ORIGINAL ARTICLE

A Comparison of N-acetyl Aspartate Concentrations between Two Main Subtypes of Multiple Sclerosis using Magnetic Resonance Spectroscopy Imaging

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Abstract

Background and Objective: Multiple sclerosis (MS) is the most common non-traumatic neurological disorder among young adults. Studies are ongoing to find efficient biomarkers for early and differential diagnosis of different MS subtypes based on neuroimaging techniques, particularly magnetic resonance spectroscopy imaging (MRSI). Relapsing-remitting MS (RRMS) and primary-progressive MS (PPMS) are the two main subtypes of MS often difficult to be differentiated. The present study aims to comparatively assess the concentrations of N-acetyl aspartate (NAA) in RRMS and PPMS patients using MRSI. The concentrations in two regions of the brain including white matter (WM) lesions (plaques) and normal-appearing WM (NAWM) are assessed. **Materials and Methods:** The MRSI (1.5 T) of the brain of MS patients ($n = 28$) was performed to determine the relative concentrations of NAA in NAWM and plaque regions and compared between two main MS subtypes of RRMS and PPMS. The images were acquisitioned with point resolved spectroscopy sequence, single voxel mode (24 mm × 24 mm × 24 mm), and repeated time (1500 ms) and echo time (35 ms). The relative concentrations of NAA in NAWM and plaque were compared between two subtypes. **Results:** The analysis of variance showed no significant difference in NAA of NAWM between PPMS and RRMS ($P = 0.06$). Similarly, there was no significant difference in NAA levels in the plaque region between PPMS and RRMS groups ($P = 0.7$). It should be noted that the difference in the concentrations of NAA in NAWM between RRMS and PPMS was higher than the difference between the plaque regions. **Conclusion:** Our findings showed that the NAA values in NAWM assessed by MRSI may be an adjunctive diagnostic index for differential diagnosis of two subtypes of MS disorder.

Key words: Magnetic resonance spectroscopy, multiple sclerosis, primary progressive, relapse remitting

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS) characterized by demyelination, inflammation, and neurodegeneration. In MS, the patient's own immune system attacks and destroys the myelin sheath which consequently damages the white matter (WM). The damage to the myelin sheath

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appear to form of plaques that causes nerve conduction disturbances.[1] Traditional magnetic resonance imaging (MRI) techniques are not reliable tools for determining specific clinical criteria for accurate diagnosis of MS. The two main drawbacks of traditional MRI in this regard are inability to characterize the pathology of MS plaques and normal-appearing WM (NAWM).[2] However, the pathologic assessments have highlighted the importance of axonal pathology in MS.[3-5] Pathological changes in NAWM are very important and directly related to the disability of MS patients.[6,7] Using other modality of MRI called magnetic resonance spectroscopy (MRS), researchers and clinicians are able to non-invasively monitor axonal damage through determining the regional changes in brain metabolite levels.[8-10] The most powerful resonance signals within the brain are N-acetyl aspartate (NAA), creatine (Cr), and choline. In normal proton spectrum of the brain, the peak of NAA dominant, the other signal peaks, as decreasing the NAA levels, is a marker for increasing the anatomical anomalies and neurological disorders in MS.[5,11] NAA is a pivotal molecule with crucial role in studies investigating the underlying mechanism of MS disorder as well as studies developing reliable biomarkers of the disorder. This molecule is present at high concentrations in the brain. The levels found in various areas of the brain can reach 10 mM or greater, making it one of the most concentrated molecules in the CNS. Recent studies have demonstrated the metabolic and neurochemical functions of NAA in progression of different neurodegenerative disorders, particularly MS. The two features make the NAA an important molecule for early or differential diagnosis of MS. First, the NAA proton signal is prominent in MRS imaging (MRSI), making NAA one of the most reliable markers for brain MRS studies. Second is the connection between the concentrations of NAA and progression of MS disorder. In addition, some initial studies have indicated that NAA may be a good biomarker for differential diagnosis of different subtypes of MS disorder.

MRSI has become a valuable tool for quantitative assessment of the metabolic abnormalities in the human brain and different organs including prostate, breast, and other organs. This technique is currently used in routine clinical imaging, particularly for cancer assessment, as well as in preclinical and clinical research studies. The parallel imaging and high-speed MRSI approaches (image acquisition sequencing) are the main advantages of this technique in assessing MS patients.

During the last two decades, MRS was performed only to restricted areas of the brain, but today, the whole-brain MRS are performed with a high resolution.[12-14] The findings of MRSI are important to differentiate subtypes of MS.[15,16] There necessarily is no link between the activities and progress of the disease with a number of lesions.[17,18] Some studies have compared MS patients with normal participants as well as different subtypes of MS through comparison of some metabolites in the brain determined using MRSI.[19,20] In most of these studies, the results showed a reduction in the NAA and Cr levels of secondary-progressive MS (SPMS) patients as compared to relapsing-remitting MS (RRMS) in NAWM.[21,22] Other similar studies that compared two RRMS and primary-progressive MS (PPMS) groups have indicated that Cr concentration was significantly increased in lesions in PPMS compared to RRMS.[16]

Most of the previous studies investigated just one region of the brain, mainly, NAWM. In addition, majority of these studies suffer small sample size as well as they compared one subtype of MS with healthy counterparts. RRMS and PPMS are the two main subtypes of MS often difficult to be differentiated. The present study aims to comparatively assess the concentrations of NAA in RRMS and PPMS patients using MRSI. The concentrations in two regions of the brain including WM lesions defined as plaques and NAWM are assessed.

MATERIALS AND METHODS

Participants

This is a case–control diagnostic study conducted from June 10, 2016, to December 30, 2016, on MS patients (n = 28). The MS patients were divided into two groups of RRMS patients (n = 16) and PPMS patients (n = 12) according to the clinical manifestations and standard diagnostic criteria. The MS patients were selected among the patients who have been referred to Golestan Hospital, Ahvaz, Iran. The MS patients were diagnosed as MS cases with two subtypes of RRMS and PPMS according to the standard McDonald's benchmark,[23] and their Expanded Disability Status Scale (EDSS) scores ranged 1-7 (mean = 2.63; standard deviation [SD] = 1.63). The patients were in age range of 22-57 years (mean = 38.02; SD = 9.2). This age range was chosen because this age group has the highest prevalence of MS.[17,24] All of the processes of this study were approved by the guidelines of Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (which were in complete accordance with the Declaration of Helsinki of ethical principles for medical research involving human subjects). All of the participants completed informed consent form prior participation in the study. The patients were examined by an expert neurologist, and after the diagnosis of RRMS, they underwent MRS of the brain.

MRS assessments

The MRSI at magnetic field strength of 1.5 T (General Electric Healthcare, Germany) of the brain of MS patients (n = 28) was performed to determine the relative concentrations of NAA in NAWM and plaque regions and compared between two main MS subtypes of RRMS and PPMS. For both groups, the same standardized protocol of 1H-MR two-dimensional MRSI was used. The MRSI measurements were performed

parallel to axial T1- and T2-weighted slices. Routine automatic adjustments were applied before data acquisition. The point resolved spectroscopy (PRESS) sequence was used and special care was taken to avoid skull and subcutaneous tissue contamination. Chemical shift selective sequence was used for water suppression. Sequence parameters included repeated time (TR)/echo time (TE) 1500/35 ms. The acquisition time of spectroscopic sequences was 15 min. The images were acquisitioned with PRESS sequence, single voxel mode (24 mm × 24 mm × 24 mm), and TR (1500 ms) and TE (35 ms). The relative concentrations of NAA were compared inter-group and intra-group of between NAWM and plaque within and between two subtypes. For all cases, MRS was implemented by identical MRI scanner with the same protocol. A single radiologist who was blinded to the subtype of the patients identified periventricular MS lesions on T2-weighted MRI. To minimize partial cerebrospinal fluid volume effects, we considered voxels that were completely within a lesion, without any apparent involvement of the ventricles or sulci. Only non-enhancing lesions that occupied at least 60% of the voxel was assessed in both groups.[25] We assessed all non-enhancing lesions fulfilling the mentioned inclusion criteria. Two voxels for each patient were selected and examined and analyzed: One voxel was selected on plaque in brain WM tissue and the other voxel placed in the NAWM. Then, the signal intensity detected on the MRS spectrum which contains signals related to NAA.

To compare continuous variables between groups, after checking the normality assumption, using Kolmogorov–Smirnov test, the *t*-test and one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to calculate *P* values between every two groups. The significance level of 0.05 was set for all statistical analyses of the study.

RESULTS

Demographic and clinical data (EDSS and disease duration) are summarized in Table 1. The gender frequency distribution and disease type distribution are presented in Tables 2 and 3, respectively. The comparisons of NAA levels in NAWM and plaque between the RRMS and PPMS groups showed that the amount of difference in NAWM was higher than the difference in plaque region, but the differences in none of the regions were not statistically significant.

The ANOVA showed no significant difference in NAA of NAWM between PPMS and RRMS ($P = 0.06$).

Similarly, there was no significant difference in NAA levels in the plaque region between PPMS and RRMS groups ($P = 0.7$).

It should be noted that the difference in the concentrations of NAA in NAWM between RRMS and PPMS was higher than the difference between the plaque regions.

Table 1: Demographic and main clinical characteristics of the patients in PPMS and RRMS groups

Statistics patients	Minimum	Maximum	Mean±SD
Age	22	57	38.12±9.09
EDSS	1.00	7.00	2.54±1.61
NAA-plaque	14	164	70.30±29.90
NAA-NAWM	10	261	84.63±43.56

The concentrations of NAA in NAWM and plaque are presented. NAA: N-acetyl aspartate, NAWM: Normal-appearing white matter, EDSS: Expanded Disability Status Scale, RRMS: Relapsing-remitting multiple sclerosis, PPMS: Primary-progressive multiple sclerosis, SD: Standard deviation

Table 2: Gender distribution of MS patients

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Male	10	35.7	35.7	35.7
Female	18	64.3	64.3	64.3
Total	28	100.0	100.0	100.0

MS: Multiple sclerosis

Table 3: The distribution of MS type for the two subtypes

Type	Frequency	Percent	Valid percent	Cumulative percent
RRMS	16	57.15	57.15	57.15
PPMS	12	42.85	42.85	42.85
Total	28	100.0	100.0	100.0

MS: Multiple sclerosis, RRMS: Relapsing-remitting multiple sclerosis, PPMS: Primary-progressive multiple sclerosis

DISCUSSION

In this research, we comparatively assessed the levels of NAA between two main subtypes of MS disorder including PPMS patients ($n = 16$) and RRMS patients ($n = 12$) using MRSI at 1.5 T. Previous studies using MRSI have shown that that PPMS differ significantly from RRMS in some metabolites.[26-28] As the number of PPMS patients is low and only make up small percentage of MS patients, a few studies have been conducted on PPMS compared with other stages of the MS. Most of the previous studies have been conducted on SPMS and compared the common metabolites with healthy subjects as well as other subtypes of MS, mainly RRMS.[22,29,30] Because NAA is only found in neurons and axons, reduction of its level implies axonal damage.[31] The present study showed that NAA concentration in the NAWM is different between two subtypes of MS, but the difference was not statistically significant. This finding is consistent with most of the previous studies.[32] However, some other studies have demonstrated opposite results,[21,33] indicating that the

NAA level increased in the NAWM of PPMS as compared to RRMS. Some of the articles have reported reduction of the NAA levels in the NAWM of MS patients,[5,29,31] whereas some other ones have reported different findings.[34,35] These different results may be due to the small size and heterogeneity in the methodology of the study.[36-40] On the other hand, NAA concentration is decreased in WM plaques compared with NAWM. However, no significant difference was observed between the NAA levels in plaque in the two subtypes of disease. Further studies are needed to shed more light on the exact trend of NAA concentrations in different subtypes of MS.

CONCLUSION

Our results showed that even in MS patients without plaque, the changes in the metabolites are detectable using MRSI. However, the amount of the changes is not significant. Our findings showed that the NAA values in NAWM assessed by MRSI may be an adjunctive diagnostic index for differential diagnosis of two subtypes of MS disorder. Considering the differences in clinical manifestations and symptoms of RRMS and PPMS patients which are relatively similar, this amount of difference could be expected. Moreover, the sample size of our study was relatively low and using larger sample size, and we will be able to reach a more conclusive criterion in this regard. In this regard, comparison of the levels of NAA and also other important metabolites between the three main subtypes of MS including RRMS, PPMS, and SPMS and healthy control using a large sample size will be of significant clinical value.

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