DOI: 10.1590/0004-282X20150066

ARTICLE

Neurodegenerative changes in the brainstem and olfactory bulb in people older than 50 years old: a descriptive study

Alterações neurodegenerativas no tronco cerebral e bulbo olfatório em indivíduos acima de 50 anos: um estudo descritivo

Francine Hehn de Oliveira, Edson Rodrigues Neto, Mariana Kumaira Fonseca, André Silvestre Reitz da Costa, Marcio Aloisio Bezerra Cavalcanti Rockenbach, Renata dos Santos Padilha, Liana Lisboa Fernandez, Arlete Hilbig

ABSTRACT

With the increase in life expectancy in Brazil, concerns have grown about the most prevalent diseases in elderly people. Among these diseases are neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Protein deposits related to the development of these diseases can pre-date the symptomatic phases by years. The tau protein is particularly interesting: it might be found in the brainstem and olfactory bulb long before it reaches the limbic cortex, at which point symptoms occur. Of the 14 brains collected in this study, the tau protein was found in the brainstems of 10 (71.42%) and in olfactory bulbs of 3 out 11. Of the 7 individuals who had a final diagnosis of Alzheimer's disease (AD), 6 presented tau deposits in some region of the brainstem. Our data support the idea of the presence of tau protein in the brainstem and olfactory bulb in the earliest stages of AD.

Keywords: neurodegenerative diseases, Alzheimer, Parkinson, tau protein, brainstem.

RESUMO

Com o aumento da expectativa de vida no Brasil e no mundo, crescem as preocupações com as doenças mais prevalentes entre os idosos, dentre elas as doenças neurodegenerativas (DN) como a doença de Alzheimer (DA) e a doença de Parkinson (DP). Sabe-se que os depósitos proteicos relacionados com o desenvolvimento destas doenças podem preceder a fase sintomática em anos. A proteína tau é de particular interesse, uma vez que parece ser encontrada no tronco encefálico e bulbo olfatório muito antes de atingir o córtex límbico, quando ocorrem os primeiros sintomas. Dos 14 encéfalos coletados neste estudo, a proteína tau foi encontrada, no tronco encefálico, em 10 (71,42%) e no bulbo olfatório em 3 de 11. Dos 7 indivíduos que tiveram diagnóstico final de DA, todos apresentavam depósitos de tau em alguma região do tronco encefálico. Nossos dados estão de acordo com a literatura mais recente, que tem confirmado a presença de proteína tau no tronco encefálico e bulbo olfatório nos estágios mais precoces da DA.

Palavras-chave: doenças neurodegenerativas, Alzheimer, Parkinson, proteína tau, tronco cerebral.

The elderly population in Brazil has increased significantly over the past several decades[1], comprising 8.6% of the country's total population in 2010 and is estimated that they will account 25% of the Brazilian population in 2050[2]. In the elderly population, there has been an increase in the frequency of neurodegenerative diseases (NDs)[3], which, from the pathologic point of view, are characterized by neuronal loss in specific regions associated with the presence of intra- or extracellular protein deposits. These pathologic changes increase in intensity and frequency with the evolution of disease, affecting different brain regions in a stereotypical manner[4].

Alzheimer's disease (AD) is the most prevalent ND[3,4]. Early clinical detection of AD is uncommon during the early years[3], and its clinical diagnosis is always based on probability. The definitive diagnosis depends on an anatomopathological exam of the post-mortem brain, conducted out through the

Universidade Federal de Ciências da Saúde de Porto Alegre, Faculdade de Medicina, Departamento de Patologia, Porto Alegre RS, Brazil.

Correspondence: Francine Hehn de Oliveira; Laboratório de Patologia da UFCSPA; Rua Sarmento Leite, 245/Anexo II; 90050-170 Porto Alegre RS, Brasil; E-mail: franhehn@gmail.com

Conflict of interest: There is no conflict of interest to declare.

Support: Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS - Rio Grande do Sul Research Support Foundation).

This study was submitted to the Postgraduate Program in Pathology of the Universidade Federal de Ciências da Saúde de Porto Alegre as a requirement for the Master's degree and was conducted in the Department of Forensic Medicine of Porto Alegre and in the Pathology Laboratory of UFCSPA.

Received 11 April 2014; Received in final form 22 February 2015; Accepted 16 March 2015.

identification of selective neuronal loss in specific areas and through the detection, by an immunoperoxidase reaction, of intracellular deposits of hyperphosphorylated tau protein and extracellular deposits of β-amyloid protein[4,5,6].

There is evidence that the pathologic processes of AD begin approximately 10 years before cognitive deficits emerge. AD is understood as a continuum, from early asymptomatic neurodegenerative stages until dementia. The deposition of tau protein is present from the beginning through the late stages of the disease and cannot be reversed[6,7]. The tau aggregates accumulate in the neurons, as pretangles (pNFTs), neurites (NTs) and tangles (NFTs)[6,8]. The process of intraneuronal disease progresses in a topographically systematic manner and can require a very long period, almost an entire life, to reach its full extent[6,8]. According to Braak *et al.*[9], the presence of tau deposits is classified in six stages, from I to VI, beginning with the transentorhinal region (stage I) and progressing until stage VI with severe pathology of the isocortex. Late phases of the disease cause recognizable symptoms and have been correlated with the clinical manifestations of AD[6,10,11]. However, studies of these phases have been laden with difficulties, due to the large number of altered nerve cells. In the early phases of the disease, the circumstances are clearer, and individuals without clinical symptoms can present pathologic changes associated with AD, such as the presence of abnormally phosphorylated tau protein in some nerve cells or even in a single neuron[12].

β-amyloid deposits in senile or neuritic plaques are also hallmarks of the pathogenesis of AD[9]. According to Thal *et al.*, there are five phases of β-amyloid deposits. In phase 1, the isocortex is involved; in phase 2, the hippocampus and the entorhinal cortex; in phase 3, the striatum and the diencephalic nucleus; in phase 4, various brainstem nuclei; and, finally, in phase 5, the cerebellum and the additional brainstem nuclei[13,14].

Parkinson's disease (PD) is the second most common ND after AD, considered a multisystem disorder with variegated non-motor deficits and neurological symptoms, including impaired olfaction, sleep disorders, gastrointestinal and urinary abnormalities and cardiovascular dysfunction, in addition to other symptoms and signs such as pain, depression and mood disorders[15]. PD has a prevalence of 0.5 to 1% among individuals 65 to 69 years old, reaching 1 to 3% in individuals 80 years old and older[4]. Sporadic PD (90% of cases) is the most frequent Lewy body disease. It is progressive and clinically manifests after the pathological stages have advanced. The anatomopathological diagnosis, both in preclinical and clinical phases, is accomplished through the identification of selective neuronal loss in the substantia nigra pars compacta and many other neuronal systems besides the presence of Lewy bodies (LB) e Lewy neuritis (LN)[16]. Damage to specific subnuclei of the substantia nigra is frequently considered the most important hallmark of PD[16]. The Lewy pathology can be found in necropsies of individuals without clinical manifestations of the motor symptoms of PD (incidental Lewy pathology)[17,18]. Currently, Braak's classification[16] is used for the pathologic staging of the disease, and it ranges from I to VI, beginning with the involvement of the medulla oblongata and the posterior pons in stage I through stage VI, with deposits in the neocortex[16].

The present study is the initial phase of a human brain bank study of neurodegenerative diseases conducted in our Institution and intend to describe the presence of pathological changes related to NDs in the brainstem (midbrain, pons and medulla oblongata) and olfactory bulb (OB) in a sample of patients from south of Brazil.

METHOD

This was a descriptive study, in which the samples were collected by convenience and with the informed consent of first-degree relatives. Fourteen brains were collected from individuals 50 years old or older who underwent death verification in the Forensics Department (FD) of Porto Alegre. Individuals who were the victims of violent deaths, whose relatives did not provide informed consent and who had acute neurological events as the cause of death were excluded.

The donors' clinical information was obtained after death through the next of kin, using a questionnaire to evaluate cognitive decline (IQCODE)[19] and a customized form to evaluate motor and sensory changes. For the IQCODE, the cut-off point for dementia was a score of 3.27 or greater, as suggested by Sanchez and colleagues[19]. After removal, the brains were fixed in a 10% formalin solution for four weeks and were weighed, sectioned, and macroscopically evaluated, in accordance with the international protocol (Figure 1). After the macroscopic examination, in addition to the specific areas described in the paragraph below, the frontal, parietal, temporal and occipital cortices, hippocampus, amygdala, basal ganglia and cerebellum were sampled for histopathological evaluation, were stained with hematoxylin-eosin (HE) and were submitted to immuno-histochemical reactions (IHC).

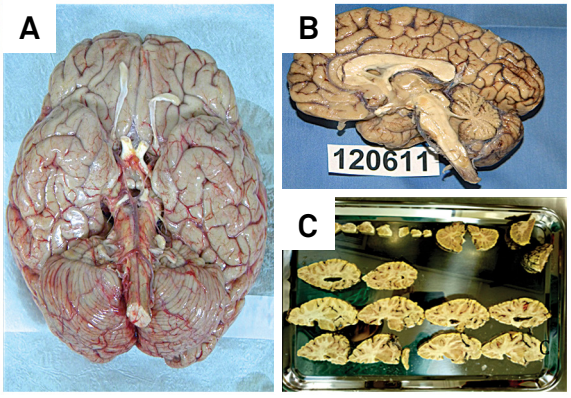


Figure 1. Case 3 (MSA patient) - Basal view of the brain after removal (A) and sagital section after four weeks in formalin 10%, showing severe atrophy of brainstem and cerebellum (B); standardized sections after fixation in formalin (C).

For this study, the following specific regions of interest were selected: the midbrain (substantia nigra at the level of the emergence of the oculomotor nerve), pons (section including segment of the locus ceruleus) and medulla oblongata (at the level of the dorsal motor nucleus of the vagus). Besides these regions, we also evaluate the olfactory bulb, which was submitted to full examination and paraffin embedded in craniocaudal orientation. These regions were also evaluated by routine HE staining and IHC.

The immuno-histochemical technique was performed in compliance with the routine protocols of the Laboratory of Pathology at UFCSPA. After deparaffination, the samples were immersed in 3% hydrogen peroxide and 10% methanol for 15 minutes to inhibit endogenous peroxidase activity. Then, they were boiled in citrate (10 mM, pH 6.0) and/or treated with 1% formic acid to recover the antigen. After washing with PBS, they were incubated with common horse serum for 1 hour and then with primary antibody at 4°C in a wet chamber overnight. The following primary antibodies were used: anti-Aβ (human monoclonal mouse antibody, DAKOCYTOMATION, clone 6F/3D, code M0872), 1:60 dilution, following 3 minutes of incubation in 1% formic acid; anti-phosphorylated tau (monoclonal mouse antibody, INNOGENETICS, clone AT-8, code 90206), 1:500 dilution, following 10 minutes of incubation in citrate; and anti-alpha-synuclein (mouse monoclonal antibody, NOVOCASTRA, clone KM51, code ASYN-L), 1:200 dilution, following 4 minutes of incubation in 1% formic acid and 20 minutes of incubation in citrate. After overnight incubation in primary antibodies, the slides were washed three times in PBS and were incubated in DAKO secondary polymer for 40 minutes, and horseradish peroxidase for 30 minutes and, finally, DAB. All of the slides were counterstained with hematoxylin for 10 seconds. All of the procedures were performed with negative and positive controls.

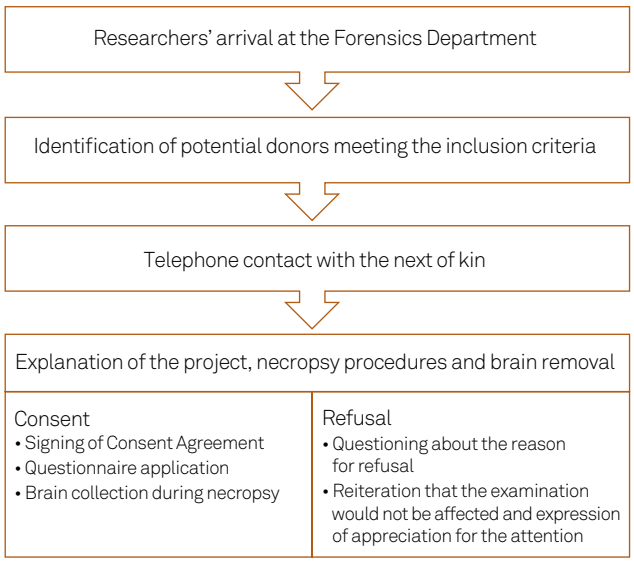
The slides were studied by light microscopy for histopathological diagnosis, aiming to identify possible changes in the morphological and anatomical structures. The presence of protein inclusions on IHC against specific antibodies was semi-quantitatively classified as follows: absent, 1+ (1 to 4 positive cells/10 HPF), 2+ (5 to 9 positive cells/10 HPF) and 3+ (more than 10 positive cells/10 HPF).

The data collected from each case were compared with the corresponding values on the IQCODE, with the evaluation form for motor changes and with clinical information.

The study was approved by the ethics committees at both of the involved institutions (CEP – UFCSPA; and CEP – DML Porto Alegre).

The data are presented in a descriptive manner, and to compare the mean ages between the demented and non-demented patients, Fisher's test was performed.

Collection flowchart



RESULTS

Sample description

The average age of the individuals in our sample was 72.07 years old (± 15.79). When the subjects were categorized in clinically demented (D) and non-demented (NoD) categories, based on the IQCODE, the average ages were 72.87 years old and 71.16 years old, respectively. No statistically significant difference was found between the groups regarding age. The demented individuals corresponded to 57% of the sample, and their average IQCODE score was 3.72. The non-demented individuals' average IQCODE score was 2.74, and there was a statistically significant difference between the two groups for this variable ($p = 0.016$, Fisher's test), as expected. Regarding the questionnaire about motor changes, only one patient presented light tremor and rigidity. A detailed sample description is provided in Table 1.

Macroscopic findings

Six of the 14 analyzed brains presented macroscopic changes, as detailed in Table 2. We highlighted the presence of mild frontal atrophy with expansion of the frontal horn of the lateral ventricles as the most frequent finding (found in 3 cases). Moreover, brain number 10 presented temporal atrophy with volumetric reduction of the hippocampus. Brain number 3 was from a patient with clinical diagnosis of

Table 1. Clinical data of patients.

	Demented (D)	Non demented (ND)
n (%)	8 (57.14)	6 (42.86)
Age (years)	72.87	71.16
Gender		
Female	4 (50%)	3 (50%)
IQCODE Mean (SD)	3.72 (0.6)	2.74 (0.7)

IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly; SD: Standard Deviation.

Arq Neuropsiquiatr 2015;73(7):569-577

Table 2. Description of 14 analyzed brains sample.

Case	Age of Death	Gender	IQCODE	Cause of Death	Microscopic Findings	β-amyloid		Tau				α-synuclein				Pathological Diagnostic
						DP	SP	NTs	pNFTs	NFTs	Gr	LB	NTs	PNIs	CGIs	
1	91	F	3.00	Pneumonia												Undefined
M						-	-	2+	1+	-	2+	-	-	-	-	Tau deposit
P						-	-	2+	-	1+	1+	-	-	-	-	
MO						-	-	1+	1+	-	1+	-	-	-	-	
OB						NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
2	79	M	3.15	Cardiac												Undefined
M				Tamponade		-	-	1+	1+	1+	-	-	-	-	-	Tau deposit
P						-	-	-	-	-	-	-	-	-	-	
MO						-	-	-	-	-	-	-	-	-	-	
OB						-	-	-	-	-	-	-	-	-	-	
3	59	F	4.52	Septic shock	Cerebellar atrophy											MSA
M					BG atrophy	-	-	-	-	-	-	-	-	3+	3+	
P						-	-	-	-	-	-	-	-	3+	3+	
MO						-	-	-	-	-	-	-	-	3+	3+	
OB						-	-	-	-	-	-	-	-	3+	3+	
4	56	M	3.00	Undetermined												NORMAL
M						-	-	1+	-	1+	1+	-	-	-	-	
P						-	-	-	-	-	-	-	-	-	-	
MO						-	-	-	-	-	-	-	-	-	-	
OB						NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
					Frontal atrophy											
5	93	F	3,33	Myocardial	Increase TH											Alzheimer Pathology
M				Infarction		-	-	3+	1+	3+	1+	-	-	-	-	Braak III/IV
P						-	-	2+	1+	3+	-	-	-	-	-	
MO						-	-	1+	1+	1+	-	-	-	-	-	
OB						NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
																Alzheimer Pathology
6	68	F	3,27	Undetermined												Braak I/II
M						-	-	1+	-	-	-	-	-	-	-	
P						-	-	-	-	-	-	-	-	-	-	
MO						-	-	-	-	-	-	-	-	-	-	
OB					Metastatic	-	-	-	-	-	-	-	-	-	-	Alzheimer Pathology
					carcinoma of the cervix											Braak III/IV

Continue

Continuation

7	89	F	3,00	Multiorgan												
M				Failure		-	-	1+	-	-	-	-	-	-	-	
P						-	-	-	-	-	-	-	-	-	-	Isolate β-amyloid
MO						-	-	1+	-	-	-	-	-	-	-	deposit
OB						-	-	-	-	-	-	-	-	-	-	
8	49	M	3,00	Undetermined												
M						-	-	-	-	-	-	-	-	-	-	
P						-	-	-	-	-	-	-	-	-	-	Alzheimer Pathology
MO						-	-	-	-	-	-	-	-	-	-	Braak I/II
OB						-	-	-	-	-	-	-	-	-	-	
9	54	M	3.86	Pneumonia												Alzheimer Pathology
M						-	-	-	-	-	-	-	-	-	-	Braak V/VI
P						-	-	-	-	-	-	-	-	-	-	
MO						-	-	-	-	-	-	-	-	-	-	
OB					Frontal and	-	-	-	-	-	-	-	-	-	-	
					Temporal atrophy											
10	62	M	4.77	Acute												Alzheimer Pathology
M				Pulmonar		1+	1+	3+	3+	3+	2+	-	-	-	-	Braak III/IV
P				edema		-	-	2+	2+	3+	1+	-	-	-	-	
MO					Frontal atrophy	-	-	2+	1+	2+	2+	-	-	-	-	
OB					Increase of LV	-	-	3+	1+	1+	-	-	-	-	-	
																Alzheimer Pathology
11	62	M	3.38	Liver												Braak V/VI
M				Cirrhosis	Frontal atrophy	-	-	2+	1+	2+	1+	-	-	-	-	
P					Increase of LV	-	-	2+	2+	1+	1+	-	-	-	-	
MO						-	-	1+	-	-	-	-	-	-	-	
OB						-	-	-	-	-	-	-	-	-	-	
																Undefined
12	81	F	3.36	Undetermined												Tau deposit
M						-	-	2+	1+	2+	1+	-	-	-	-	
P						-	-	1+	-	1+	-	-	-	-	-	
MO						-	-	-	-	1+	-	-	-	-	-	
OB						-	-	2+	1+	1+	1+	-	-	-	-	

Continue

Continuation

						Alzheimer Pathology										
						Braak V/VI										
13	62	F	3.00	Undetermined												
M						-	-	-	-	-	-	-	-	-	-	-
P				Parieto-occipital infarction		-	-	-	-	-	-	-	-	-	-	-
MO						-	-	-	-	-	-	-	-	-	-	-
OB						-	-	-	-	-	-	-	-	-	-	-
14	17	M	3.28	Cardiac Tamponade												
M						-	-	2+	1+	1+	-	-	-	-	-	-
P						-	-	2+	1+	2+	-	-	-	-	-	-
MO						-	-	-	-	-	-	-	-	-	-	-
OB						-	-	3+	3+	1+	-	-	-	-	-	-

DP: diffuse plaque; SP: senile plaque; NTs: neurites; pNFTs: pre-tangles; NFT: tangles; Gr: granules; LB: Lewy Bodies; PNIs: Perineuronal Nuclear Inclusions; CGIs: Cytoplasmic Glial Inclusions; M: Midbrain; P: Pons; MO: medulla oblongata; OB: olfactory bulb; BG: basal ganglia; NA: not available; MSA: multiple system atrophy; TH: temporal horn; LV: lateral ventricle.

multiple system atrophy (MSA) and showed severe cerebellar atrophy, predominantly in the white matter, putamen, pons basis and cerebellar peduncle, as well as slight paleness of the substantia nigra (Figure 2).

Microscopic findings

Hematoxylin-eosin (HE)

In case 1, we found an old 0.3 cm infarction in the pons. In the case of the clinic diagnosis of MSA (case 3), microscopy confirmed the atrophic areas described in the macroscopic exam, in addition to neuronal loss and areas of demyelination, mainly in the pons and cerebellar white matter.

Another common finding, observed in 11 of 14 brains, was the presence of corpora amylacea, ranging from rare in some cases to numerous in others, predominantly localized in the subpial and periventricular regions. This observation did not appear to be related to the patient's age or to the presence of protein deposits associated with the ND, because they were present both in younger and older patients, as well as in patients with and without protein deposits.

In 11 of 14 cases, the OB was available, and no histological alterations were identified.

Immuno-histochemical findings

Histopathological diagnosis and classification of AD according to Braak and Braak[9]

In addition to the protein evaluation in the four areas proposed in this study, we evaluated the same proteins in the areas previously described by Braak *et al.*[12], including the hippocampus and the entorhinal, frontal, parietal, and occipital cortices, to establish anatomopathological diagnoses.

Of the 10 patients who presented tau protein deposits in some location of the brainstem, 8 also had tau in the cortical areas in association with β-amyloid protein deposits, characterizing Alzheimer-type pathology. Of these 8 patients, 3 met the criteria for stage V/VI of Braak's Classification for AD and were also considered demented patients according to the IQCODE evaluation; 3 were classified as stage III/IV and 2 as stage I/II. In the case in which the IQCODE score was less than 3.27 (case 7), the

Figure 2. Case 3 - Anterior view of the brainstem showing pons basis atrophy (A); sagital section also shows pons basis atrophy and severe atrophy of the cerebellum (B).

individual was classified with Alzheimer-type pathology, stage V/VI, without clinical correlation.

The other 3 cases presented only tau deposits in cortical neurons without beta-amyloid. In all of these cases, the IQCODE score was less than 3.27 (cases 1, 2 and 13).

Beta-amyloid protein (Tables 2 and 3)

Only 1 of the 14 brains (case 10) presented two beta-amyloid deposits in the brainstem, in the form of diffuse and senile plaque (Figure 3) localized in the mesencephalic-diencephalic transition of the midbrain. Considering that this case had also beta-amyloid deposits in isocortex, hippocampus, entorhinal, cortex and striatum, we could classify it as stage IV, according to Thal. et al.[13] classification.

Phosphorylated tau protein (Tables 2 and 3)

The data on the distribution and quantity of tau protein deposits are detailed in Table 2. Ten of 14 brains presented several tau deposits in one of the four areas evaluated. In these cases, the most common forms of protein deposits were pretangles (pNFT) (Figure 4A) and dystrophic neurites (DN) (Figure 4B) and, less frequently, tangles (NFT) (Figure 4C) and granules (Figure 4D). Of the cases in which the OB was available for analysis (n = 11), three presented diffuse and strong positivity to tau antibody, under the form of dystrophic neuritis (DN), mainly in the external plexiform layer (Figure 5).

Alpha-synuclein protein (Tables 2 and 3)

Deposits of α-synuclein were only found in one case, in the individual with a clinical diagnosis of probable MSA in a distribution compatible with the disease, thus confirming the diagnosis. In this case, α-synuclein deposits in the form of cytoplasmic glial inclusions (CGIs) (Figure 6A) and perineuronal nuclear inclusions (PNIs) (Figure 6B) were identified and mainly observed in the white matter of the cerebellum (Figure 6C), in the pons, medulla oblongata, and midbrain. Many CGIs and PNIs were also identified in the putamen, and several were identified in the cortex.

Table 3. Brainstem location of protein deposits.

Case	Beta-amyloid	Tau	Alfa-synuclein
Midbrain	1/14	10/14	1/14
Substantia Nigra	1/14	10/14	1/14
Periaqueductal gray matter	0	4/14	1/14
Pons	0	6/14	1/14
Locus ceruleus		6/14	1/14
Reticular formation		4/14	1/14
Medulla Oblongata	0	5/14	1/14
Dorsal motor nucleus of the vagus		4/14	1/14
Hypoglossal nucleus		3/14	1/14

Total number of brainstem segments: 14.

DISCUSSION

We interviewed 30 relatives and 16 of them (53.3%) did not consent to the donation. The main reasons offered were disagreement with the necropsy procedure that the subject would undergo (25%; n = 4), philosophical or religious issues (12.5%; n = 2), objections from other relatives (12.5%; n = 2) and consideration for the deceased person's wishes expressed when alive (12.5%; n = 2). Most commonly, no reason for refusal was given (31.2%; n = 5)[20].

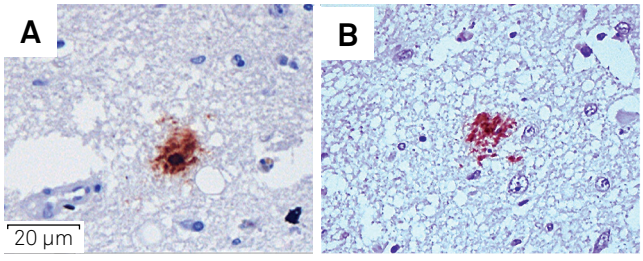


Figure 3. Case 10 - senile plaques of β-amyloid deposit in the midbrain (A and B) (β-amyloid, 600X).

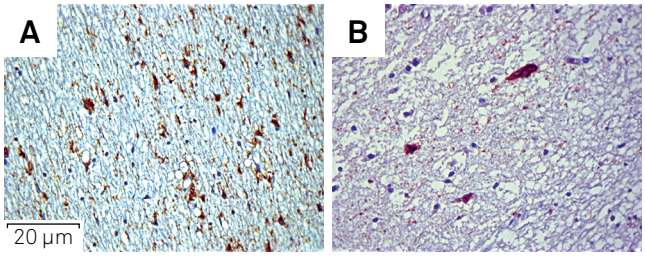


Figure 5. Case 14 - Pretangles and dystrophic neurites (A) and tangles (B) in the olfactory bulb (Tau, 400X).

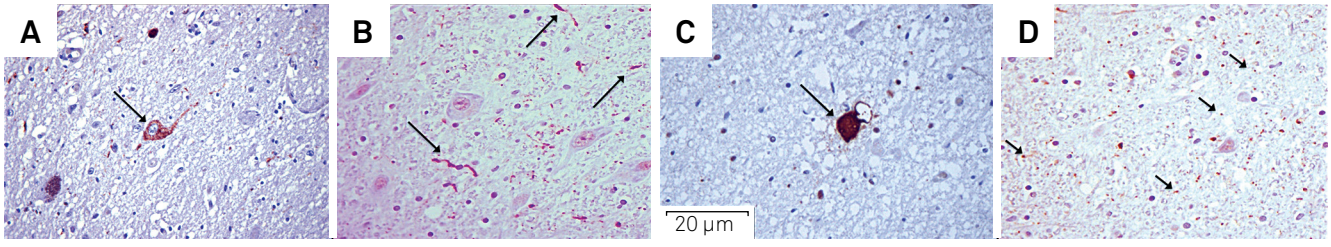


Figure 4. Cases 5 and 10 - pretangles in the medulla oblongata (A); dystrophic neurites in the midbrain (B); tangle in the pons (C) and granules in the pons (C) (Tau, 400X).

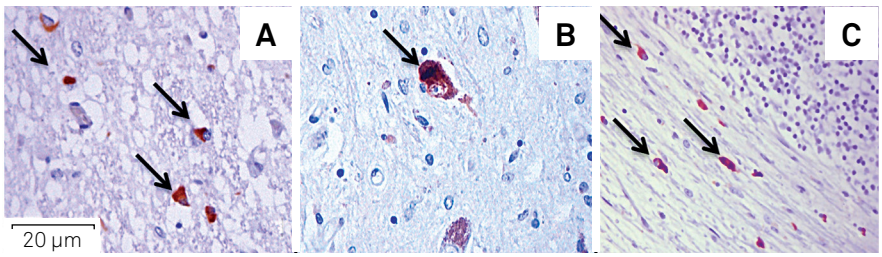


Figure 6. Case 3 - Cytoplasmic glial inclusions in the pons (A); perinuclear neuronal inclusion in the midbrain (B) and cytoplasmic glial inclusions in the cerebellar white matter (C) (α-synuclein, 600X).

To assess mental status of the patients, we used IQCODE as a screening method and we believe that despite this questionnaire does not define the etiology of dementia, it has demonstrated utility as a good tracking instrument for cognitive changes and has been strongly correlated with anatomopathological findings on deceased people.

We categorized the patients into two groups: non-demented (NoD) and demented (D). We found that the same number of men and women were affected, which could be explained by the small sample size. Another noteworthy finding was that the majority of our sample (57%) was classified, according to the IQCODE, in the demented group. It is possible that families who suffer from the difficulties of this disease are more amenable to donation.

Although we found a significant number of individuals (n = 11) with a protein deposit in at least one brainstem segment or in the OB, when we compared demented to non-demented cases, our data did not show statistically significant differences regarding deposits. Researchers have attempted to find clinical signs of early ND and these changes could be the result of protein deposits in the brainstem and OB, because, according to more recent studies, these segments are affected long before the cortical areas[21]. No association was found between protein deposits in the brainstem and clinical changes as related by the next of kin through the IQCODE and the motor alteration questionnaire.

Some authors have demonstrated that the brainstem nuclei present NFTs before the transentorhinal region. According to Grinberg *et al.*[22], in all of the cases in which the transentorhinal region presented NFTs, they were also found in the dorsal raphe nucleus (DRN). Moreover, 22% of the individuals who did not present NFTs in the cortical regions (Braak 0) exhibited NFTs in the DRN, suggesting that the brainstem was affected by AD before the supratentorial regions[22]. Our findings agreed, in general, with this idea. Of the 8 cases in which we found Alzheimer-type pathology, seven (87.5%) presented tau protein deposits in some brainstem segment or in the OB, and in 10% of the cases (1/10) in which we found tau deposits in the brainstem, this protein was not found in cortical areas, a finding that could be related to the early stages of AD.

It has been well documented that olfactory dysfunction (hyposmia or anosmia) is a very common characteristic of various NDs, occurring particularly in patients with AD and PD[23]. In many cases, an olfactory deficit is an early event in these diseases, and it can precede the typical clinical manifestations by many years. Deposits of α-synuclein, tau and β-amyloid proteins were present in many ND patients' OBs and olfactory epithelia[24], and it has been suggested that the OB could be the first neuronal structure in which these deposits appear during NDs[23]. We found NFTs and NTs in the OBs of three of the 11 individuals (27,2%) whose OBs were available for analysis (cases 10, 12 and 14). Mundiñano *et al.* found NFTs in 42.9% of the OBs of patients with confirmed diagnoses of AD, exactly the same percentage that we found[23].

We found no cases of sporadic PD. Regarding α-synuclein deposits, only the case that had a clinical diagnosis of MSA presented this protein in all the segments of the brainstem, as well as in the OB. Alpha-synuclein is the main component of CGIs in the MSA[25].

Only one case was positive to beta-amyloid deposits in the brainstem, more specifically in the midbrain, and this individual had stage V/VI AD, according to Braak's classification. According to Thal *et al.*[13], we could classify this case in phase 4, which include various brainstem nuclei.

This study revealed interesting findings regarding protein deposits in the brainstems and OBs of people with and without dementia, but due to the very small sample size, better interpretation of the results was precluded. For the first time, a research group reproduces these findings in a population from the south of Brazil.

Acknowledgements

The authors would like to thank the Department of Forensic Medicine of the General Institute of Medical Forensic Analyses of Rio Grande do Sul and the staff of the pathology laboratory for their support.

References

1. Carvalho J, Garcia A. [The aging process in the Brazilian population: a demographic approach]. Cad Saúde Pública. 2003;19(3):725-33. Portuguese. http://dx.doi.org/10.1590/S0102-311X2003000300005
2. Fagundes SD, Silva MT, Silva MFR, Pereira MG. Prevalence of dementia among elderly Brazilians: a systematic review. Sao Paulo Med J. 2011;129(1):46-50. http://dx.doi.org/10.1590/S1516-31802011000100009
3. Prado M, Caramelli P, Ferreira ST, Cammarota M, Izquierdo I. Envelhecimento e memória: foco na doença de Alzheimer. Revista USP. 2007;75:42-9.
4. Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. N Engl J Med. 2003;348(14):1356-64. http://dx.doi.org/10.1056/NEJM2003ra020003
5. Braak H, Braak E. Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. Brain Pathol. 1991;1(3):213-6. http://dx.doi.org/10.1111/j.1750-3639.1991.tb00661.x
6. Braak H, Del Tredici K. Alzheimer's disease: pathogenesis and prevention. Alzheimers Dement. 2012;8(3):227-33. http://dx.doi.org/10.1016/j.jalz.2012.01.011
7. Braak H, Del Tredici K. Alzheimer's pathogenesis: is there neuron-to-neuron propagation? Acta Neuropathol. 2011;121(5):589-95. http://dx.doi.org/10.1007/s00401-011-0825-z
8. Bancher C, Brunner C, Lassmann H, Budka H, Jellinger K, Wiche G et al. Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res. 1989;477(1-2):90-9. http://dx.doi.org/10.1016/0006-8993(89)91396-6
9. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-59. http://dx.doi.org/10.1007/BF00308809
10. Nelson PT, Jicha GA, Schmitt FA, Liu H, Davis DG, Mendiondo MS et al. Clinicopathologic correlations in a large Alzheimer disease center autopsy cohort: neuritic plaques and neurofibrillary tangles "do count" when staging disease severity. J Neuropathol Exp Neurol. 2007;66(12):1136-46. http://dx.doi.org/10.1097/nen.0b013e31815c5efb
11. Sabbagh MN, Cooper K, DeLange J, Stoehr JD, Thind K, Lahti T et al. Functional, global and cognitive decline correlates to accumulation of Alzheimer's pathology in MCI and AD. Curr Alzheimer Res. 2010;7(4):280-6. http://dx.doi.org/10.2174/156720510791162340
12. Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. Acta Neuropathol. 2011;121(2):171-81. http://dx.doi.org/10.1007/s00401-010-0789-4
13. Thal DR, Rüb U, Orantes M, Brask H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58(12):1791-800. http://dx.doi.org/10.1212/WNL.58.12.1791
14. Montine TJ, Phelps CH, Beach TG, Bifio EH, Cairns NK, Dickson DW et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012;123(1):1-11. http://dx.doi.org/10.1007/s00401-011-0910-3
15. Ferrer I, Martinez A, Blanco R, Dalfó E, Carmona M. Neuropathology of sporadic Parkinson disease before the appearance of parkinsonism: preclinical Parkinson disease. J Neural Transm. 2011;118(5):821-39. http://dx.doi.org/10.1007/s00702-010-0482-8
16. Braak H, Del Tredici K, Rüb U, Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197-211. http://dx.doi.org/10.1016/S0197-4580(02)00065-9
17. Braak H, Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett. 2006;396(1):67-72. http://dx.doi.org/10.1016/j.neulet.2005.11.012
18. Duyckaerts C, Dickson D. Neuropathology of Alzheimer's Disease and its variants. In: Dickson D, Weller R, editors. Neurodegeneration: the molecular pathology of dementia and movement disorders. 2nd ed. Hoboken: Wiley-Blackwell; 2011. p. 62-91.
19. Sanchez MA, Lourenco RA. Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): cross-cultural adaptation for use in Brazil. Cad Saúde Pública. 2009;25(7):1455-65. Portuguese. http://dx.doi.org/10.1590/S0102-311X2009000700003
20. Fonseca MK, Rodrigues Neto E, Costa AS, Rockembach MA, Padilha RS, Fernandez LL et al. Assessing families' and patients' attitudes toward brain donation for research purposes in a Brazilian population sample. Cell Tissue Bank. 2014. http://dx.doi.org/10.1007/s10561-014-9465-6
21. Grinberg LT, Rueb U, Heinsen H. Brainstem: neglected locus in neurodegenerative diseases. Front Neurol. 2011;2:42. http://dx.doi.org/10.3389/fneur.2011.00042
22. Grinberg LT, Rüb U, Ferretti RE, Nitrini R, Farfel JM, Polichiso L et al. The dorsal raphe nucleus shows phospho-tau neurofibrillary changes before the transentorhinal region in Alzheimer's disease: a precocious onset? Neuropathol Appl Neurobiol. 2009;35(4):406-16. http://dx.doi.org/10.1111/j.1365-2990.2008.00997.x
23. Mundiñano IC, Caballero MC, Ordóñez C, Hernandez M, DiCaudo C, Marcilla I et al. Increased dopaminergic cells and protein aggregates in the olfactory bulb of patients with neurodegenerative disorders. Acta Neuropathol. 2011;122(1):61-74. http://dx.doi.org/10.1007/s00401-011-0830-2
24. Bloch A, Probst A, Bissig H, Adams H, Tolnay M et al. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol. 2006;32(3):284-95. http://dx.doi.org/10.1111/j.1365-2990.2006.00727.x
25. Duda JE, Giasson BL, Gur TL, Montine TJ, Robertson D, Biaggioni I et al. Immuno-histochemical and biochemical studies demonstrate a distinct profile of alpha-synuclein permutations in multiple system atrophy. J Neuropathol Exp Neurol. 2000;59(9):830-41.