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Value of MRS in the Evaluation of Deep Grey Matter in Multiple Sclerosis

Background/Objective: In this study, our purpose was to evaluate the thalamic magnetic resonance (MR) spectroscopic changes in multiple sclerosis (MS) patients.
Patients and Methods: Forty-three relapsing-remitting MS (RRMS) patients and 23 control subjects were included in this study. Routine brain MR and MR spectroscopy (MRS) using single voxel technique were carried out in all the patients and the controls. The N-Acetylaspartate (NAA), choline (Cho) and creatine (Cr) peaks were calculated. The thalamic relative metabolite ratios of the RRMS patients were compared to those of the control subjects by using the statistical measures (Mann-Whitney U test). The level of statistical significance was set at 0.05.
Results: The thalamic NAA/Cr and NAA/Cho ratios were lower while the thalamic Cho/Cr ratio was higher in the MS patients than those of the control subjects. But these differences in the spectroscopic relative metabolite ratios were not statistically significant between the groups (p=0.34, p=0.22 and p=0.1, respectively).
Conclusion: The spectroscopic relative metabolite ratios were of limited value in determining the presence of metabolic changes in the deep grey matter of MS patients. New studies about the presence of the deep grey matter damage in MS patients are necessary.

Keywords: Multiple Sclerosis, Brain Metabolites, Magnetic Resonance Spectroscopy, Neuronal Loss, Thalamus

Introduction

Multiple sclerosis (MS), commonly seen in young adults, is a disease characterized by chronic demyelination and degeneration of myelin. Relapses may be seen either during relapsing remitting (RR), secondary progressive or primary progressive MS patients.[1-4] Relapses show the new onset or continuing disease activity in the central nervous system (CNS).[2] Magnetic resonance imaging (MRI) provides additional information about MS patients supplementary to their clinical evaluations.[3] MRI shows both presence and localization of demyelinating plaques in the CNS and the differences in their characteristics during the disease course.[4] Moreover, MRI may offer valuable data about the determination of the effect of treatment modalities used in MS patients.[3-7]

Initially, MS was regarded primarily as a white matter (WM) disease and most studies focused on this aspect. However, some studies also suggested significant involvement of grey matter (GM).[1,8-10] The cortical atrophy observed in MS patients has been linked to the neuronal injury resulting from the inflammation in the white matter.[9-11] The volumetric and/or MR spectroscopic evaluation of the cortical GM has some technical difficulties because of its complex shape.[8,11] On the other hand, thalami offer an easier way for the proton MR spectroscopy (MRS) to determine the deep GM (DGM) injury. Some eloquent studies performed recently concentrated on the injury of the DGM in MS.[8,11-14] These studies with different methodologies all showed neuronal loss in the thalamus in MS patients due to various reasons. Instead of using absolute concentration values which have been used by all the aforementioned studies, we aimed to evaluate the thalamic structural changes in a group of MS patients using relative metabolite ratios.

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Patients and Methods

Subjects

A total of 43 patients (24 female, 19 male), aged 20-60 years (mean, 37 years) with a disease duration of 3-17 years (mean, 5 years) and the Expanded Disability Status Scale (EDSS) of 0-5.0 (mean, 2) were included in the study. From December 2006 to December 2008, the patients were all diagnosed as RRMS based on McDonald's MS criteria in the out-patient clinic of the neurology department of our hospital when they were admitted with the complaint of new onset or sign and symptom worsening. An experienced neurologist, blinded to the MRI findings assessed the clinical status, neurological examination and physical disability of the patients by EDSS within three weeks of cranial MRI and MRS acquisition.[15]

A total of 23 subjects (8 female, 15 male), aged 20-58 years (mean, 43 years) with headache without any other pathological findings or additional illnesses constituted the control group. Their neurological examinations and cranial MRI results were normal. Informed consents were obtained from all the participants and the university ethics committee approved the study protocol.

MRI and Analysis

In all patients, routine brain MRI were obtained with a 1.5 T MR unit (Magnetom Vision Plus, Siemens, Erlangen, Germany) using a standard birdcage head coil according to the following protocol: sagittal-axial T1-weighted (W) spin-echo (SE) sequences with magnetization transfer technique (TR/TE 550/18), three planes T2W turbo spin-echo (TSE) (TR/TE 5400/99), and sagittal-axial fluid attenuated inversion recovery (FLAIR) (TR/TE/TI 8400/114/2150) sequences were obtained. Imaging parameters were as follows: slice thickness: 1 mm; field of view (FOV): 24 cm; 256×256; matrix: 5 mm; and number of excitations (NEX): 2.[6] Following those sequences, MRS using PRESS sequence was performed by placing an 8cm^3 VOI (volume of interest) in bilateral thalami (TE/TR: 135/2000, NEX: 136) (Fig. 1). The VOI included the thalami without exceeding their borders (as soon as possible). To avoid placement of VOI beyond the borders of the thalami, control of VOI was carefully done with three planes T2W or T1W images before MRS examination. The total examination duration for routine MR sequences and MRS was approximately 25 minutes.

Following acquisition of all images, MRS findings in addition to the routine MRI findings were evaluated by an experienced radiologist blind to the clinical and laboratory data at the workstation of our MRI unit. The peak values of N-Acetylaspartate (NAA), Choline (Cho) and Creatine (Cr) and relative ratios of these metabolites were calculated.

All the statistical analyses were done with SPSS 13.0 for windows (SPSS Inc., Chicago, IL, USA). Since study parameters showed non-normal distribution, nonparametric statistics were used. Mann-Whitney U test was performed to determine between groups. The level of statistical significance was set at 0.05.

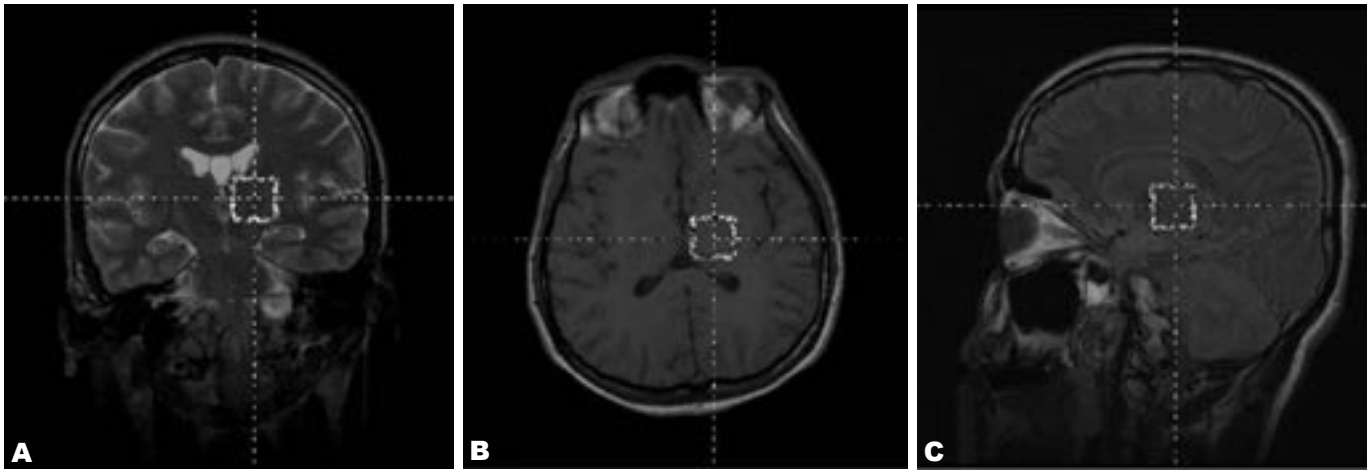

Fig. 1. MR images of a 37-year-old man (a control subject) showing the position of MRS examination area. **A.** Coronal T2W. **B.** Axial T1W. **C.** Sagittal FLAIR. In all images, the white rectangles indicate the volume of interest (VOI) for MR spectroscopy. To achieve a reproducible position, the VOIs were placed in the same regions in all patients and control subjects

Results

Totally, one-hundred ninety-eight MS plaques in different parts of the brain were observed on T2W and FLAIR images of the patients (Fig. 2). There were T2 hypo-intensities in the basal ganglia of 12 cases (28%). Diffuse cortical atrophy was present in four (9%) of the cases. The patients had no other pathologies other than MS plaques in the WM. None of the RRMS patients had MS plaque in the GM. There were nonspecific periventricular WM hyper-intensities in 10 of the control cases without any additional pathology.

Both groups were homogeneously distributed in terms of age and sex so there was no confounding variable in our study. Statistically compared parameters (age, sex and relative spectroscopic ratios) were homogeneously distributed between groups. There was no statistical significance between groups in terms of age and sex ($p>0.05$).

The mean Cho/Cr, NAA/Cho and NAA/Cr ratios in the patients were 1.04±0.04, 1.82±0.09, and 1.79±0.06 respectively. These ratios in the control group were 0.91±0.04, 2±0.11, and 1.82±0.06, respectively (Fig. 3). The spectroscopic metabolite ratios in the patient group were not significantly different from the control subjects ($p>0.05$). The results of MRS examinations with median and inter-quartile range values are shown in Table 1.

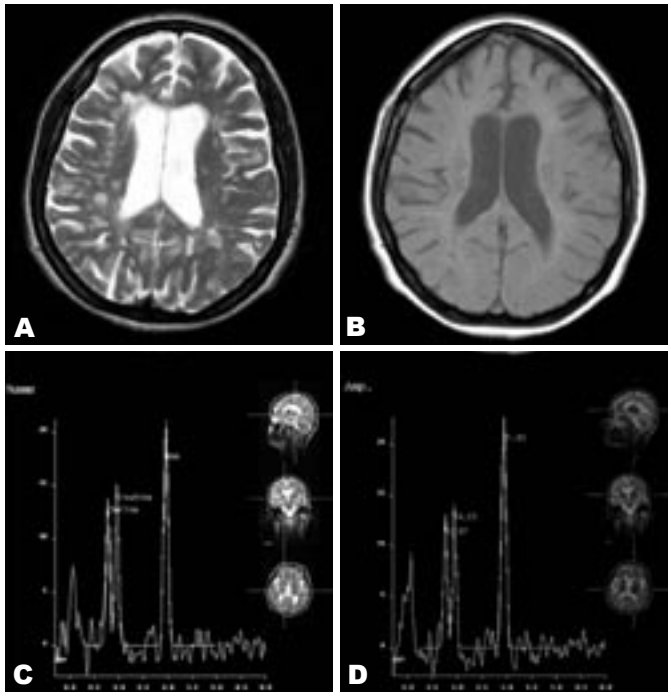

Fig. 2. Axial MRI and MRS images of a 42-year-old female patient with RRMS. In axial T2W **(A)** and T1W **(B)** images, multiple periven-tricular MS plaques and cortical atrophy are seen. In proton MR spectra of this patient, locations of N-acetylaspartate (NAA), choline (Cho) and creatine (Cr) peaks are observed **(C)**. The NAA/Cho, NAA/Cr and Cho/Cr ratios of this patient were 1.69, 1.55 and 0.92, respectively **(D)**.

Discussion

Proton MRS, a noninvasive technique assessing the metabolites and their changes within the brain, is commonly used in the differential diagnosis of different pathologies like tumor, infarction or abscess in the CNS.[5,16,17] Any decrease in NAA which is a neuronal marker shows any neuronal injury and/or loss of neurons.[16-18]

There are a limited number of studies evaluating the role of MRS determining DGM injury of MS patients in the literature.[8,11,13,14] Inglese et al.[13] reported 7% lower NAA and 14% higher Cho concentrations in the DGM nuclei of eleven RRMS patients compared with nine control subjects ($p<0.02$ for both). In this study, the difference between patients and control subjects with respect to Cr concentrations did not exhibit significant difference across the DGM nuclei. Cifelli et al.[8] and Wyelezinska et al.[11] reported decrease either in the NAA concentrations or the thalamic volumes in two different studies, each including 14 MS patients (secondary progressive MS and RRMS cases, respectively) compared with control subjects. Wyelezinska et al.[11] demonstrated a decrease in the NAA/Cr ratio of RRMS cases. Meanwhile, Geurts et al.[14] found a marked decrease in the primary progressive MS compared to a mild decrease in RRMS cases in the thalamic NAA concentrations of MS patients. There was no statistical significance in terms of absolute Cho and Cr concentrations in any of these studies (Geurts et al.,[14] Cifelli et al.[8] and Wyelezinska et al.[11]) comparing MS patients with control cases.

In our study, NAA/Cr and NAA/Cho ratios were lower while the Cho/Cr ratio of RRMS patients was higher than the control subjects. But these differences did not reach a statistical significance ($p=0.3$, 0.2 and 0.1, respectively). That is the reason we concluded that MRS has limited value in the evaluation of thalamic or DGM injury in MS patients. The decrease in NAA/Cr and NAA/Cho ratios, although statistically not significant, which might be attributed to mild NAA decrease,

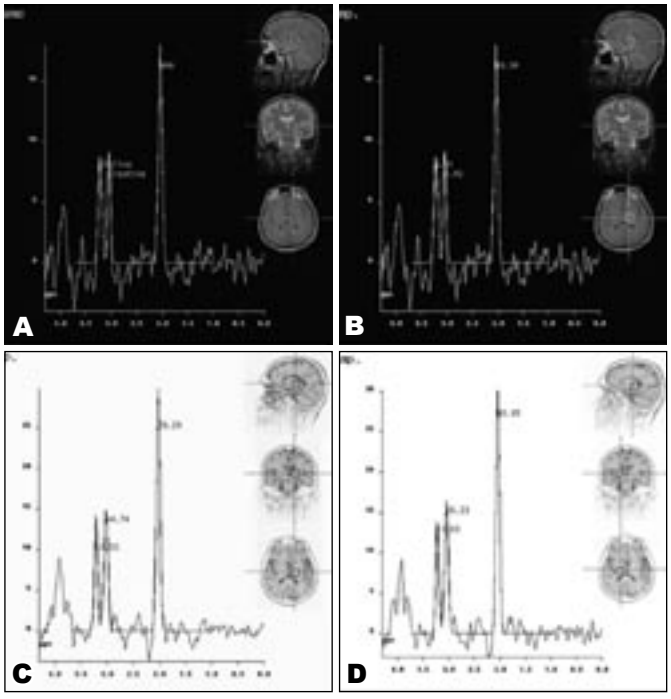

Fig. 3. Proton MR spectra images in a 29-year-old woman control subject **(A, B)** and a 26-year-old male patient with RRMS **(C, D)**. N-Acetylaspartate (NAA), choline (Cho), and creatine (Cr) peaks are observed **(A)**. The NAA/Cho, NAA/Cr and Cho/Cr ratios are 2.24, 2.08 and 0.95, respectively **(B)**. These values in the left thalamus of a RRMS patient are 2.16, 1.99 and 0.92, respectively **(C)**, while in the right thalamus they are 2.26, 1.9 and 0.84, respectively **(D)**.

may represent mild neuronal injury in the thalami in the early-moderate phases of MS. On the other hand, the increases in the Cho/Cr ratio may be the result of myelin damage and gliosis which have been reported to occur in MS.[18,19] As a difference from the above mentioned studies, the relative metabolite ratios were used in our study instead of the absolute concentration values whose measurement necessitates complicated calculations and/or sophisticated software making it unpractical.[11] Therefore, relative metabolite ratio measurements are preferred in clinical practice. Our study pointed out that use of thalamic single voxel MRS with PRESS sequence alone in the evaluation of DGM in MS patients was not enough so findings of routine cranial MRI should be considered to determine the GM injury in MS patients.

The first limitation of our study was the use of a single voxel technique to measure the metabolite ratios. If we could have evaluated the correlation of absolute and relative metabolite concentrations in the WM and GM in a more global way, additional data might have been found offering better understanding of MS etiopathogenesis. Second, the relatively long TE value used in the MRS acquisition (rather than TE 30-35) hindered us from the examination of some other metabolites like myoinositol and citrulline. Myoinositol is a marker of gliosis.[14] Citrulline has been more frequently found in WMs of MS patients than in healthy subjects.[18] Evaluation of citrulline and myoinositol levels in the deep GM of MS patients in new studies with a lower TE is needed. As a third limitation, the comparisons of pre- and post-treatment MRS examinations were missing in our study. Therefore, new studies showing the reversibility of the thalamic metabolite ratios after treatment may be planned. The last limitation was the presence of few control subjects. There were MRS examinations of cases with normal MRI and normal neurological evaluation belonging to the previously conducted studies. The control subjects were age-matched with our patients (20-60 years) and were chosen from our archives. Control patients out of this age range were excluded resulting in a limited number of control age matched cases. But statistical analysis showed that the number of control patients was enough to carry out the measurements. So we did not perform any more new MRS on new controls.

Table 1. MRS Findings of Patients and Controls

Groups		Cho/Cr	NAA/Cho	NAA/Cr
Patients	Case Number	43	43	43
	Median	0.99	1.75	1.79
	Inter-Quartile Range	0.24	0.63	0.51
Controls	Case Number	23	23	23
	Median	0.9	1.75	1.89
	Inter-Quartile Range	0.29	0.87	0.26
	P Value (α=0.05)	0.10	0.22	0.34

In conclusion, the spectroscopic relative metabolite ratios were of limited value in determining the presence of metabolic changes in the DGM of MS patients. One of the reasons for this may be the difficulty in differentiation of patients with earlier and reversible neuronal damage from long-lasting cases with irreversible neuronal loss. New studies about the presence of the DGM damage in MS

patients are necessary.

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