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ALZHEIMER'S DISEASE AND PROTON MAGNETIC RESONANCE SPECTROSCOPY OF LIMBIC REGIONS

A suggestion of a clinical-spectroscopic staging

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ABSTRACT - *Objective:* To compare magnetic resonance proton spectroscopic with clinical data and to propose a spectroscopic staging of Alzheimer's disease (AD). *Method*: Subjects (n=46), normals (12) and with AD (34), paired to age (CDR0-CDR3); AD diagnosis according to DSM-IV/NINCDS-ADRDA criteria; ^{1}H-MRS with Signa Horizon LX-GE, 1.5T; single voxel at hippocampal region/HCR and posterior cingulate area/PCA. *Results:* Statistically significant decrease ($p<0.01$) only of Naa/Cr - at HCR among the CDR0, CDR1+CDR2, and CDR3, and at PCA between CDR0 and CDR1+CDR2 in relation to CDR3. *Conclusion:* The HCR is the first to show Naa reduction (CDR1). The PCA suffers later (CDR3). These values decline progressively according to the severity stages. Considering the disparities between the HCR and PCA it is possible to suggest a spectroscopic (metabolite) staging (MS) of AD, as follows: MS0 (~CDR0)=both normal HCR and PCA, MS1-2 (~CDR1-2)=abnormal HCR and normal PCA, and MS3 (~CDR3)=both abnormal HCR and PCA. These results make possible the early diagnosis, to follow the degenerative process throughout the course, and to suggest a spectroscopic staging related to the clinical stages of AD.

KEY WORDS: Alzheimer disease, ^{1}H-MRS, Naa, disease staging.

Doença de Alzheimer e espectroscopia de prótons por ressonância magnética de regiões límbicas: sugestão de um estadiamento clínico-espectroscópico

RESUMO - *Objetivo:* Comparar dados de espectroscopia de prótons por ressonância magnética com clínicos e propor um estadiamento espectroscópico da doença de Alzheimer (DA). *Método:* Sujeitos (n=46), normais (12) e com DA (34), emparelhados por idade (CDR0-CDR3); diagnóstico de DA de acordo com os critérios DSM-IV/NINCDS-ADRDA; ^{1}H-MRS com Signa Horizon LX-GE, 1.5T; voxel único em região hipocampal/RHC e área posterior do cíngulo/APC. *Resultados:* Redução estatisticamente significativa ($p<0.01$) apenas de Naa/Cr - na RHC entre CDR0, CDR1+CDR2 e CDR3, e na APC entre CDR0 e CDR1+CDR2 em relação a CDR3. *Conclusão:* A RHC é a primeira a apresentar redução de Naa (CDR1). A APC é acometida mais tardiamente (CDR3). Esses valores declinam progressivamente de acordo com os estágios de gravidade. Considerando as disparidades entre a RHC e a APC é possível sugerir um estadiamento espectroscópico (metabólico) (MS) da DA como segue: MS0 (~CDR0)=RHC e APC ambos normais, MS1-2 (~CDR1-2)=RHC anormal e APC normal e MS3 (~CDR3)=RHC e APC ambos anormais. Esses resultados permitem um diagnóstico precoce, o seguimento do processo degenerativo ao longo da evolução e sugerir um estadiamento espectroscópico relacionado aos estágios clínicos da DA.

PALAVRAS-CHAVE: doença de Alzheimer, ^{1}H-MRS, Naa, estadiamento da doença.

Alzheimer's disease (AD) is the most common degenerative dementia. It progressively affects cortical areas and subcortical structures, undermining the normal function, and leads to progressive cognitive and functional decline and to the appearance of behavioral disorders. Considering the severity and the prevalence of this disease there is a growing interest focused on its underlying processes and the possibility of early diagnosis. The brains of AD patients exhibit neuropathologic changes (senile plaques, neurofibrillary tangles, neuronal loss, glial reaction). Considering the neurofibrillary degeneration, the degenerative process observed in AD presents a sequential pattern as determined by Braak and Braak's[1] neurofibrillary tangle/NFT and Delacourte's and al.[2] paired helical

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filament/PHF-tau immunostaining stages. It begins in the primary limbic areas, hippocampal region (HCR), and spreads to associative limbic areas for instance, posterior cingulate area (PCA), and finally to the heteromodal areas, temporo-parieto-frontal cortex. Both groups of authors have shown that the (HCR) degenerates at an earlier stage (beginning at NFT I-II/PHF-tau 1-3 stages and progressing), whereas at the PCA the degeneration occurs later (beginning at NFT III-IV/PHF-tau 6-7 stages and progressing).

The degenerative process, centered by neuronal loss and followed by corollary changes, such as tissue dysmetabolism, atrophy and gliosis, can be observed 'in vivo' with functional imaging techniques. Proton magnetic resonance spectroscopy (^{1}H-MRS) permits the identification of neurometabolites related to several pathways of the intermediate metabolism. N-acetylaspartate (Naa) is an aminoacid found only in neurons in the adult CNS, and is used as a measure of neuronal density. However, it can also be considered a marker of neuronal function or viability, since Naa depletion is not always irreversible. Choline (Cho) is associated with membrane breakdown and turnover. Myo-inositol (mI) is an organic osmolyte, also involved in second messenger system functioning, and represents also a putative glial cell marker. Creatine (Cr) refers to the sum of creatine and phosphocreatine, reflecting energy use. The Cr peak is thought to be relatively stable between individuals and in most brain areas, therefore it is often used as an internal reference. Thus the technique can be used 'in vivo', in a nonivasive way, to evaluate neuronal integrity, glial reaction and energy metabolism[3-7].

The ^{1}H-MRS studies of post-mortem AD brains, as well as 'in vivo', have shown consistent reduction of Naa. This corresponds to the primary neurometabolite change in AD brain, reflecting neuronal dysfunction/loss. Changes of other metabolites are probably corollary occurrences[7-13]. Several studies have addressed this question aiming the hippocampal region[9,11,14-18] in mild-moderate AD patients. The posterior cingulum was also studied in mild-moderate AD and MCI patients in comparison to normal controls - significantly lower values of Naa/Cr were found in AD patients compared both to MCI and normal control subjects[19]. Correlations of neurometabolites with cognitive assessment (ex., Mini-Mental State Examination/MMSE, Alzheimer's Disease Assessment Scale-cognitive section (ADAS-cog) were found in only a few AD studies[7]. Studies correlating Naa and cognition were mostly performed aiming the medial temporal region on mild[20,21] and mild-moderate AD patients[9,22]. Other cortical regions thought to be important in memory circuits, like the posterior cingulum, were less studied[19]. Longitudial studies were performed by Adalsteinsson et al.[23] and Dixon et al.[24] over the course of one year and by Jessen et al.[22] over 2 years, on mild-moderate AD patients. Adalsteinsson's et al.[23] reported an overall decline of Naa concentration over time, but the medial temporal lobes were not included in the analysis; Dixon's et al.[24] study of the hippocampus showed that the decrease of Naa was not significant after one year follow-up, and Jessen's et al.[22] study was performed on patients who participated in a former cross-sectional study. In this follow-up (with a mean interval of 23 months) a correlation of Naa/Cr decrease in the medial temporal lobe with cognitive decline was observed. Considering that the ^{1}H-MRS metabolite studies rely on the degenerative process, and that there is a chronological difference of the degeneration between hippocampal region and posterior cingulate area, it is rational to compare the spectroscopic changes of these regions in each clinical severity stage to look for a possible spectroscopic staging of the degenerative process of AD 'in vivo'.

The aim of the present study is to follow the sequential degenerative process at the hippocampal region and posterior cingulate area in AD patients, from mild to severe clinical stages in comparison to normal controls, to possibly identify a spectroscopical staging of AD. Preliminary results with this approach were already presented[16-17].

METHOD

Subjects – Normal controls (n=12) and probable AD patients (n=34), totalizing 46 subjects constituted the sample. Two neurologists (EE and JLSC) and a psychiatrist (JL) evaluated the controls and patients at an outpatient clinic. Normal subjects had no cognitive impairment, psychiatric complaints or ADL problems. The diagnosis of probable AD was established according the DSM-IV and NINCDS-ADRDA criteria. The ischemic score[25] was ≤4 and the MMSE[26] scores were obtained. The clinical staging was according to the Clinical Dementia Rating (CDR)[27]. The characteristics of the sample are shown in Table 1.

Technique – MRI and single voxel ^{1}H-MRS studies were performed on a 1.5T scanner (Signa Horizon LX-GE). The spectroscopic studies were performed with the automated PROBE-P, using Cr as the reference metabolite.

PRESS pulse sequence with TR1500ms/TE30ms. The volumes of interest (VOI) studied were localized in the left and right medial temporal lobes (hippocampal region = entorhinal area + subiculum + hippocampus proper + dentate gyrus) (HCR, l&r) and posterior cingulate area (mainly part of Brodmann's area 23) (PCA, bilateral). The following metabolites expressed as ratios were studied: N-acetylaspartate (Naa/Cr), choline (Cho/Cr) and myo-inositol (mI/Cr). A qualified specialist (DMM) supervised all neuroimaging procedures.

Statistics – Data analysis was performed using ANOVA and post-hoc calculation (Tukey)[28,29]. Descriptive results of mean and sd were expressed in the tables till the centesimal order.

Ethics – The study was approved by the local Ethics Committee (CEP-IPUB/UFRJ)/informed consent signed.

RESULTS

The present data relate to the metabolite ratios obtained for the HCR and the PCA. The results of the HCR were obtained from the pooled data of each CDR stage sample. The data for the Naa/Cr are shown on Table 2.

There was statistical significance in the difference of Naa/Cr values among stages as shown by the ANOVA summary and the Tukey test.

For the other metabolite ratios, Cho/Cr and mI/Cr, there was no statistical significance among stages as shown by ANOVA and Tukey test, for both hippocampal region and posterior cingulate area.

In view of the results and considering that the Naa changes are the most important findings to evaluate neuronal degeneration, only the Naa/Cr ratio was taken in account.

There was no statistical significance between Naa results of CDR1 and CDR2 patients at the hippocampal region, and among CDR0, CDR1 and CDR2 at the posterior cingulate area. Additionally, Naa values of CDR1 and CDR2 stages were related to the normal values found at the posterior cingulate area, and are also frequently put together in clinical trials (mild-moderate AD). Thus, these stages could be joined for a further analysis. The results obtained are shown on Table 3.

In other words, the Naa data at the hippocampal region were statistically significant for CDR0 vs CDR1+CDR2 vs CDR3 stages, and at the posterior cingulate area only between CDR2 vs CDR3 stages.

DISCUSSION

The increasing prevalence rate of AD and the upcoming new specific therapeutic possibilities lead to the need of earlier and more accurate diagnosis of the disease. ^{1}H-MRS of the brain may be considered a valuable investigative and clinical tool for early diagnosis of AD as it permits to know the metabolite composition of normal and pathologic nervous tissue sample(s) 'in vivo', in a noninvasive way. It also permits a better understanding of physiological and pathophysiological mechanisms in normal state and in disease condition[4-6]. The choice of the VOIs is very important if one considers early diagnosis and disease progression studies. Based on neuropathological studies, it was already shown that the hippocampal region is the best early target and that the posterior cingulate area is a suitable target for later assessment[1,2].

Some spectroscopic studies relied on functional results (SPECT and PET techniques) to underpin the choice of the VOIs. Excluding the well-known

Table 1. Characteristics of the sample.

	normal		Alzheimer	
stages	CDR0	CDR1	CDR2	CDR3
n	12	12	12	10
male/female	3/9	3/9	7/5	3/7
age (years±sd) (range)	74.08±5.53 (65-80)	76.75±8.5 (66-93)	76.00±4.97 (70-81)	76.9±6.71 (68-82)
schooling				
years±sd	12.75±3.5	10.92±4.11	10.83±3.97	8.1±2.56
MMSE(*)				
score±sd	27.92±1.93	24.10±2.5	18.5±2.94	7.9±3.21

(*) p=0.0001 among the stages.

Table 2. Values for the Naa/Cr metabolite ratio at HCR and PCA - normal subjects (CDR0) and probable AD patients (CDR1, CDR2, CDR3).

Naa/Cr	normal	Alzheimer		ANOVA	
	CDR0	CDR1	CDR2	CDR3	F	*p*
HCR	1.50±0.13	1.31±0.15	1.23±0.14	1.14±0.17	17.1	<0.0001
PCA	1.53±0.13	1.51±0.11	1.49±0.13	1.31±0.14	7.9	0.00053

HCR, hippocampal region; PCA, posterior cingulate area.

results of hypoperfusion/hypometabolism of the temporo-parietal cortical region, considered consistent and characteristic of AD beyond the earliest stages, these studies need some comments. The anatomofunctional focus of such studies was the distributed brain network pertaining to memory systems, constituted basically by the hippocampal region, mammilary bodies, thalamus, and posterior cingulum[30-34]. Minoshima's et al.[32] study is frequently referred to justify the selection of the posterior cingulate cortex for spectroscopic studies for early diagnosis of AD and MCI. This group described a metabolic reduction (glucose metabolism with PET) in the posterior cingulate cortex in very early AD, but remarked that in spite of technical difficulties, the metabolic activity of the inferior temporal lobe appeared decreased. Other studies, such as Johnson's et al.[31] (perfusion with SPECT) for preclinical prediction of AD, Fakhri's et al.[30] (MRI-guided quantitative SPECT and volumetric MRI) to identify prodromal phase of AD, and Nestor et al.[33] (MRI combined with PET), in patients with mild AD, showed in a general way that regional decreases in perfusion were more prominent, in order of discriminating power, at the hippocampal region, the amygdala, the posterior cingulate, the thalamus, and the anterior cingulate.

These studies confirm that AD is associated with dysfunction of networks implicated in human amnesia. Coherent with this is the finding of hypometabolism in the hippocampal complex. Earlier negative results were possibly due to a lack of spatial resolution and/or poor localization of the neuroimaging techniques. Thus, the hippocampal region can be considered as the earliest structure to show hypoperfusion or hypometabolism, and that other structures of the memory network are also affected. Some of these structures (for instance, the

Table 3. Values for the Naa/Cr metabolite ratio at HCR and PCA - normal subjects (CDR0) and probable AD patients (CDR1+CDR2 and CDR3), and Tukey test.

Naa/Cr	normal	Alzheimer	ANOVA			
	CDR0	CDR1+CDR2	CDR3	F	p	
HCR	1.50±0.13	1.27±0.15	1.14±0.17	23.32	<0.0001	
PCA	1.53±0.13	1.50±0.12	1.31±0.14	10.91	0.000147	
pair-wise comparisons via Tukey HSD (VassarStats) - HCR						
	CDR0	CDR1-2	CDR3		CDR1-2	CDR3
Mean	1.50	1.27	1.14	CDR0	p<0.01	p<0.01
N	15	40	18	CDR1-2		p<0.01
MS=0.02;df=70						
HSD.^{05}=0.11; HSD.^{01}=0.13						
pair-wise comparisons via Tukey HSD (VassarStats) - PCA						
	CDR0	CDR1-2	CDR3		CDR1-2	CDR3
mean	1.53	1.50	1.31	CDR0	n/s	p<0.01
n	12	24	10	CDR1-2		p<0.01
MS=0.02;df=43						
HSD.^{05}=0.13; HSD.^{01}=0.17						

HCR, hippocampal region; PCA, posterior cingulate area; n/s, non-significant.

Table 4. Criteria for a spectroscopic staging of AD.

Clinical severity	CDR stage	Naa/Cr ratio		MS stage
		HCR	PCA	
Normal	0	normal	normal	MS0
mild-moderate	1-2	decreased	normal	MS1-2
Severe	3	decreased	decreased	MS3

HCR, hippocampal region; PCA, posterior cingulate area; MS, spectroscopic (metabolite) stage (^{1}H-MRS).

caudal portion of the anterior cingulate and the posterior cingulate) probably suffer deafferentation due to earlier lesion of the hippocampal region and its projection to the cingulate gyrus as shown by morphological and experimental studies[34-36], and do not reflect primary and early injury. Therefore it is possible to suggest that spectroscopy may offer better data on the degenerative situation of a given region in comparison to the isotopic functional methods that show regional changes of perfusion or metabolism, reflecting probably not only local changes, but also (and mostly, depending on the stage of the disease) the repercussion (deafferentation) from distant affected structures.

Several studies on the hippocampus[9,11,14,16-18,20] and one on the cingulum[19] with mild or mild-moderate AD patients were found in the literature. However, no studies were found in which spectroscopic data of these limbic regions are compared and related to the clinical severity stages of AD and normal controls. The analysis of the studies, already discussed formerly, in comparison to the present one show the consistent reduction of Naa/Cr, in both the hippocampus[9,11,14] and posterior cingulum[19], in a progressive way[20-24]. There were no changes in other metabolites regarding the present objective.

There is a clear correlation between the present spectroscopic findings and the CDR staging of AD. The progressive decrease of Naa runs in parallel with the increasing severity of the disease (Table 2). Such results have not been reported in other studies. In the same way, there is a correlation between the present spectroscopic findings and cognition. The progressive decrease of Naa also runs in parallel with the decline of the MMSE scores (compare Table 1 and Table 2) obtained in each clinical stage. Such observation has been reported in only a few AD studies[9,20-22].

The sequential degeneration of the hippocampal region and of the posterior cingulate area shows a chronological disparity that can be used to follow the progression of the degenerative process and allow for a spectroscopic staging of the disease. The hippocampal region is the first to show reduction of Naa values (a specific neuronal marker) beginning at Braak's/Delacourte's NFT I-II/PHF-tau 1-3 stages (~CDR1), and declining progressively as the disease worsens (CDR2 and CDR3). The Naa values at posterior cingulate area decrease later, from Braak's/Delacourte's NFT III-IV/PHF-tau 6-7 stages (~CDR3).

Thus, considering the Naa/Cr ratio, it is possible to follow the degenerative process throughout the several clinical stages, from CDR1 to CDR3, as well as to suggest a ^{1}H-MRS metabolite staging of the disease in relation to the clinical stages as shown in Table 4.

These results can be used as a tool for diagnosing the initial phase of AD, considering the early changes at the HCR. It also permits to follow the HCR degeneration throughout the course of the disease. In addition, considering the chronological disparity of the degenerative process between the hippocampal region and the posterior cingulate area, a spectroscopic staging of the disease can be suggested.

In conclusion, the hippocampal region, the first to present neuropathological changes in AD, is also the first to show Naa reduction (from CDR1 on). These values show a progressive declining trend according to the severity stages. The posterior cingulate area suffers later neuropathological as well as spectroscopical changes (CDR3). There is a clear correlation between the Naa reduction, CDR stages and cognitive decline, as assessed with the MMSE. The present study adds to the knowledge that the AD degeneration follows a chronologically different tempo, and correlates with cognitive de-

cline and CDR worsening. These results make possible (i) the early diagnosis of AD using the data of the metabolite changes at the hippocampal region; (ii) to follow the degenerative process throughout the course of the disease, and (iii) to suggest, considering the Naa/Cr ratio, a spectroscopic (metabolite) staging of the disease related to the clinical severity stages.

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