brazilian validated version [23]. The severity of depression was measured using the Brazilian validated version of the Hamilton Depression Scale [HAM-D; 24–26]. This scale was used only for the MDD group.

CSF Samples and Laboratory Procedures

CSF samples were obtained by lumbar puncture in the L3–4 or L4–5 interspace and no serious adverse events were reported. All lumbar punctures were performed in the morning to limit potential circadian fluctuation in CSF protein concentrations. The sam-

ples were analyzed within 30 min after collection and cell-free supernatants were stored in polypropylene tubes, and immediately frozen at -80°C until analysis to ensure the stability of the CSF marker during the storage period. All archived CSF samples were analyzed at the CSF Laboratory, Rio de Janeiro.

Measurement of CSF P-tau₁₈₁, T-tau and A β 42 was performed using commercial available enzyme-linked immunosorbent assays (ELISA INNOTEST p-tau-181, INNOTEST htau, INNOTEST β -amyloid [1–42]–Ag-kits, respectively; Innogenetics, Gent, Belgium) according to the manufacturer's protocol [27–29].

Statistical Methods

Means and standard deviations (SD) or Median and interquartiles were calculated for all variables as applicable. We have also calculated the A β 42/P-tau and A β 42/T-tau ratios, following previous studies which have shown that this could be a more sensitive measure of change in early disease stage [30,31]. Because of the skewed characteristics of the biomarkers results, we have conducted the analysis for statistical significance of differences in the results using the Kruskal–Wallis test with a *P*-value < 0.05 .

This study was approved by the ethics committee, and all the patients signed the informed consent before any procedure.

Results

Age and education level did not differ among the groups. As expected, AD patients presented lower scores of MMSE than those of depressed and healthy subjects, whereas MMSE scores of healthy and depressed subjects did not differ significantly. The sociodemographic data are summarized in Table 1. CDR scores showed that four AD patients were at the mild, 12 at the moderate and five at the severe stage of the disease. The HAM-D mean-score was 13.9 ± 2.96 and 7 patients were mildly depressed (HAM-D = 8–13), 13 were at the moderate stage (HAM-D = 14–18) and one showed a severe depression (HAM-D = 19–22). Some MDD patients ($n = 15$) complained of subjective cognitive problems, but no objective impairment was observed. All patients with MDD were treated with antidepressants after the baseline assessments. After a 4-months follow up, 5 patients remitted (HAM-D ≤ 8) and 15 of patients with MDD showed a good response ($>50\%$ reduction in baseline depressive symptoms).

CSF A β 42 levels of depressed patients and normal controls were significantly higher than in AD, whereas there was no difference between MDD and controls. There was not any significant difference in measures of P-tau among the groups. T-tau, however, showed to be significantly different among the groups, with higher measures in the AD group. There was no significant difference between healthy controls and MDD, though the crude numbers show a trend from healthy controls to MDD and to AD. These data are depicted in Table 2 and Figure 1(A)–(C).

It is worthwhile to mention that higher levels of P-tau were observed in four MDD patients compared with controls based on the previously established cut-off value of 61 pg/mL [12]. One of

Table 1 Sociodemographic data of the sample

	HC n = 8 (male = 2; female = 6)	MDD n = 20 (male = 1; female = 19)	AD n = 12 (male = 9; female = 3)	Kruskal–Wallis test	P- value
Age (years) ^a	70.7 ± 6.34 (62–81)	71.3 ± 6.10 (59–83)	72.1 ± 8.45 (59–83)	0.45	0.79
Education, (years) ^a	7.90 ± 5.10 (2–16)	4.19 ± 3.09 (0–8)	4.81 ± 4.76 (0–16)	4.25	0.12**
MMSE score ^a	27.1 ± 1.28 (25–29)	25.5 ± 2.43 (19–30)	12.7 ± 6.22 (3–23)	35.8	<0.01****

AD, Alzheimer disease; HC, healthy control; MDD, major depressive disorder; MDD, MMSE, mini-mental state examination.

^aData presented as mean ± SD (range); **P* = 0.05 HC vs. MDD; ***P* = 0.07 HC vs. AD; ****P* = 0.10 HC vs. MDD; *****P* ≤ 0.01 HC vs. AD.

Table 2 Concentrations of Aβ42, total tau (T-tau), and phosphorilated tau (P-tau) in cerebrospinal fluid

CSF biomarkers	HC	MDD	AD	Kruskal–Wallis test	P- value
Aβ42 (pg/mL) ^a	771 (574–1138)	984 (689–1110)	412 (328–504)	26.2	<0.01*
P-tau (pg/mL) ^a	40.3 (18.4–51.3)	40.5 (15.5–60.4)	46.2 (34.1–69.1)	1.94	0.38**
T-tau (pg/mL) ^a	156 (108–219)	169 (141–276)	359 (229–587)	12.3	<0.01***
Aβ42/P-tau ^a	22.4 (19.6–31.8)	21.6 (15.9–32.7)	8.9 (6.3–12.6)	20.6	<0.01****
Aβ42/T-tau ^a	5.5 (5.0–6.4)	4.4 (2.7–6.9)	1.4 (0.6–2.4)	28.9	<0.01*****

AD, Alzheimer disease; HC, healthy control; MDD, major depressive disorder; CSF, cerebrospinal fluid.

^aData presented as median (interquartile range);

P* < 0.01 MDD vs. AD; **P* < 0.01 HC vs. AD; **P* = 0.64 HC vs. MDD; *P* = 0.33 MDD vs. AD; ***P* = 0.18 HC vs. AD; ***P* = 0.70 HC vs. MDD; ****P* < 0.01 MDD vs. AD; ****P* < 0.01 HC vs. AD; ****P* = 0.20 HC vs. MDD; *****P* < 0.01 MDD vs. AD; *****P* < 0.01 HC vs. AD; *****P* = 0.67 HC vs. MDD; ******P* < 0.01 MDD vs. AD; *P* < 0.01 HC vs. AD; *P* = 0.20 HC vs. MDD.

them also had a lower level of Aβ42. The complete profile of MDD patients is depicted in Table 3.

We have also looked into the correlation of the biomarkers and the MMSE and HAM-D scores. A significant and moderate correlation was found for the Aβ42 and MMSE (positive correlation), whereas there was a significant and moderate negative correlation between the t-tau and MMSE. No other significant correlations were found with regard to the HAM-D and the biomarkers.

Conclusion

As expected, CSF Aβ42 levels were significantly lower and T-tau levels were significantly higher in AD patients as compared to MDD and control groups. However, we were not able to replicate the results from previous studies which showed that P-tau was significantly higher in AD as compared to healthy and depressed elderly [9,32], despite the presence of a numerical trend.

Figure 1 Biomarker levels in Healthy controls (HC), Alzheimer's disease (AD), and Major Depression Disorder (MDD) patients.

Figure 2 Correlation between biomarkers and Mini Mental State Examination scores (MMSE).

Figure 3 Correlation between biomarkers and Hamilton Depression Scale scores (HAM-D).

Table 3 Characteristics of the depressed patients (n = 21)

P	Age (years)	E (years)	MMSE score	CDR rate	HAMILTON scale	Aβ42 (pg/mL)	P-tau (pg/mL)	T-tau (pg/mL)
01	61	8	24	0	14	1044	33.7	106
02	63	4	26	0	17	1123	51.9	214
03	74	4	25	0	12	464	60.4	257
04	65	1	25	0	19	625	15.5	141
05	77	8	26	0	14	1100	39.5	159
06	63	0	24	0	14	783	70.1	322
07	74	4	24	0	14	1063	70.5	276
08	64	1	26	0	15	689	37	156
09	73	0	19	0	16	870	88.5	608
10	75	2	25	0	16	1085	68.4	471
11	73	8	29	0	12	1222	41.2	143
12	75	0	22	0	16	1296	60.4	289
13	61	3	28	0	10	1273	15	139
14	73	4	28	0	14	500	15.3	214
15	77	1	22	0	14	700	35.5	167
16	76	8	30	0	9	824	50.4	307
17	75	4	22	0	18	1124	15.5	232
18	71	8	27	0	18	1110	15.5	126
19	78	4	27	0	10	984	40.5	85
20	68	8	28	0	10	520	44.8	117
21	81	8	28	0	10	520	14.9	169

P, patients; E, education; CDR, clinical dementia rating; MMSE, mini-mental state examination.

This may be attributable to the relatively small sample size of our study.

Furthermore, A β 42/T-tau and A β 42/P-tau ratios were significantly lower in AD patients as compared to the MDD and healthy groups. Overall, there were no significant differences in the comparison between MDD and control groups.

There is enough evidence to state that the CSF markers may be valuable to help early diagnosis of AD, especially in the phase before clinically overt dementia, that is, in patients with MCI [26–28, 30]. Moreover, increased CSF T-tau and P-tau are typically found in preclinical AD patients and they can differentiate this condition from geriatric major depression whose patients present lower levels of T-tau [9,30,31,33,34]. Recently, these data have been presented as new clinical and preclinical criteria to diagnose AD [19,35,36]. To the best of our knowledge, our results are the first to show the potential of CSF A β 42, T-tau, and P-tau levels to differentiate between AD and depression in a Brazilian sample. Aside from this study, there are a few ones which assess whether depression and dementia may be recognized by different biomarker “signatures” [9,15,32]. Overall, these studies have concluded that patients with depression could be differentiated from preclinical AD based on P-tau and T-tau levels. However, in line with a previous report [9], we observed that MDD patients tended to present higher P-tau levels than the healthy subjects. In addition, four depressed patients presented higher levels of P-tau based when compared to the previously established cut-off value [14].

This study has some limitations which ought to be acknowledged. Several studies have reported a wide variability in CSF measures according to the assay employed and to certain other different characteristics among centers. Although all of them come to the conclusion that A β 42 is lower, and T-tau and P-tau are higher in AD, cut-offs may differ among different centers [33,37]. Our mean measures are within the ranges shown in several studies [9,13,15,32], but caution should be taken in generalizing the interpretation of our findings, mainly due to the cross-sectional design of this report. Also, the small sample size in our study may be held responsible for some nonsignificant differences in the comparison.

Further prospective studies, as the one intended in a second phase of the present report, are needed to assess the relationship between the A β 42, T-Tau, and P-Tau levels as distinctive biomarkers for AD and geriatric MDD. We suggest that CSF markers should be investigated in large populations to elucidate the possible association between geriatric major depression and neurodegeneration, as it was done by a multicenter collaborative study in 2009 for MCI [31]. Although this is still an expensive procedure, the upcoming future strategies to treat AD will demand that the diagnosis of incipient AD be performed early on the course of the disease [19,35,36]. Differential diagnosis with depression is still an issue to be resolved and might be answered by CSF A β 42, T-tau and P-tau measurements, among other techniques.

Author Contributions

Taylor Reis: clinical evaluations, data analysis/interpretation, and drafting article.

Carlos Otávio Brandão: CSF analysis, data analysis /interpretation, and drafting article.

Evandro da Silva Freire Coutinho: statistics and critical revision of article.

Elias Engelhardt: critical revision of article and approval of article.

Jerson Laks: concept/design, data analysis/interpretation, critical revision of article, and approval of article.

Acknowledgments

To the Conselho Nacional de Pesquisa [Brazilian National Council of Research CNPq] for the support of Evandro Coutinho and Jerson Laks.

Disclosures

None.

Conflicts of Interest

The authors declare no conflict of interest.

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