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Monitoring interferon β treatment response with magnetic resonance spectroscopy in relapsing remitting multiple sclerosis

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Abstract

The aim of this study is to compare the white matter of multiple sclerosis (MS) patients with healthy controls and to monitor the response to the treatment with magnetic resonance spectroscopy (MRS).

Fifteen healthy controls and 36 recently diagnosed MS patients never treated with interferon β were included in this study. In the patient group, MRS was performed before treatment, at 6th and 12th month after the initiation of treatment and once in control group. Patient group was divided into 3 interferon groups randomly. Physical examination findings were recorded as Expanded Disability Status Scale scores before treatment, at 6th and 12th month of interferon treatment.

At the end of 1 year follow up, 26 of 36 patients completed the study. In patients' white matter lesions, N-acetylaspartate/creatine (NAA/Cr) ratios were lower than control group's white matters. NAA/Cr ratios were higher in control group's white matter than patient's normal appearing white matter but this difference was not statistically significant. There was no difference in choline/creatine (Cho/Cr) ratios between 2 groups. In follow-up period, NAA/Cr and Cho/Cr ratios obtained from patients' white matter lesions and normal appearing white matter did not change statistically.

This study showed that in MS patients' white matters, especially in white matter lesions, neuron viability is reduced compared with healthy controls' normal white matter; and in the patients treated with interferon β NAA/Cr ratios remained stable. These stable levels of metabolite ratios in the patients who received interferon β therapy can be explained with either the shortness of the follow-up period post-treatment or may reflect a positive effect of the beta interferon therapy on the progress of MS.

Abbreviations: Cho = choline, CNS = central nervous system, Cr = creatine, EDSS = Expanded Disability Status Scale, IFN = interferon, MRS = magnetic resonance spectroscopy, MS = multiple sclerosis, NAA = N-acetylaspartate.

Keywords: choline, creatine, EDSS, interferon β, magnetic resonance spectroscopy, multiple sclerosis, N-acetylaspartate

1. Introduction

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS). It affects between 250,000 and 350,000 people in the United States.[1] The incidence peaks at 30 years old and the prevalence peaks at 50 years. Patients present with neurologic findings disseminated in space and time, that is affecting differing locations and occurring over multiple episodes.[2] MS is characterized by acute episodes of demyelination, axonal degeneration, and progressive neurodegeneration of the CNS leading to long-term disability.[3] MS has been classically considered a white matter disease, but recent pathology and imaging studies have reinforced the notion that the gray matter is also affected by these pathological changes. Conventional magnetic resonance imaging (MRI) techniques are highly sensitive in demonstrating the spatial and temporal dissemination of demyelinating plaques in the brain and spinal cord.[4,5]

Conventional MRI techniques are used currently to diagnose and follow patients with MS. With technological advances, nonconventional MRI techniques are now able to detect changes not seen on conventional MRI and even predict disability.[2]

Proton MR spectroscopy (1H-MRS) has a unique capability to provide chemical-pathological characterization of MR-visible lesions and normal-appearing brain tissues.[6] Metabolic abnormalities in patients with MS are not restricted to lesion sites, normal appearing white and gray matter are also affected even in early stages of disease.[7] The peaks in MRS data quantify specific neurometabolites, which may reflect specific MS-related events, such as demyelination, inflammation, and axonal/neuronal dysfunction.[8] Since N-acetylaspartate (NAA) is detected almost exclusively in neurons and their processes in the normal mature brain, decreases in this metabolite have been interpreted as a measure of axonal injury reflecting neurodegeneration. Changes in the resonance intensity of choline (Cho) represent increased levels of membrane phospholipids which are released during active myelin breakdown.[9] The vast majority of 1H-MRS studies have reported decreases in NAA and NAA/Cr in lesions and in normal appearing tissues in patients with the different forms of MS.[10]

Because of MRS's ability to monitor the cerebral metabolite changes in relatively small volumes of interest, a few studies have now used MRS to evaluate immunomodulatory therapies in Relapsing Remitting Multiple Sclerosis (RRMS).[8] Previous

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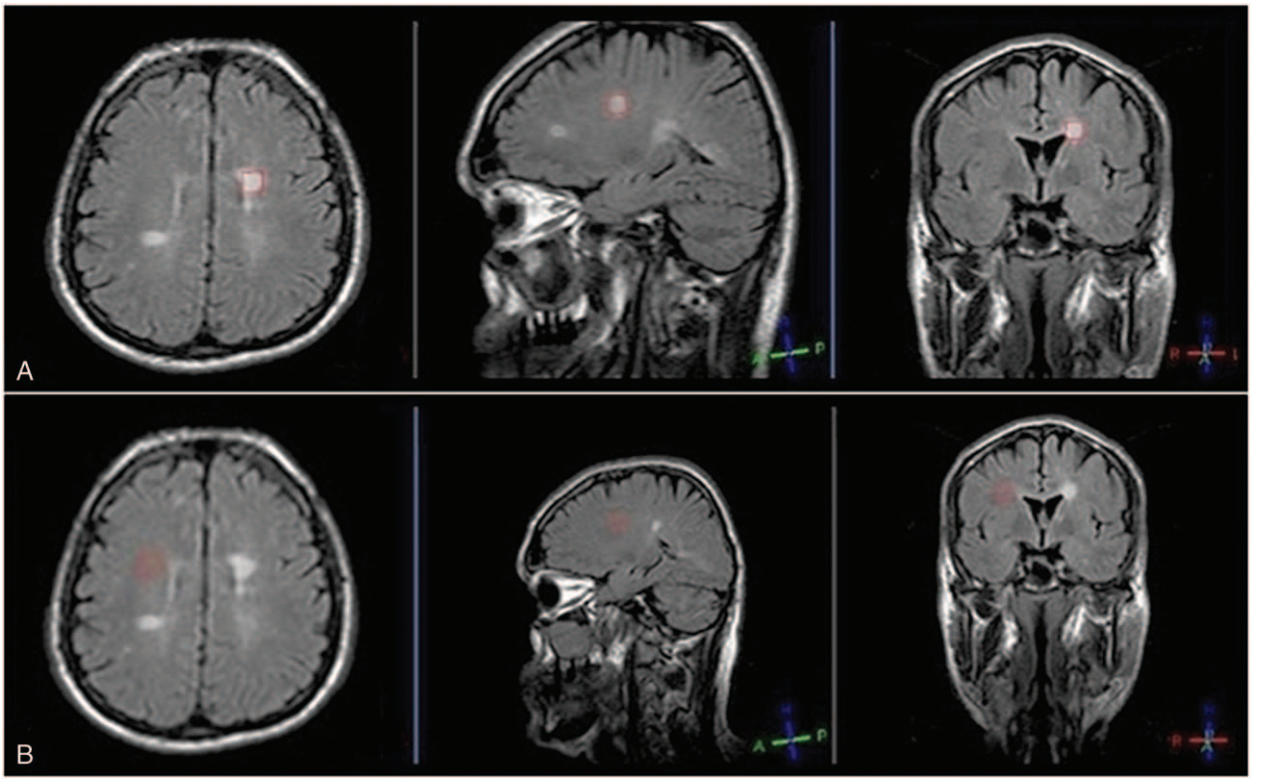

Figure 1. In patient group, MRS voxel was placed in periventricular largest lesion (A) and contralateral symmetric normal appearing white matter (B). MRS = magnetic resonance spectroscopy.

longitudinal 1H-MRS studies have shown that NAA levels increase or remain stable with interferon β (IFN-β) treatment. The aim of this study is to compare the white matter of recently diagnosed MS patients with healthy controls and to monitor the patient's treatment response with serial 1H-MRS.

2. Materials and methods

2.1. Standard protocol approvals and patient consents

The study was approved by the ethics committee of the Erciyes University, and written informed consent was obtained from all participants.

2.2. Participants

MS participants were recruited from the Erciyes University, Department of Neurology MS Center. Individuals recently diagnosed as RRMS and never treated with IFN-β before were enrolled. Individuals who met the following inclusion criteria were enrolled in this study: an age older than 18 years, absence of any systemic disease, having mild or moderate disability, mild or moderate T2 burden in brain MRI. Participants were excluded if they had experienced an MS relapse in the prior 30 days, severe disability, high burden of T2 lesion in MRI, and prior IFN-β treatment history. Participants in study group did not receive any routine immunomodulatory or immunosuppressive treatment except IFN-β. However, if participants experienced relapse at MRI acquisition time (e.g., 6th or 12th month of treatment), participants were treated with high-dose intravenous steroids and MRI acquisition was postponed for a month to prevent the inflammatory effect of relapses on normal appearing white matter (NAWM) and white matter lesions (WML).

A cohort of age-matched healthy volunteers was also recruited. Fifteen age- and sex-matched healthy volunteers were enrolled as control group. Inclusion criteria for control groups were absence of any systemic or neurologic disease; normal T2-FLAİR brain MRI which was routinely acquired before MR Spectroscopy sequences; absence of contraindication to MRI acquisition (e.g., claustrophobia, pacemakers, implants); no history of cognitive difficulties. Participants in control group did not receive any medication such as steroids.

Thirty-six patients recently diagnosed as RRMS were enrolled in this study. Patients were randomized to 3 IFN-β treatment groups (Avonex, Biogen, Cambridge, MA, USA; Betaferon, Schering AG, Berlin, Germany; Rebif, Serono, Geneva, Switzerland). Patient demographics and baseline Expanded Disability Status Scale (EDSS) scores were recorded. Individuals were evaluated by a neurologist before treatment (T0), at 6th (T6) and 12th (T12) month of treatment. EDSS scores and number of relapses were recorded.

2.3. MRI protocol and image analysis

MRI was performed with a 1.5 Tesla, Achieva; Philips Medical Systems, Best, The Netherlands device. To quantify the lesion load and number of active lesions, MRI examination was performed in a separate session preceding MRS. For MRS protocol, initially sagittal, axial, and coronal fluid attenuated inversion recovery (FLAIR) or T2 image were acquired to determine the MRS voxel localization. MRS measurements were performed using Release 11.1.1 software system. MRS values were acquired after voxel placement with automatic shimming. (TE 144 and TR 2000 ms, slice thickness = 3 mm, interslice distance = 0.5 mm, voxel size ranged from 2 to 4 mL).

Table 1

Demographic profile of participants.

	Patient	Control	*P*
Age	34.08 ± 7.4	32.40 ± 8.2	0.15
Gender, male	8/26	6/15	0.22
Education, y	11.8 ± 3.9	11.1 ± 4.2	0.32
Smoking status	6/26	4/15	0.11
WBC, 10^9 cells/L	7.2 ± 2.4	6.2 ± 1.8	0.18
Hb, g/dL	12.3 ± 2.1	12.7 ± 1.9	0.29
MCV, fL	89.1 ± 3.9	88.2 ± 4.4	0.08
MPV, fL	8.96 ± 1.2	9.31 ± 1.9	0.16
PLT, 10^9 cells/L	248.7 ± 51.2	269 ± 39.9	0.62
AST	21.4 ± 4.2	19.4 ± 3.3	0.12
ALT	19.9 ± 3.9	20.7 ± 2.8	0.46

ALT = alanine aminotransferase, AST = aspartate aminotransferase, Hb = hemoglobin, MCV = mean corpuscular volume, MPV = mean platelet volume, PLT = platelet count, WBC = white blood cell.

In patient group, MRS voxel was placed in periventricular largest lesion and contralateral symmetric NAWM (see Fig. 1). MRS voxels were placed in same localization in the T0, T6, and T12 MRS imaging. During follow-up period, the patient was excluded if an MS lesion appears in the NAWM area of interest.

In the control subjects, the same areas identified in patients were assessed with MRS once, for the white matter measurement of metabolite concentration. The values were compared with patient's WML and NAWM.

2.4. Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 21.0 package. Shapiro–Wilk test was used to check the normality of distribution and the homogeneity of variances. In normal distribution, variance analysis was performed in repeated variables. Friedman test was performed if variable distribution was not normal. Intergroup differences (treatment groups) were evaluated with variance analysis. Patient and control groups were compared with chi-square in respect of sex. And *t* test was performed in respect of age and other numeric variables. The relationship between numeric variables was analyzed with the Spearman correlation test. Statistical significance was assumed at a $P < 0.05$.

3. Results

From January 2010 to September 2013, a total of 36 RRMS patients were recruited for the present study. At the end of 1 year follow up, 10 patients (2 pregnancies, 2 switches to second-line treatment, 3 left the interferon treatment, 3 quit follow up) were excluded and 26 patients completed the study. The dataset consisted of data from 26 RRMS patients (18 female, 8 male, mean age: 34.08 ± 7.4, mean time interval from first relapse to diagnosis was 2.21 ± 1.4 years, mean EDSS was 1.0) responding both at baseline and follow-up 6 months and 1 year later (see Table 1). There was no statistically significant difference in age and sex between patient and control groups.

3.1. MRS results

Participant's baseline and follow-up MRS metabolite ratios are summarized at Table 2. At baseline, NAA/Cr ratios acquired from patient's WMLs were significantly lower than control group's white matter ($P < 0.05$). NAA/Cr ratios acquired from patient's NAWM were lower than control group's white matter but this difference was not statistically significant ($P > 0.05$). Other MRS findings were not statistically different between patient and control groups.

3.2. Follow up

In the patient group, no significant variations were found in the values of the metabolite ratios in both WMLs and NAWM during the 12 months of the treatment period. Table 3 display the values of metabolite peaks in WML and NAWM of patients with MS evaluated at each stage of the study.

There was no significant difference between 3 interferon groups (Avonex, Betaferon, Rebif) in the values of the metabolite ratios in both WMLs and NAWM during the 12 months of the treatment.

3.3. EDSS results

Patient group's mean initial EDSS was 1.0. There was no significant difference between interferon groups in terms of EDSS ($P > 0.05$). In RRMS patients, EDSS did not vary significantly over the entire period of the study and treatment groups were not

Table 2

Metabolite ratios of control subjects and patients with multiple sclerosis.

Time	Metabolite ratios	Patient	Control	*P*	*P*†
Baseline	NAA/Cr (NAWM)	1.75 ± 0.37	1.92 ± 0.42	0.192	0.078
6th mo	NAA/Cr (NAWM)	1.74 ± 0.34	—	—	
12th mo	NAA/Cr (NAWM)	1.73 ± 0.38	—	—	
Baseline	NAA/Cr (WML)	1.65 ± 0.32	1.92 ± 0.42	0.026*	0.035*
6th mo	NAA/Cr (WML)	1.61 ± 0.35	—	—	
12th mo	NAA/Cr (WML)	1.69 ± 0.34	—	—	
Baseline	Cho/Cr (NAWM)	0.91 ± 0.33	0.92 ± 0.21	0.870	0.716
6th mo	Cho/Cr (NAWM)	0.94 ± 0.32	—	—	
12th mo	Cho/Cr (NAWM)	0.95 ± 0.27	—	—	
Baseline	Cho/Cr (WML)	1.08 ± 0.37	0.92 ± 0.21	0.100	0.253
6th mo	Cho/Cr (WML)	0.86 ± 0.31	—	—	
12th mo	Cho/Cr (WML)	0.95 ± 0.39	—	—	

Cho = choline, Cr = creatine, NAA = N-acetylaspartate, NAWM = normal appearing white matter, WML = white matter lesion.

* $P < 0.05$

† Significance value adjusted by age and gender.

Table 3

Patient group's mean values of metabolite peaks in WMLs and normal appearing white matter.

Metabolite	Baseline	6th mo	12th mo	*P*
NAWM NAA/Cr	1.75 ± 0.37	1.74 ± 0.34	1.73 ± 0.38	0.98
NAWM Cho/Cr	0.91 ± 0.33	0.86 ± 0.31	0.95 ± 0.39	0.80
WML NAA/Cr	1.65 ± 0.32	1.61 ± 0.35	1.69 ± 0.34	0.68
WML Cho/Cr	1.08 ± 0.37	0.86 ± 0.31	0.95 ± 0.39	0.13

Cho = choline, Cr = creatine, NAA = N-acetylaspartate, NAWM = normal appearing white matter, WML = white matter lesion.

significantly different in terms of EDSS ($P > 0.05$). On the other hand, there were no significant correlation between EDSS scores and MRS metabolite ratios (see Table 4).

4. Discussion

Proton MR Spectroscopy is the first nonconventional MR technique used in MS and has proved to be particularly informative by revealing metabolic abnormalities related to the 2 primary pathologic processes of the disease. These are active inflammatory demyelination and neuronal/axonal injury in both T2-visible lesions and in brain regions that are not associated with evident structural abnormalities on conventional MR imaging, the so-called normal-appearing brain tissue.[4,11] MRS offers a wealth of data on the biochemistry of a selected brain tissue volume, which represent potential surrogate markers for the pathology underlying MS. In particular, the NAA peak in an MR spectrum is a putative marker of neuronal and axonal integrity, and the Cho peak appears to reflect cell-membrane metabolism.[7,8]

MRS studies comparing lesions and NAWM in RRMS and SPMS measured the following metabolites: NAA, which has been suggested to be a marker for neuron viability and axonal density in the brain, creatine (Cr), which represents a combination of Cr and phosphocreatine, a marker of gliosis and Cho thought to be a marker associated with membrane phospholipids, in which greater concentrations of Cho are highly indicative of active inflammatory disease. In particular, NAA or the NAA/Cr ratio is a good surrogate marker for the monitoring of neuroprotection in therapeutic studies. Most of the MRS studies of RRMS show reduced NAA/Cr in WMLs and NAWM.[12,13]

In this study, MRS was performed before treatment, at 6th and 12th month after initiation of treatment in recently diagnosed RRMS patients and once in control group. At baseline, RRMS patient's NAWM MRS metabolites revealed low NAA/Cr and Cho/Cr ratios compared with healthy controls. But this difference was not statistically significant; on the other hand, patient's WML NAA/Cr ratios were significantly lower than healthy controls and Cho/Cr ratios were not significantly different.

Recent 1H-MRS studies have shown that metabolic abnormalities in MS patients are not restricted to WML's, but NAWM and normal appearing gray matter were affected. In the early stages of disease, NAA decreases were reported in the NAWM indicating axonal damage and become more evident in advanced disease stages.[6] Correspondingly, our study revealed low NAWM NAA/Cr ratios even if in recently diagnosed RRMS patients. Anik et al[14] reported low NAA/Cr ratios in NAWM of MS patients compared with healthy volunteer's white matters like most of the MRS studies. However, in another study, NAA/Cr ratios acquired from RRMS patient's NAWM were similar to healthy control's white matter but SPMS patient's NAWM NAA/Cr ratios were significantly lower.[15]

On the other hand, one should keep in mind that Cr is increased in NAWM. Therefore, attention should be paid to the NAA/Cr ratio, as it might be falsely low due to an increased Cr.[16] Vrenken et al[17] revealed elevated quantitative Cr levels in MS patient's NAWM compared with healthy controls; however, NAA levels were not significantly different. Likewise Kirov et al[18] reported NAWM Cr, Cho, and mI levels were increased in 21 RRMS patients but NAA levels were similar as controls.

Table 4

Correlation between baseline, 6th month, and 12th month MRS metabolites and EDSS scores.

Metabolite	Baseline EDSS	6th mo EDSS	12th mo EDSS
NAA/Cr (NAWM)			
Baseline	Rho = 0.253, *P* = 0.212	—	—
6th mo	—	Rho = 0.249, *P* = 0.220	—
12th mo	—	—	Rho = 0.083, *P* = 0.689
NAA/Cr (WML)			
Baseline	Rho = 0.103, *P* = 0.617	—	—
6th mo	—	Rho = 0.340, *P* = 0.870	—
12th mo	—	—	Rho = 0.150, *P* = 0.463
Cho/Cr (NAWM)			
Baseline	Rho = −0.369, *P* = 0.064	—	—
6th mo	—	Rho = 0.025, *P* = 0.903	—
12th mo	—	—	Rho = 0.136, *P* = 0.506
Cho/Cr (WML)			
Baseline	Rho = −0.135, *P* = 0.510	—	—
6th mo	—	Rho = 0.047, *P* = 0.819	—
12th mo	—	—	Rho = −0.012, *P* = 0.953

Cho = choline, Cr = creatine, EDSS = Expanded Disability Status Scale, MRS = magnetic resonance spectroscopy, NAA = N-acetylaspartate, NAWM = normal appearing white matter, Rho = Spearman correlation coefficient, WML = white matter lesion.

In this study, there was an insignificant difference in terms of NAA/Cr ratios in between RRMS patient's NAWM and healthy control's WM. This might be explained by insufficient number of the patient group or the subtle axonal damage in the recently diagnosed RRMS patients. Probably this gap will be more prominent due to progressive axonal loss in the following years. Pathologic NAWM changes were reported even in clinically isolated syndrome (CIS) patients. Wattjes et al[19] reported decreased quantitative NAWM NAA levels in RRMS and CIS patients compared with healthy controls.

Numerous studies likewise our study normalizes signal intensities to an intravoxel standard such as Cr, in order to obtain an estimate of relative concentration. The main limitation of this approach is that Cr may not remain unaffected by MS pathology. Changes in Cr could contribute to any changes in NAA/Cr. Since Cr is present in both neurons and glial cells, Cr changes could be associated with neuroaxonal alteration, oligodendroglial disturbance, and astrocytic proliferation. It has therefore been suggested that it would be more accurate to interpret decreases of brain NAA/Cr as markers of a less specific disturbance in the cerebral tissue integrity.[12,20] The majority of MRS studies evaluating WMLs in MS revealed decreased quantitative NAA levels and NAA/Cr ratios in concordance with our study.[10] Decreased NAA/Cr ratios can be explained by axonal loss and demyelination and elevated Cr due to gliosis.

In general, acute inflammatory demyelinating lesions, which usually enhance with contrast on T1-weighted images, show increases in Cho and lactate (Lac) resonances during the first 6 to 10 weeks following lesion development. Changes in the resonance intensity of Cho can be interpreted as a measure of membrane phospholipids released during active myelin breakdown, whereas Lac increases mainly seem to reflect the metabolism of inflammatory cells or neuronal mitochondrial dysfunction. There is a progressive return of Lac to normal levels within weeks, whereas Cho and lipids decrease for some months, but do not always return to normal values.[4,21] MRS studies revealed controversial Cho results. Some researchers reported elevated WML Cho/Cr ratios indicating increased membrane cell turnover and activation of glial and inflammatory cells.[22] On the other hand, other researchers expressed similar Cho levels compared with healthy controls.[13,19] This study showed elevated Cho/Cr ratios acquired from WML compared with healthy controls but this difference was not significant. MRS imaging was acquired from MS patients at remission periods in this study. Therefore, reduced membrane turnover might affect Cho levels. Alternatively elevated Cr levels due to gliosis might affect Cho/Cr levels.

Conventional MR (cMR) imaging techniques have become established as the most important paraclinical tool for monitoring the efficacy of disease-modifying treatments. Also, cMR imaging is limited in the detection of subtle, disease-related changes in the NAWM. There is now growing interest in developing new imaging strategies to more specifically monitor the neurodegenerative, irreversible component of the disease. However, the high technical demands of MRS have generally limited its use in research studies, and currently available data do not suffice to support its use as a routine imaging modality of the neurodegenerative process of MS in clinical practice.[4,21]

Longitudinal MRS studies monitoring β-IFN treatment response revealed controversial results. Narayanan et al[23] studied 10 patients with RRMS before and 12 months after starting treatment with subcutaneous β-IFN and found that there was a significant increase in the NAA/Cr ratio in the treated group with a nonsignificant decrease in the NAA/Cr ratio in a group of matched untreated RRMS patients, therefore suggesting that treatment with β-IFN may partially reverse sub-lethal axonal injury. However, Schubert et al[24] expressed stable levels of quantitative NAA, Cr and Cho levels acquired from MS patient's WML and NAWM during β-IFN treatment.

There was no significant change in EDSS during follow-up period likewise NAA/Cr ratios but no significant correlation was detected between EDSS and MRS metabolites. Longitudinal MRS studies demonstrated controversial disability results. Tiberio et al[25] reported increased quantitative NAA levels in 20 MS patient's NAWM but there was no correlation between MRS metabolites and EDSS scores in terms of EDSS and Multiple Sclerosis Functional Composite. Conversely another 24 months follow-up study revealed correlation between NAA/Cr ratios and MSCF scores.[26]

The principal finding of longitudinal part of this study is that NAA/Cr and Cho/Cr ratios acquired from patient's WML and NAWM remain stable during 1 year follow-up period. These stable levels of metabolites may indicate that follow-up period is not long enough to determine the treatment effect or β-IFN treatment might slow the progression of disease. In particular, steady NAA/Cr levels may indicate that treatment might maintain neuron viability and might reduce gliosis, and steady Cho/Cr levels may indicate the anti-inflammatory effect of treatment.

There are several limitations of this study, including the relatively low number of subjects in each group. The inability to detect a significant difference between MS patient's NAWM and control's WM might be related to the small sample size. Additionally, signal intensities were normalized to an intravoxel standard such as Cr, consequently metabolite ratios were assessed but we could not be able to evaluate the single metabolite levels and the concentration of Cr may vary in different pathological conditions and at different age.[15] On the other hand, MRS imaging obtains crucial data from a limited brain region in terms of voxel capacity but these data are not always applicable for the whole brain. For ethical reasons, we were unable to study a group of untreated patients with RRMS matched for relapse frequency, MRS parameters and disability. Thus the lack of untreated MS control group does not allow us to conclude that steady levels of NAA/Cr levels briefly indicate the neuroprotective effect of β-IFN treatment. Finally in this study follow-up period may not be long enough to detect neurodegenerative changes in patients NAWM and WML in time.

In summary, in this study, recently diagnosed RRMS patients were observed with MRS imaging during 1 year to monitor β-IFN treatment response. During this follow-up period, NAA/Cr and Cho/Cr ratios acquired from RRMS patient's NAWM and WMLs remain stable. These results may indicate that β-IFN treatment slows the neurodegenerative course of the disease. Additional long-term follow-up studies are necessary to evaluate whether MRSI is a useful paraclinical tool to monitor immunomodulatory treatment response in MS.

References

[1] Keegan BM, Noseworthy JH. Multiple sclerosis. Annu Rev Med 2002;53:285–302.
[2] Files DK, Jausurawong T, Katrajian R, et al. Multiple sclerosis. Prim Care 2015;42:159–75.
[3] Rubin SM. Management of multiple sclerosis: an overview. Dis Mon 2013;59:253–60.

[4] Rovira A, Alonso J. 1H magnetic resonance spectroscopy in multiple sclerosis and related disorders. Neuroimaging Clin N Am 2013;23: 459–74.
[5] Londono AC, Mora CA. Nonconventional MRI biomarkers for in vivo monitoring of pathogenesis in multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2014;1:e45.
[6] De Stefano N, Filippi M. MR spectroscopy in multiple sclerosis. J Neuroimaging 2007;17(suppl 1):31S–5S.
[7] Llufriu S, Kornak J, Ratiney H, et al. Magnetic resonance spectroscopy markers of disease progression in multiple sclerosis. JAMA Neurol 2014;71:840–7.
[8] Narayana PA. Magnetic resonance spectroscopy in the monitoring of multiple sclerosis. J Neuroimaging 2005;15(suppl):46S–57S.
[9] Rovira À, León A. MR in the diagnosis and monitoring of multiple sclerosis: an overview. Eur J Radiol 2008;67:409–14.
[10] De Stefano N, Bartolozzi ML, Guidi L, et al. Magnetic resonance spectroscopy as a measure of brain damage in multiple sclerosis. J Neurol Sci 2005;233:203–8.
[11] Currie S, Hadjivassiliou M, Craven IJ, et al. Magnetic resonance spectroscopy of the brain. Postgrad Med J 2013;89:94–106.
[12] De Stefano N, Filippi M, Miller D, et al. Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. Neurology 2007; 69:1942–52.
[13] El-Rahman HMA, Hasan DI, Selim HA, et al. Clinical use of H1 MR spectroscopy in assessment of relapsing remitting and secondary progressive multiple sclerosis. Egyptian J Radiol Nucl Med 2012;43: 257–64.
[14] Anik Y, Demirci A, Efendi H, et al. Evaluation of normal appearing white matter in multiple sclerosis: comparison of diffusion magnetic resonance, magnetization transfer imaging and multivoxel magnetic resonance spectroscopy findings with Expanded Disability Status Scale. Clin Neuroradiol 2011;21:207–15.
[15] Aboul-Enein F, Krssak M, Hoftberger R, et al. Reduced NAA-levels in the NAWM of patients with MS is a feature of progression. A study with quantitative magnetic resonance spectroscopy at 3 Tesla. PLoS ONE 2010;5:e11625.
[16] Mader I, Rauer S, Gall P, et al. (1)H MR spectroscopy of inflammation, infection and ischemia of the brain. Eur J Radiol 2008;67:250–7.
[17] Vrenken H, Barkhof F, Uitdehaag BM, et al. MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. Magn Reson Med 2005;53:256–66.
[18] Kirov II, Patil V, Babb JS, et al. MR spectroscopy indicates diffuse multiple sclerosis activity during remission. J Neurol Neurosurg Psychiatry 2009;80:1330–6.
[19] Wattjes MP, Harzheim M, Lutterbey GG, et al. Axonal damage but no increased glial cell activity in the normal-appearing white matter of patients with clinically isolated syndromes suggestive of multiple sclerosis using high-field magnetic resonance spectroscopy. AJNR Am J Neuroradiol 2007;28:1517–22.
[20] Miller DH. Magnetic resonance spectroscopy: a possible in vivo marker of disease progression for multiple sclerosis? JAMA Neurol 2014;71:828–30.
[21] Sajja BR, Wolinsky JS, Narayana PA. Proton magnetic resonance spectroscopy in multiple sclerosis. Neuroimaging Clin N Am 2009;19: 45–58.
[22] Simone IL, Tortorella C, Federico F, et al. Axonal damage in multiple sclerosis plaques: a combined magnetic resonance imaging and 1H-magnetic resonance spectroscopy study. J Neurol Sci 2001;182: 143–50.
[23] Narayanan S, De Stefano N, Francis GS, et al. Axonal metabolic recovery in multiple sclerosis patients treated with interferon beta-1b. J Neurol 2001;248:979–86.
[24] Schubert F, Seifert F, Elster C, et al. Serial 1H-MRS in relapsing-remitting multiple sclerosis: effects of interferon-beta therapy on absolute metabolite concentrations. MAGMA 2002;14:213–22.
[25] Tiberio M, Chard DT, Altmann DR, et al. Metabolite changes in early relapsing-remitting multiple sclerosis. A two year follow-up study. J Neurol 2006;253:224–30.
[26] Bellmann-Strobl J, Stiepani H, Wuerfel J, et al. MR spectroscopy (MRS) and magnetisation transfer imaging (MTI), lesion load and clinical scores in early relapsing remitting multiple sclerosis: a combined cross-sectional and longitudinal study. Eur Radiol 2009;19:2066–74.