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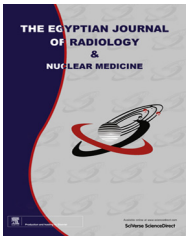

ORIGINAL ARTICLE

In vivo neurobiochemical changes of the posterior cingulate gyrus in patients with Alzheimer's disease detected by multivoxel proton magnetic resonance spectroscopy

Rania E. Mohamed [a,*], **Ashraf A. Aboelsafa** [b], **Dina M. Abo-Sheisha** [a]

[a] *Radiodiagnosis Department, Tanta University, Egypt*
[b] *Neurology Department, Tanta University, Egypt*

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KEYWORDS

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Abstract *Aim of the work:* To study the neurobiochemical changes in patients with Alzheimer's disease (AD) by multivoxel 1H-MRS.
Materials and methods: Twenty-five patients with probable AD and 12 age- and sex-matched normal controls were subjected to assessment of cognitive functions by the Mini Mental State Examination (MMSE) and imaging with multivoxel 1H-MRS for measuring the NAA/Cho, NAA/Cr, Cho/Cr, MI/NAA and MI/Cr ratios in the posterior cingulate gyrus (PCG) bilaterally.
Results: Patients with AD showed significant decrease in NAA/Cho and NAA/Cr, significant increase in MI/NAA and MI/Cr and non-significant increase in Cho/Cr ratios compared to control. Also, the severity of cognitive impairment was significantly associated with these changes. The NAA/Cho ratio at a cut-off value $\leqslant 1.14$ showed accuracy (94%), the NAA/Cr ratio at a cut-off value $\leqslant 1.40$ showed accuracy (97%), the Cho/Cr ratio at a cut-off value > 1.29 showed accuracy (85%), the MI/NAA ratio at a cut-off value > 0.60 showed accuracy (98%), and the MI/Cr ratio at a cut-off value > 0.83 showed accuracy (97%).

Abbreviations: MMSE, Mini Mental State Examination; 1H-MRS, proton magnetic resonance spectroscopy; AD, Alzheimer's disease; PCG, posterior cingulate gyrus; ACG, anterior cingulate gyrus; MCI, mild cognitive impairment; NAA, N-acetylaspartate; Cho, choline; Cr, creatine; MI, myo-inositol; Lac, lactate

* Corresponding author. Tel.: +20 1011756464/01003201025.
E-mail address: Rany1997@yahoo.com (R.E. Mohamed).

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Conclusion: The multi-voxels 1H-MRS of the PCG is sensitive to biochemical changes in AD. The 1H-MRS peak metabolite concentration ratios may be useful as markers for the progression of AD.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in elderly people. It is a progressive neurodegenerative disease that affects cortical and subcortical structures leading eventually to irreversible loss of neurons, particularly in the cortex (1). In the early stages, the most common symptom is the difficulty in remembering recent events, which is often mistakenly thought to be age-related concerns (2). As the disease advances, symptoms can include confusion, irritability, aggression, mood swings, language problems and long-term memory loss. Patients often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death (3). AD develops for an unknown and variable amount of time before becoming fully apparent, and it can progress undiagnosed for years (4). Patients with AD rely on others for assistance, placing a great burden on caregivers; with eventual social, psychological, physical and economic effects of caregiver's life (5). In developed countries, AD is one of the most costly diseases to the society (6).

Neuroimaging techniques may have an important role in the clinical evaluation of AD for early diagnosis, differential diagnosis and monitoring of disease activity. The proton magnetic resonance spectroscopy (1H-MRS) is a non-invasive imaging modality for biomarkers in AD, which is important for both early diagnosis and evaluating treatment effects. This imaging technique may provide a window into the biochemical changes associated with the loss of neuronal integrity and other neurodegenerative pathology that involves the brain before the manifestations of cognitive impairment in patients who are at risk for AD (7).

The neural network correlates of consciousness are unevenly distributed neuronal aggregates involved in conscious awareness. A critical node in this network is the posterior cingulate gyrus (PCG), which is the backmost part of the cingulate cortex lying behind the anterior cingulate gyrus (ACG), the upper part of the limbic lobe. The cingulate cortex is made up of an area around the midline of the brain. The PCG is commonly affected by neurodegenerative disease. Reduced metabolism in the PCG is an early sign of AD and is frequently present before clinical diagnosis (8). Furthermore, 1H-MRS of the PCG was more sensitive to the biochemical changes in patients with mild cognitive impairment (MCI) and AD than 1H-MRS of other neocortical regions in the brain (9).

With the aim of further contributing to the knowledge of the in vivo neurobiochemical changes of normal aging and AD, we carried out the present study performing multi-voxel 1H-MRS on the PCG of patients with AD and age-matched controls.

2. Materials and methods

2.1. Study participants

This study, performed from March 2012 to August 2013, included 25 patients (15 males and 10 females; age ranged from 64 to 85 years; mean 72.3 ± 10.4 years) referred to the Neurology Department of our institution fulfilling the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD (10). The Mini Mental State Examination (MMSE) test was used to evaluate the degree of cognitive impairment (11). Twelve normal subjects (8 males, 4 females; age ranged from 51 to 92 years; mean 71.2 ± 11.4 years) were selected amongst relatives or caregivers of the studied patients and served as the control group. All of them had a clinical history negative for neurological disease, mental disorder, head trauma or other relevant pathologies such as stroke, and scored >26 on MMSE. Illiterate subjects, subjects with physical problems interfering with test interpretation (for example, physically unable to hear or read instructions properly), or subjects with motor deficits affecting writing and drawing skills were ruled out. Additionally, we excluded patients with any metabolic illnesses that affect the cerebral peak metabolite concentration ratios such as diabetes mellitus as it was proved that myo-inositol/creatine (MI/Cr) ratio increases in the brains of patients with diabetes mellitus (12). Neither patients nor controls were taking any medications at the time of examinations. An informed consent from subjects enrolled in the study and/or their caregivers was obtained. An official permission to carry out the study was also obtained from the responsible authorities.

The MMSE is a brief 30-point questionnaire test that is commonly used to screen for dementia. It is also used to estimate the severity and follow the course of cognitive impairment. In the MMSE, any score greater than or equal to 26 points (out of 30) indicates a normal cognition. Below this, scores can indicate severe (⩽9 points), moderate (10–18 points) or mild (19–25 points) cognitive impairment (13). So, patients were further divided into three subgroups according to their points on the MMSE into mild, moderate and severe AD.

2.2. Imaging procedures

Study participants were examined initially with MRI and then with multivoxel 1H-MRS by using 1.5-Tesla MR unit, which had a spectroscopy capability (Signa Horizon SR 120; General Electric Medical Systems, Milwaukee, WI, USA) using a standard quadrature head coil (28 cm quadrature birdcage resonator). The MRI studies comprised the following sequences: multiplanar axial T1-weighted fast spin-echo (T1WFSE) with repetition time/echo time/number of excitations (TR/TE/NEX) of 500/14/2, multiplanar axial T2-weighted fast spin-echo (T2WFSE) with TR/TE/NEX of 4000/126/2, and axial fluid-attenuated inversion recovery (FLAIR) with TR/TE/NEX of 8000/142/1 and inversion time (TI) of 2200 ms (ms).

2.3. 1H-MRS protocol

The 1H-MRS was performed by using two-dimensional multivoxel long-echo (TE of 144 ms) point-resolved spatially

localised spectroscopy (PRESS) to assess the relative concentrations of metabolites with biological importance including: N-acetylaspartate (NAA), choline (Cho), creatine (Cr), lactate (Lac) and myo-inositol (MI). The PCG was examined bilaterally in both patients and control groups by using a spin-echo (SE) mode sequence. In all patients, the obtained spectra were displayed as grids of localised voxels with nominal size of $10 \times 10 \times 10$ mm, which were overlaid on the conventional MR images (either axial T2-weighted images or axial FLAIR images).

The localised voxels of interest (VOIs) were located in the posterior cingulate region in which they were placed below the cingulate sulci and above the parieto-occipital sulci, covering the PCG and inferior precunei bilaterally as regions of interest (ROIs). Water resonance suppression was optimally achieved by using the chemical shift selective water suppression (CHESS) technique. In all patients, the used parameters were TR 1000, TE 144 and 35 ms, FOV 24 cm, 18×18 phase encoding matrices, 1.0 cm section thickness, 2500 Hz spectral width and 2048 data points. The MRS scan was initiated if the line width reported by the pre-scan process was less than 8 Hz. The MR spectra obtained with a long TE of 144 ms and an additional short TE of 35 ms were utilised to confirm the phase inversion associated with J-coupled metabolites of lactate, and amino acids, but not of lipids, which may be helpful to discriminate lactate or amino acid signals from lipid signals. The acquisition time for each sequence was 5 min and 54 s. The Off-line spectral post-processing was carried out by using a semi-automated software (Functool, Version 2.33, GE Medical Systems, Milwaukee, WI, USA).

The main metabolite resonances were limited to 2.02 ppm (ppm) for NAA, 3.02 ppm for Cr, 3.20 ppm for Cho and 3.56 ppm for MI. As a result of difficulties in the calculation of the absolute metabolite concentrations, their relative concentration ratios were assessed by using the method of relative quantification with calculation of peak ratios of the NAA/Cr, NAA/Cho, Cho/Cr, MI/NAA and MI/Cr in each PCG separately in all patients and controls. The means of these peak ratios were then calculated in each of the three subgroups of patients and compared with those of the control group.

2.4. Statistical analysis

The data were collected and revised. Quantitative data were expressed as mean ± standard deviation (SD). The Receiver Operating Characteristic (ROC) curves for the mean peak metabolite concentration ratios were obtained and plotted by using a maximum likelihood curve-fitting algorithm. Relative diagnostic accuracy was estimated for each ratio by using the individual area under the ROC curve to identify the optimal cut-off values. The student *t*-test and *chi*-square test were used for comparison between two groups and the analysis of variance (ANOVA) test was used for correlation of the data. The SPSS for Windows version 18.0 software package (SPSS Inc., Chicago, IL) was used for statistical data analysis. *P*-value < 0.05 was considered statistically significant.

3. Results

This study included 25 patients with AD and 12 age- and sex-matched normal controls. The MMSE score in AD patients ranged from 8 to 22 with a mean of 14.7 ± 5.4 that was significantly lower than that of controls ($P < 0.001$); (Table 1). The duration of dementia ranged from 12 to 96 months (mean 60 ± 12.5 months).

The peak metabolite concentration ratios were bilaterally measured in the PCG of all study participants. Patients with AD showed significant decrease in the mean peak metabolite concentration ratios of both NAA/Cho ($P < 0.001$) and NAA/Cr ($P < 0.001$). Also, they showed a significant increase in the mean peak metabolite concentration ratios of MI/NAA ($P < 0.001$) and MI/Cr ($P < 0.001$). Although, the mean concentration ratio of Cho/Cr increased in patients with AD compared to that of the control group, this difference was not significant ($P = 0.91$); (Table 2).

The relationship between the means of the peak metabolite ratios and the degree of cognitive impairment evaluated by the MMSE was assessed. Severity of cognitive impairment in patients with AD was significantly associated with decreased NAA/Cho and NAA/Cr ratios ($P < 0.001$). On the other hand, it was significantly associated with increased MI/NAA and MI/Cr ratios ($P < 0.001$). However, a non significant increase in the mean Cho/Cr ratio was observed amongst different stages of AD in the group of patients ($P = 0.60$); (Table 3).

Table 4 and Figs. 1 and 2 demonstrated the results of the ROC curve analysis of the studied peak metabolite concentration ratios in patients with AD and their accuracy in distinguishing such patients from cognitively normal elderly. The NAA/Cho ratio at a cut-off value $\leqslant 1.14$ showed sensitivity (96%), specificity (91.7%), PPV (96%), NPV (91.7%) and accuracy (94%). The NAA/Cr ratio at a cut-off value $\leqslant 1.40$ showed sensitivity (100%), specificity (92%), PPV (96.2%), NPV (100%) and accuracy (97%). The Cho/Cr ratio at a cut-off value > 1.29 showed sensitivity (84%), specificity (90.9%), PPV (95.5%), NPV (73.3%) and accuracy (85%). The MI/NAA ratio at a cut-off value > 0.60 showed sensitivity (100%), specificity (91.9%), PPV (96.2%), NPV (100%) and accuracy (98%). The MI/Cr ratio at a cut-off value > 0.83

Table 1 Age, sex and MMSE in patients versus controls.

Parameter		Patients ($n = 25$)	Controls ($n = 12$)	Test value	*P*-value
Age (in years)	Range	64–85	51–92	$t = 0.292$	0.772
	Mean ± SD	72.3 ± 10.4	71.2 ± 11.4		
Sex	Female; n	10	4	$X^2 = 0.001$	0.976
	Male; n	15	8		
MMSE	Range	7–22	26–30	$t = 8.933$	< 0.001*
	Mean ± SD	14.7 ± 5.4	28.9 ± 1.25		

MMSE, Mini Mental State Examination; n, number.
* Significant.

Table 2 The mean metabolite concentration ratios in patients versus control.

Metabolite ratio	Patients (n = 25)	Controls (n = 12)	T-test	
	Mean ± SD	Mean ± SD	t	P-value
NAA/Cho	1.02 ± 0.22	1.46 ± 0.29	−4.71	<0.001*
NAA/Cr	1.05 ± 0.26	1.74 ± 0.44	−5.09	<0.001*
Cho/Cr	1.29 ± 0.20	1.15 ± 0.25	1.77	0.91
MI/NAA	0.80 ± 0.21	0.46 ± 0.12	6.30	<0.001*
MI/Cr	1.04 ± 0.16	0.53 ± 0.28	5.38	<0.001*

* Significant <0.05.

Table 3 Relationship between the mean metabolite concentration ratios and the degree of cognitive impairment assessed by the MMSE.

Metabolite ratio	Mini Mental State Examination (mean ± SD)			ANOVA	
	Mild (n = 8)	Moderate (n = 10)	Severe (n = 7)	F	P-value
NAA/Cho	1.23 ± 0.08	1.02 ± 0.12	0.77 ± 0.16	26.34	<0.001*
NAA/Cr	1.25 ± 0.14	1.09 ± 0.18	0.75 ± 0.18	17.25	<0.001*
Cho/Cr	1.25 ± 0.10	1.27 ± 0.17	1.35 ± 0.31	0.52	0.60
MI/NAA	0.69 ± 0.07	0.72 ± 0.13	1.06 ± 0.20	17.11	<0.001*
MI/Cr	0.91 ± 0.05	1.04 ± 0.09	1.19 ± 0.21	8.89	<0.001*

* Significant <0.05.

Table 4 Sensitivity, specificity, predictive values and accuracy of the metabolite ratios in patients with AD (n = 25).

Metabolite ratio	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
NAA/Cho	⩽1.14	96.0	91.7	96.0	91.7	94
NAA/Cr	⩽1.40	100.0	92.0	96.2	100.0	97
Cho/Cr	>1.29	84.0	90.9	95.5	73.3	85
MI/NAA	>0.60	100.0	91.9	96.2	100.0	98
MI/Cr	>0.83	100.0	91.6	96.2	100.0	97

PPV, positive predictive value; NPV, negative predictive value.

showed sensitivity (100%), specificity (91.6%), PPV (96.2%), NPV (100%) and accuracy (97%).

3.1. Cases

The figures (from 3 to 6) demonstrate a sample of selected cases of our study; in which each figure outlines only one case including: a normal control (Fig. 3), a case with mild AD (Fig. 4), a case with moderate AD (Fig. 5) and a case with advanced AD (Fig. 6).

4. Discussion

Alzheimer disease is an acquired disorder of cognitive and behavioural impairment that markedly interferes with social and occupational functioning (14). It is an incurable disease having a long and progressive course with major public health problems (15). 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 (7). Amongst diagnostic imaging modalities, the 1H-MRS is considered as a valuable tool for early diagnosis of AD, as it allows, in a non-invasive manner, determination of regional concentration ratios of a number of critical neurochemicals in both normal and pathologic nervous tissues in vivo (1).

The NAA is an amino acid present primarily within neurons in the central nervous system as well as in dendrites and axons. It is an acetyl donor in lipid synthesis, which is present in neurons and is actively involved in myelin synthesis. Therefore, it is a very specific marker for viable neurons, axons and dendrites. The diagnostic utility of NAA is based on its ability to quantify neuronal injury or loss on a regional basis; therefore it is widely used as a marker of neuronal density and as an in vivo marker of neurometabolic fitness. Reduced NAA concentration in the PCG has been described in patients with AD using 1H-MRS (9). Moreover, Cr is an important indicator of the energy metabolism and is used as an internal reference to calculate the concentration of the other metabolites. In addition, Cho is generally used as a marker of cellular density and membrane turnover, so it reflects the damaged cholinergic neurons in AD. Furthermore, MI, which is a sugar-alcohol, is primarily located in glial cells and often interpreted as a marker of gliosis and glial cell numbers, as it exists in a considerably higher concentration within glial cells (16).

The PCG is a part of the limbic system involved in memory retrieval and evaluation of information (17). Abnormalities within the PCG are associated with the earliest pathologic

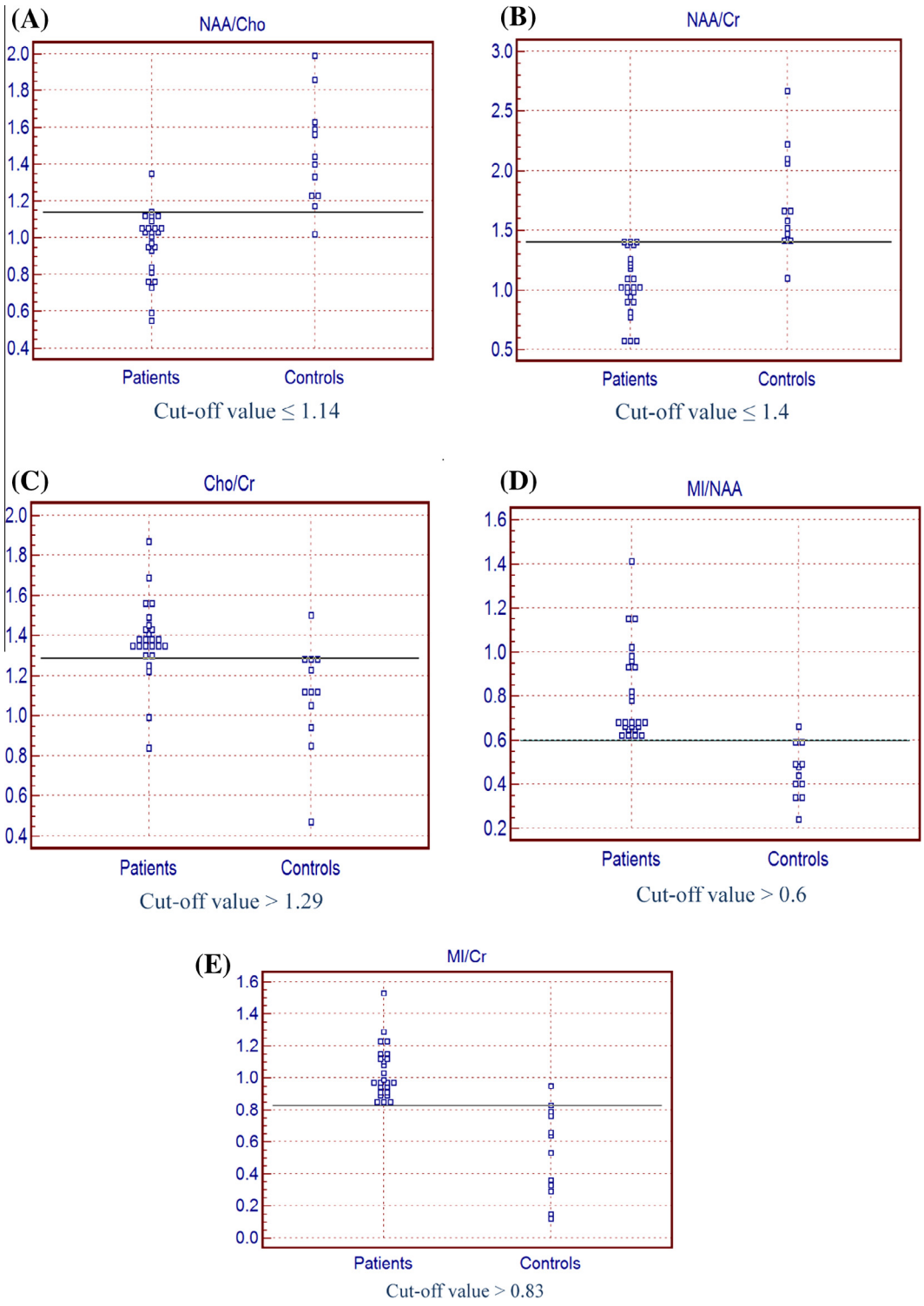

Fig. 1 (A–E) Demonstrates the NAA/Cho (A), NAA/Cr (B), Cho/Cr (C), MI/NAA (D) and MI/Cr (E) values, which were obtained from patients and controls.

changes involving the entorhinal and the hippocampal cortices. The hippocampus and PCG are considered as the earliest structures to show hypoperfusion or hypometabolism. Furthermore, degeneration of the hippocampus and PCG can be used to follow the progression of the degenerative process and allows for a spectroscopic staging of AD (18).

In the current study, we used multivoxel 1H-MRS to study changes in the metabolite concentration ratios of the NAA/Cho, NAA/Cr, Cho/Cr, MI/NAA and MI/Cr of the PCG in patients with AD and age- matched normal controls aiming to study the neurochemical pattern of normal aging and AD. As in previous studies (7,9,18,19), although done on different

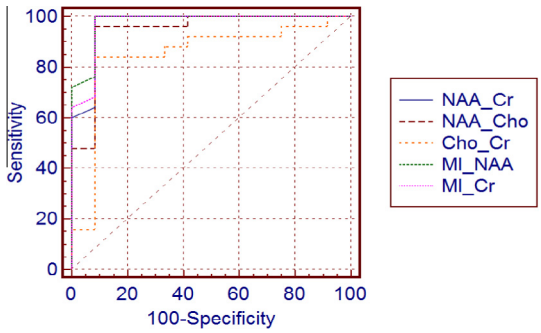

Fig. 2 Demonstrates the ROC curve analysis of peak metabolite concentration ratios of NAA/Cho (at cut-off value ⩽1.14 with sensitivity 96% and specificity 91.7%), NAA/Cr (at a cut-off value ⩽1.40 with sensitivity 100% and specificity 92%), Cho/Cr (at a cut-off value >1.29 with sensitivity 84% and specificity 90.9%), MI/NAA (at a cut-off value >0.60 with sensitivity 100% and specificity 91.9%), and MI/Cr (at a cut-off value >0.83 with sensitivity 100% and specificity 91.6%) between patients and controls.

brain regions, we found significant reduction of the mean metabolite concentration ratios of NAA/Cho ($P < 0.001$) and NAA/Cr ($P < 0.001$) in the PCG of patients with AD, whilst the mean metabolite concentration ratios of MI/NAA and MI/Cr significantly increased ($P < 0.001$). Also, we found non-significant increase of the mean Cho/Cr ratio in patients with AD compared to normal control ($P = 0.91$). This latter finding was also observed by Kantarci (20) who suggests that Cho/Cr elevation might be a preclinical marker for AD in elderly. The reduction of NAA observed in patients with AD could be attributed to neuronal loss or dysfunction, and the elevation of MI to gliosis (19). In addition, Liu et al. (21), observed a reduction in NAA/Cr ratio in patients with MCI when compared to normal control. Furthermore, Zhoua et al. (18), observed increased MI/Cr peak intensity ratio, with decreased NAA/Cr, in patients with early probable AD but they did not find changes in the Cho/Cr ratio. Also, Kizu et al. (22), reported non-significant changes in Cho/Cr ratios in the PCG in AD patients. The rise in grey matter Cho levels has been associated with decline in memory function and regional cerebral metabolism (23). However, Block et al. (24), found no significant changes in the Cho resonance in subjects with AD. Moreover, Loos et al. (16), reported that a fall in NAA/Cr, with an increase in MI/Cr, favours the diagnosis of AD. Furthermore, Lim et al. [25], concluded that both the decreased NAA/Cr of the PCG and the increased MI/Cr of the ACG may reflect biochemical changes in AD according to the posterior-dominant progression of AD pathology. However, Menezes et al. (26), who studied changes in the metabolite concentration ratios in the hippocampus of patients with MCI and early mild AD as well as normal aging individuals, concluded that the ratio value of metabolites NAA/Cr, MI/Cr, Cho/Cr and MI/NAA did not show any significant differences between controls versus MCI or mild AD patients.

The MMSE is a useful and widely used clinical screening tool for dementia. It is one of the criteria recommended by the National Institute for Clinical Excellence in following clinical status after treatment of AD with cholinesterase inhibitors. The association between MMSE score and MI elevation in the posterior cingulate region of the brain is considered as a relatively specific marker for AD. Additionally, the presence of a correlation between NAA/Cr and MMSE in the AD is a key finding in its diagnosis (27).

In the present study, we recognised that the mean MMSE score in the group of patients significantly reduced compared to that of the control group ($P < 0.001$). Moreover, we classified the severity of AD in the group of patients based on MMSE into mild, moderate and severe, and observed that the severity of cognitive impairment in patients with AD was significantly ($P < 0.001$) associated with decreased NAA/Cho and NAA/Cr mean metabolite ratios as well as increased MI/NAA and MI/Cr mean metabolite ratios in the PCG. This is in harmony with the results of prior studies (18,20,25,28) who stated that, the total MMSE scores obtained for MCI and AD significantly decreased compared to the control group ($P < 0.05$). Also, they observed a significant association between NAA/Cr and MI/NAA ratios in the PCG and the MMSE in patients with AD and they depicted that the progressive decrease of NAA in such patients runs parallel with the decline in the MMSE scores. Additionally, we recognised a non-significant increase in the mean concentration ratio of Cho/Cr with increased severity of cognitive impairment in AD patients ($P = 0.60$). This is in accordance with Modrego et al. (29), who observed that the increased metabolite concentration ratio of Cho/Cr in different stages of AD in the posterior cingulate area was statistically not significant.

1H-MRS is sensitive to biochemical changes during the pathologic progression of AD before there is a significant loss of neuronal integrity. In addition, it is useful for predicting and monitoring different pathological stages in the course of AD (9). Currently, we used ROC curve analysis of the studied metabolite concentration ratios in the PCG of patients with AD to evaluate their accuracy in distinguishing such patients from cognitively normal elderly. We observed that MI/NAA, NAA/Cr and MI/Cr metabolite concentration ratios had highest sensitivity, specificity and accuracy in prediction and evaluation of patients with AD. Our results showed that the NAA/Cho ratio at a cut-off value ⩽1.14 had sensitivity (96%), specificity (91.7%), PPV (96%), NPV (91.7%) and accuracy (94%). Moreover, NAA/Cr ratio at a cut-off value ⩽1.40 showed sensitivity (100%), specificity (92%), PPV (96.2%), NPV (100%) and accuracy (97%). The Cho/Cr ratio at a cut-off value >1.29 showed sensitivity (84%), specificity (90.9%), PPV (95.5%), NPV (73.3%) and accuracy (85%). Additionally, the MI/NAA ratio at a cut-off value >0.60 depicted sensitivity (100%), specificity (91.9%), PPV (96.2%), NPV (100%) and accuracy (98%). Finally, the MI/Cr ratio at a cut-off value >0.83 showed sensitivity (100%), specificity (91.6%), PPV (96.2%), NPV (100%) and accuracy (97%).

Kantarci et al. (30), demonstrated that NAA/Cr measured from the PCG added predictive value for conversion to dementia. Furthermore, Modrego et al. (29), demonstrated that ROC curve analysis for NAA/Cr < 1.61 predicted conversion of MCI into AD with 100% sensitivity, 75% specificity, PPV of 83% and NPV of 100% with area under the curve of 0.91. Similarly, Fayed et al. (31), showed that NAA/Cr < 1.40 in the PCG predicted conversion of MCI to probable AD with sensitivity of 82% and specificity of 72% with area under the curve of 0.82. Finally, Modrego et al. (32), showed that NAA/Cr < 1.43 in posteromedial parietal cortex predicted conversion to probable AD with 74% sensitivity and 84% specificity

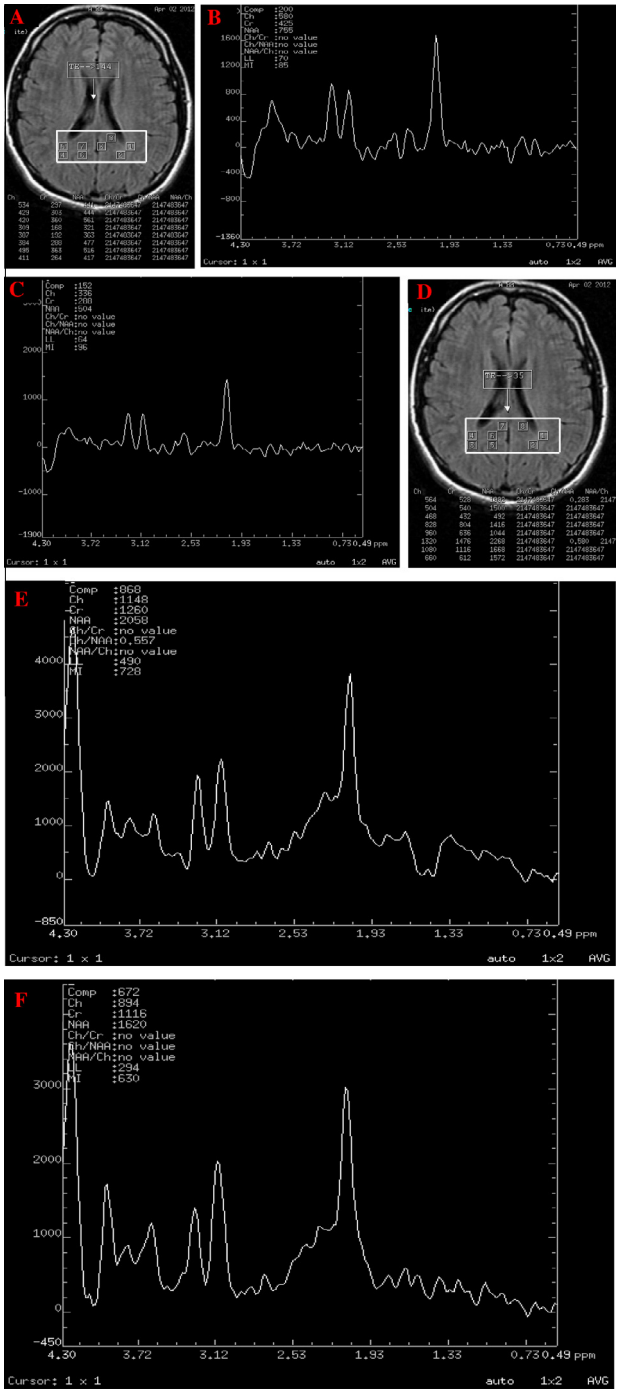

Fig. 3 (A–F) Demonstrates a sixty-five year old healthy elderly male volunteer with MMSE score of 28. The 1H-MRS multivoxel picture (A), and MRS spectrum of the right (B) and left (C) posterior cingulate gyri at a long TE of 144 ms, in addition to 1H-MRS multivoxel picture (D), and MRS spectrum of the right (E) and left (F) posterior cingulate gyri at a short TE of 35 ms reveal normally average NAA/Cr, NAA/Cho, Cho/Cr, Lac/NAA and MI/NAA peak metabolite concentration ratios of both posterior cingulate gyri. These ratios are (NAA/Cr = 1.78, NAA/Cho = 1.30, Cho/Cr = 1.36, MI/NAA = 0.35 and MI/Cr = 0.57) in the right PCG (B and E) and (NAA/Cr = 1.75, NAA/Cho = 1.5, Cho/Cr = 1.17, MI/NAA = 0.38 and MI/Cr = 0.56) in the left PCG (C and F).

with area under the curve of 0.84. Noteworthy, NAA/Cr measurements could predict cognitive decline and monitor disease activity in patients with clinically established AD [20]. Moreover, MI/NAA and NAA/Cr ratios can be considered as early predictors for prodromal AD in people with MCI (9).

We thought that our study might provide an important close idea about the AD and the changes of brain metabolite concentration ratios, which might help for early diagnosis of the disease in a preclinical stage and hence early management. We obtained the MR spectra at two different echo times, a long TE of 144 ms and an additional short TE of 35 ms which were used to prove the phase inversion associated with J-coupled metabolites of lactate and amino acids, but not of lipids in order to distinguish lactate or amino acid signals from lipid signals. Additionally, we considered the use of PCG for placement of 1H-MRS voxels to involve PCG and inferior precunei bilaterally, as a strengthening point of our study. The PCG is a limbic cortical region that is affected relatively early in the pathologic progression of AD. Furthermore, the midline location of the posterior cingulate voxels allows high quality spectra and reproducible voxel placement with easy evaluation of bilateral posterior cingulate gyri in the same patient (18), as well as appropriate evaluation of the alterations in the metabolite concentration ratios at different stages of the disease. Additionally, we used the ROC curve analysis of the studied different metabolite concentration ratios in the posterior cingulate gyri of patients with AD and we evaluated the optimal cut-off values of these ratios with best sensitivity, specificity, predictive values and accuracy in assessment of the severity of the disease and prediction of progress of cognitive impairment with better differentiation between patients with AD and cognitively normal elderly.

Regrettably we faced some limitations. Although, the location of the posterior cingulate voxel was relatively free of air-bone-brain interface susceptibility problems, we considered the un-examination of the anteromedial temporal lobe, including hippocampus and entorhinal cortex as a limitation. However, we were incapable to constantly acquire technically satisfactory quality spectra from this region as a result of the proximity of magnetic susceptibility artifacts created by the tissue-air interface near the petrous bone. Moreover, obtaining spectra from a voxel small enough to sample the anteromedial temporal lobe structures without partial voluming could not be achieved. Thus, the most technically robust and functionally appropriate regions to study are the posterior cingulate gyri (9). Also, previous studies (18,25) observed that the posterior cingulate voxel was better than that in other regions in the brain, such as superior temporal lobe and medial occipital lobe. Moreover, the small number of patients in the current study was another limitation. So, further extended studies on a larger number of patients utilising a standardised enrolment and diagnosis criteria, data acquisition methods, should be conducted to set up normative values.

In summary, AD is associated with dysfunction of networks implicated in human amnesia. The multi-voxels 1H-MRS at 1.5 Tesla of the PCG is sensitive to the biochemical changes in AD. Therefore, this non-invasive technique has a great potential in becoming an adjunct to the clinical evaluation and management of such disease. The 1H-MRS peak metabolite concentration ratios may be useful as markers for the progression of AD. In view of our study, we observed that MI/NAA (at cut-off value >0.06), NAA/Cr (at cut-off

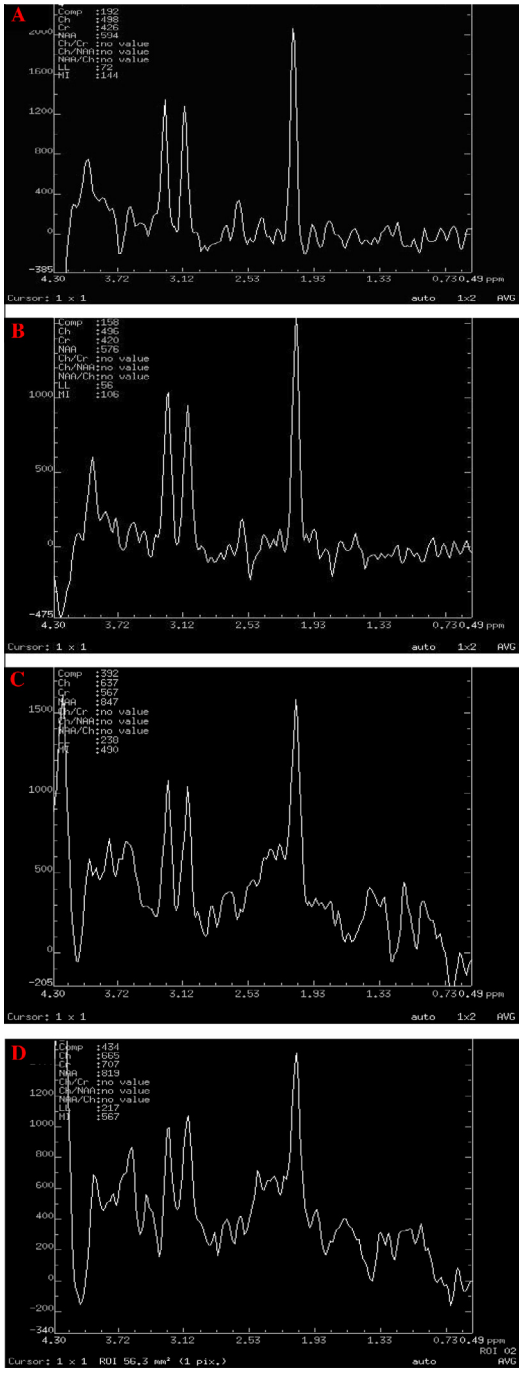

Fig. 4 (A–D) Demonstrates a sixty-seven year old female patient with mild degree of AD and her MMSE score is 20. The MRS spectrum of the right (A) and left (B) posterior cingulate gyri at a long TE of 144 ms, in addition to MRS spectrum of the right (C) and left (D) posterior cingulate gyri at a short TE of 35 ms reveal significant reduction in the NAA/Cr, NAA/Cho peak metabolite concentration ratios of both posterior cingulate gyri. Additionally, there is significant increase in the MI/NAA and MI/Cr as well as non significant increase in the Cho/Cr peak metabolite concentration ratios of both posterior cingulate gyri. These ratios are (NAA/Cr = 1.39, NAA/Cho = 1.19, Cho/Cr = 1.17, MI/NAA = 0.58 and MI/Cr = 0.86) in the right PCG (A and C) and (NAA/Cr = 1.37, NAA/Cho = 0.96 and Cho/Cr = 1.18, MI/NAA = 0.58 and MI/Cr = 0.86) in the left PCG (B and D).

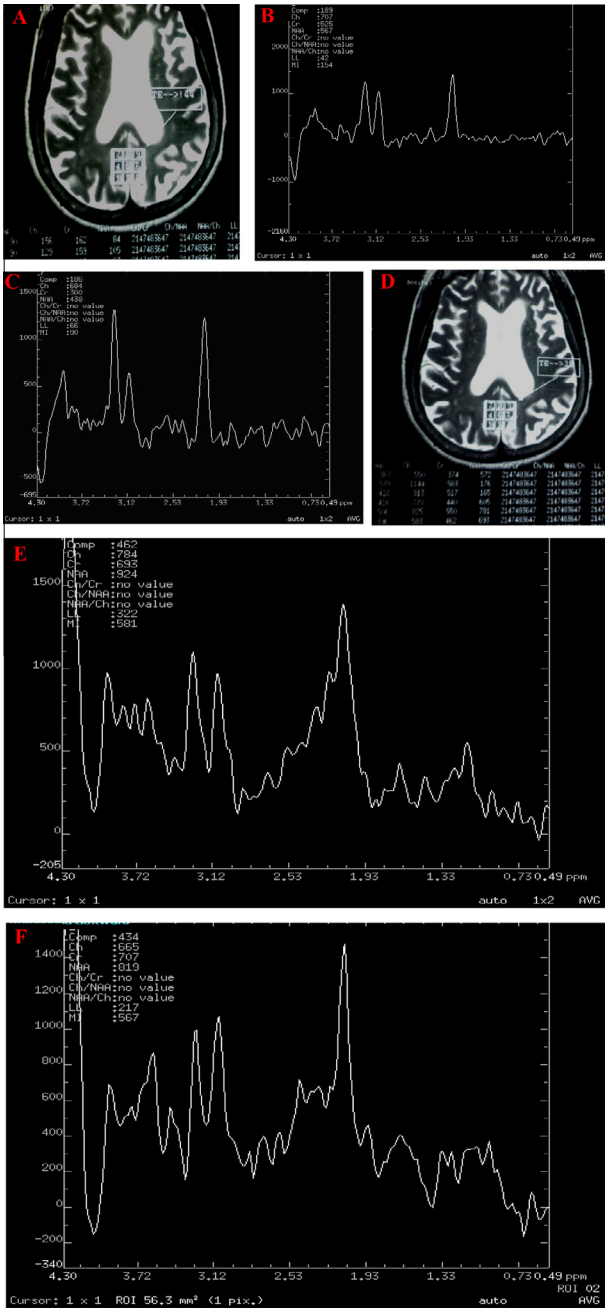

Fig. 5 (A–F) Represents a seventy-eight year old male patient with moderate degree of AD and his MMSE score is 12. The 1H-MRS multivoxel picture (A) as well as MRS spectrum of the right (B) and left (C) posterior cingulate gyri at a long TE of 144 ms, in addition to 1H-MRS multivoxel picture (D) as well as 1H-MRS spectrum of the right (E) and left (F) posterior cingulate gyri at a short TE of 35 ms reveal significant reduction in the NAA/Cr, NAA/Cho peak metabolite concentration ratios of both posterior cingulate gyri. Additionally, there is significant increase in the MI/NAA and MI/Cr as well as non significant increase in the Cho/ Cr peak metabolite concentration ratios of both posterior cingulate gyri. These ratios are (NAA/Cr = 1.08, NAA/Cho = 0.80, Cho/Cr = 1.34, MI/ NAA = 0.62 and MI/ Cr = 0.84) in the right PCG (B and E) and (NAA/Cr = 1.05, NAA/Cho = 0.972 and Cho/Cr = 1.083, MI/ NAA = 0.69 and MI/Cr = 0.80) in the left PCG (C and F).

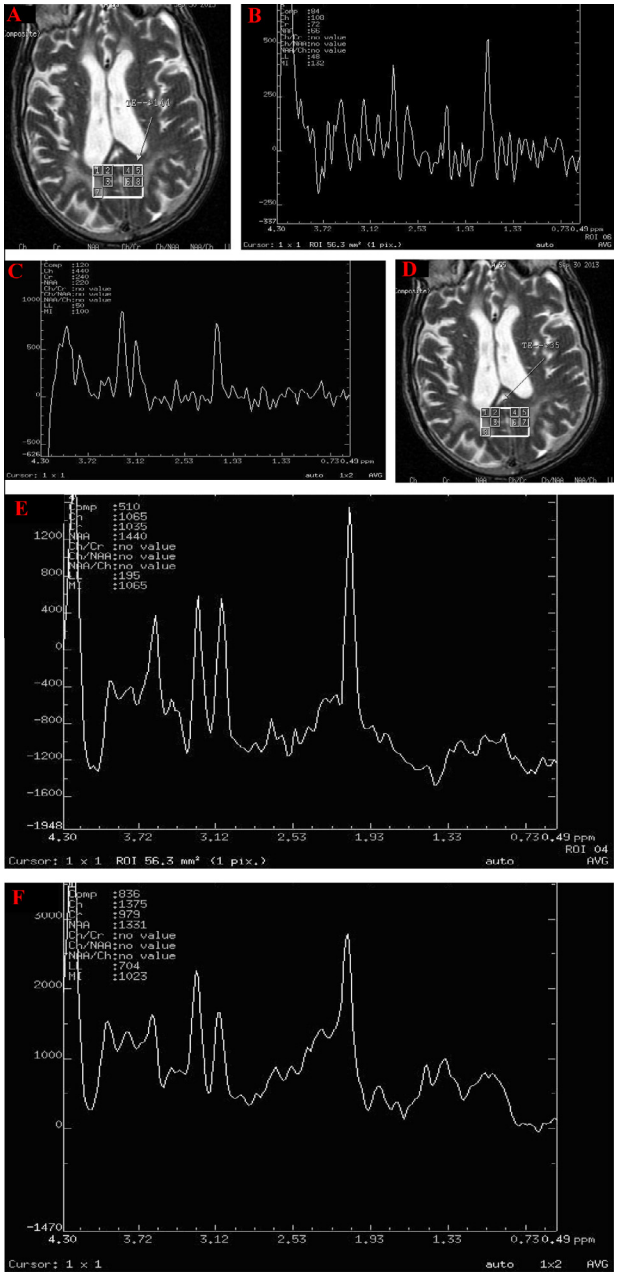

Fig. 6 (A–F) Demonstrates a seventy year old male patient with severe degree of AD and his MMSE score is 8. The 1H-MRS multivoxel picture (A), and MRS spectrum of the right (B) and left (C) posterior cingulate gyri at a long TE of 144 ms, in addition to 1H-MRS multivoxel picture (D), and MRS spectrum of the right (E) and left (F) posterior cingulate gyri at a short TE of 35 ms reveal significant reduction in the NAA/Cr, NAA/Cho peak metabolite concentration ratios of both posterior cingulate gyri. Additionally, there is significant increase in the MI/NAA and MI/Cr as well as non significant increase in the Cho/Cr peak metabolite concentration ratios of both posterior cingulate gyri. These ratios are (NAA/Cr = 0.9, NAA/Cho = 0.61, Cho/Cr = 1.5, MI/NAA = 0.74 and MI/Cr = 1.03) in the right PCG (B and E) and (NAA/Cr = 0.92, NAA/Cho = 0.50, Cho/Cr = 1.83, MI/NAA = 0.77 and MI/Cr = 1.04) in the left PCG (C and F).

value $\leqslant$1.40) and MI/Cr (at cut-off value $>$0.83), had highest sensitivity, specificity and accuracy in prediction and evaluation of patients with AD. Also, we observed that the severity of cognitive impairment in patients with AD was significantly ($P < 0.001$) associated with decreased NAA/Cho and NAA/Cr peak metabolite concentration ratios as well as increased MI/NAA and MI/Cr peak metabolite concentration ratios in the PCG of patients with AD.

Conflict of interest

None.

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