MAGNETIC RESONANCE SPECTROSCOPY OF NORMAL APPEARING WHITE MATTER IN EARLY RELAPSING-REMITTING MULTIPLE SCLEROSIS

Enas El Sayed Mohamed Ahmed, Mahmoud Ashraf Zaiton* , HananAbd ElAzim Hassan*, Wael Mahmoud ElSayed* , Khalid Mohamed Shawki***

*Neurology * and Radiolgy** departments faculty of Medicine ; zagazig university.*

ABSTRACT

Back ground: Multiple sclerosis (MS) is an inflammatory disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged .MS is a chronic disease of the central nervous system characterised by multicentral inflammation and myelin destruction .

Magnetic resonance imaging (MRI) is now widely used for diagnosing MS and detecting clinically silent lesions. However, correlations found between conventional techniques, such as T2-weighted lesion load, and disability, are weak or absent and therefore, neither the magnetic resonance techniques nor clinical measurements are gold standard to assess disease or disability.Alteration of NAWM is of great importance because its true patho-physiological significance is not completely understood. Decrease in N-Acetyl Aspartate (NAA) has been used as an marker of axonal damage or loss that presumably appears secondary to inflammation or demyelination ,although primary axonal damage is not excluded .

Objectives : Demonstrate the limitation of convenential magnetic resonance techniques.

Observation the pathological processes in NAWM by MRS.

Correlation between the results of MRS and the physical disability of the patients using EDSS .

Subjects and methods: This study was carried out on 23 patients with clinically definite multiple sclerosis patients, who met the criteria of clinically definite MS according to McDonald criteria 2010, selected from Neurology Department, Zagazig University Hospitals [10].

Results: We found that the mean value of NAA and the mean ratios of NAA/CR and NAA/ CHO were significant lower in the Cases but the mean values of MI and GLX are significant higher. . Also there was significant negative correlation between the mean value of NAA in MS plaque by MRS and EDSS score as a measure of disability.

There was significant negative correlation between the mean value of NAA in NAWM and EDSS score .

Discussion: We have clearly demonstrated a correlation between an MRS measure and EDSS. EDSS seems to reflect the existence of irreversible disability probably related to axonal degeneration.

Key wards

Multiple sclerosis; Magnetic resonance imaging;Normal appearing white mater;Proton magnetic resonance spectroscopy;Expanded Disability Status Scale

Corresponding author: Enas El Sayed Mohamed Ahmed Mobile:01001200386

Email:dr_enasneuro@yahoo.com

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated inflammatory disease that attacks myelinated axons in the central nervous system (CNS), destroying the myelin and the axon in variable degrees. In most cases, the disease follows a relapsing-remitting pattern, with short-term episodes of neurologic deficits that resolve completely or almost completely. A minority of patients experience steadily progressive neurologic deterioration [1].

MS distribution

It is recognised that MS is unevenly but non-randomly distributed throughout the world and that environmental factors play a significant role in the onset of MS. Many epidemiology studies have been performed to investigate these phenomena. Several of these studies support the existence of a gradient of MS

prevalence, which increases with distance from the equator in both northern and southern hemispheres [2].

Etiology

The cause of MS is unknown, but it is likely that multiple factors act in concert to trigger the disease. It has been hypothesized that MS results when an environmental agent or event acts in concert with a genetic predisposition to immune dysfunction[3].

Axonal Damage in Multiple Sclerosis

Historically, axonal damage has been recognized as a histopathological hallmark of MS since the very early descriptions of the disease by Charcot in the late 1800s [4]. More recent clinical, histopathological, and neuroimaging evidence has shed new light on these early findings and supported the concept that the debilitating disease course and long-term disability in MS patients was consequent to axonal loss possibly consequent to demyelination [5].The features of axonal damage in MS, however, were similar to those detected in other neurological diseases lacking demyelination, such as amyotrophic lateral sclerosis (ALS), and suggested the possibility that axonal damage in MS might be concurrent to demyelination, but not necessarily consequent to myelin destruction [6,7].

Clinical Rating Scales

A patient may be rated according to several clinical disability scales, on the basis of findings on the history and physical examination. The most widely accepted of these is the 10-point Kurtzke Expanded Disability Status Scale (EDSS), which was developed originally in 1955 as the Disability Status Scale and has been revised over the years [8].

The EDSS assigns a severity score to the patient's clinical status that ranges from 0-10 in increments of 0.5. The scores from grades 0-4 are determined using functional systems (FS) scales that evaluate dysfunction in the following 8 neurologic systems:

- Pyramidal,Cerebellar ,Brainstem,Sensory,Bladder and bowel, Vision, Cerebral, Other

Advantages of the EDSS are that it is widely used clinically, is easy to administer, and requires no special equipment.

Proton magnetic resonance (MR) imaging plays an essential role in the management of patients with a wide range of neurological conditions. While conventional MR imaging provides important structural information, data on underlying brain function is often limited. The generation of a spectrum of brain metabolites by MR spectroscopy provides the clinician with information on the regional chemical environment [9].

Proton MR Spectroscopy

Over the years, scientists have obtained MR spectra using a variety of nuclei[10] .However, the high sensitivity of the hydrogen (^{1}H) nucleus, its abundance within certain neurometabolites and the fact that the technique can be performed using standard clinical MR imaging machines makes hydrogen the principal nucleus applicable to spectroscopic investigation associated with clinical imaging of the brain [11].

The proton MR spectrum comprises a set of resonances (peaks) distributed along the x-axis, labelled in parts per million (ppm) . The amplitude of the resonances is measured on the y-axis typically using an arbitrary scale. Three prominent peaks are consistently seen: N-acetyl aspartate (NAA) at 2.02; Creatine (Cr) at 3.02; and Choline (Cho) at 3.2 .Although the positions of the resonances along the x-axis are constant, the relative heights of the resonances can differ depending on various MR imaging parameters [12].

SUBJECTS AND METHODS

This study was carried out on 23 patients with clinically definite multiple sclerosis patients, who met the criteria of clinically definite MS according to McDonald criteria 2010, selected from Neurology Department, Zagazig University Hospitals [13].

Ten healthy volunteers with similar age and gender distribution as control group were included in this study. The study was approved by the Ethical Committee of our Faculty and

informed written consent was obtained from patients and controls.

Patients were classified according to clinical course as having recurrent relapse with partial remission and no progression between attacks (relapsing remitting course)

The patients group:

This group included 23 Patients with clinically definite relapsing remitting MS according to the Mc Donald criteria[14] **.** Their ages ranged from 17 year to 45 year, with disease duration ranged from 1 to 12 years

The control group:

Ten apparently healthy unmedicated subjects, of matched age and gender served as a control group.

All patients were subjected to the following:

(1) History and examination:

- Detailed history taking about age of onset, duration of disease, number of relapse and current treatment(disease modifying therapy).
- The duration of the disease (measured in years). Patient's age at first symptoms ..
- Assessment of disease severity by the Expanded Disability Status Scale (EDSS)[15]**.**

(2)Routine laboratory investigations:

(3) Radiological evaluation was performed in Zagazig University Radiology MRI Unit: MR images were acquired using a 1.5T Philips using a standard quadrature head coil.

Prior to H-MRS examination each patient's disability was measured using EDSS . The H-MRS was performed the same day as the neurological examination by the same radiologist at the same center using the same MRI scanner and following the same protocol.

The radiologist was blinded to the neurological examination results and all the neurologists were blinded to the MRS results.

At the beginning of the study MRI and H-MRS were also performed on 10 healthy, age matched control subjects with not known systemic or neurological disease by the same radiologist using the same MRI scanner and following the same protocol as well.

These conventional MR images were used to position a spectroscopic volume of interest (VOI) in one or two areas with demyelinating lesions and one area of normal appearing white matter in MS patients and in one area of normal white matter in the healthy control group

The number of peaks fitted included the chemical shift ranges:

- *N*-acetylasparate (NAA) at 2.0 ppm.
- Creatine/phosphocreatine (Cr) at 3.0 ppm.
- Choline compounds (Cho) at 3.2 ppm.
- Myo-inositol (mI) at 3.5 ppm.
- Glutamine–glutamate–GABA complex (Glx) between 2.1 and 2.5 ppm

RESULTS

Table (1) : Comparison between the different metabolic values of MS plaques and that of NAWM of the patient group by MRS .

	Patient group		p
	MS plaques Mean ±SD	**NAWM Mean ±SD**	
NAA	**1.9860 ±0.172**	**1.968±0.2048**	**0.891**
CR	**3.0266± 0.0118**	**3.0278± 0.01389**	**0.2 70**
CHO	**3.2155±0.0135**	**3.2160± 0.012**	**0.376**
MI	**3.9488 ± 0.247**	**4.1053± 0.01475**	**0.005***
GLX	**3.6874 ± 0.0654**	**3.7670± 0.0 4318**	**0.256**
NAA/CR	**1.4815 ± 1.0438**	**1.1539± 1.08437**	**0.112**
NAA/CHO	**1.1582 ± 0.7565**	**0.9070± 0.77619**	**0.162**

This table shows that the mean value of MI was significant higher in the NAWM than in the MS plaque in the same patient .

Table (2): Comparison between the different metabolic values of NAWM of the patient group and that of the control group by MRS .

	Patient group NAWM (N=23)	Control group (N=10)	p
NAA	1.968±0.2048	2.0275±0.00288	0.003*
CR	3.0278± 0.01389	3.0237± 0.00356	0.366
CHO	3.2152±0.1437	3.2160± 0.012	0.881
MI	4.1053± 0.01475	3.5887± 0.09979	0.00**
GLX	3.7670± 0.0 4318	3.6343± 0.08474	0.005*
NAA/CR	1.1539± 1.08437	2.235± 0.30725	0.0008*
NAA/CHO	0.9070± 0.77619	1.7360± 0.17335	0.001*

This table shows that the mean value of NAA and the mean ratios of NAA/CR and NAA/ CHO were significant lower in the Cases than the control ($p<0.005$) but the mean values of MI and GLX are significant higher than the control group.

Table (3): Correlation between EDSS and clinical data of the patients.

Clinical data of the patients	EDSS	
	r	p
Age	0.200	0.361
Age of onset	0.074	0.738
Disease duration	0.416*	0.048
No of relapse	0.667**	0.001

This table shows that there was significant positive correlation between the duration of the disease and EDSS score as a measure of disability and number of ralapses and EDSS score .

Table (4): Correlation between EDSS and metabolic values of MS Plaques in MRS.

Metabolic values	EDSS	
	r	p
NAA	-0.613**	0.002
CR	-0.307	0.154
CHO	-0.277	0.201
MI	-0.051	0.818
GLX	-0.121	0.581
NAA/CR	0.50	0.820
NAA/CHO	-0.366	0.086

This table shows there was significant negative correlation between the mean value of NAA in MS plaque by MRS and EDSS score as a measure of disability.

Table (5):Correlation between EDSS score and the metabolic values of NAWM in MRS.

	EDSS	
	r	p
NAA	- 0.764**	0.454
CR	-0.347	0.104
CHO	-0.283	0.190
MI	-0.295	0.172
GLX	-0.231	0.288
NAA/CR	-.013*	0.953
NAA/CHO	-0.179	0.419

This table shows that there was significant negative correlation between the mean value of NAA in NAWM by MRS and EDSS score also there was significant negative correlation between the mean ratio of NAA/CR and EDSS.

DISCUSSION

The major goal of this study was to investigate specific metabolite changes, particularly NAA alterations, in the different areas of NAWM of MS patients using MRS technique and to conduct an analysis of spectroscopy markers of axonal integrity, astrogliosis, and demyelination in vivo and correlation with the clinical disability of the patients using EDSS score. Our results showed the metabolite values and ratios of MS patients (in NAWM and in WML) determined by H-MRS. MS patients in this study from the early stages of the disease, showed higher values for MI in NAWM compared with the MS plaques. These results coincide with others published earlier, using similar techniques [16,17,18,19,20] .

In contrast to our results, **Fernando et al ,2004** [21] reported higher absolute concentrations of NAA in NAWM and no significant decreased NAA level in patients with definite MS. The Cr and Cho in this study were increased in RRMS patients in plaques and NAWM but there was not statistically significant

when compared to the controls. On contrary,other authors found in their study significant elevation in Cr in RRMS group compared to controls [22] .Other studies demonstrated increased Cho levels in MS plaques and NAWM in RRMS, as well as decreased NAA concentrations [24] , presenting inflammatory changes and significant axonal damage.

Metabolic ratios are less sensitive than absolute concentrations of individual metabolites [23] . When comparing NAA/Cho and NAA/Cr ratios in the lesions, NAWM in patients versus controls, they were clearly reduced, consistent with results obtained in previous studies . The observationis that the magnitude of the reduction varies with the type and clinical form of the disease . Since it was first described, MS has been considered a demyelinating disease of the central nervous system, and until recently axonal loss had not been studied in depth. The N-Acetyl Aspartate levels detected by H-MRS could provide a precise marker of axonal loss [25] .

Although the function of myoinositol (mI) is uncertain, we know that it is involved in the polyphosphoinositol second messenger cascade and that it is relatively concentrated in glial

cells when compared with neurones as was shown by multinuclear spectroscopy techniques performed in rats . So an increase in mI may reflect neuroglial homeostasis and gliosis **.** This finding is in agreement with other study that showed significant increase in NAWM Mi. the results provide evidence that M I is increased in NAWM in early clinical stage of multiple sclerosis [26] **.**

Another observation in this work was significant elevation of glutamate in NAWM when compared with the control group .This was in agreement with the findings of **Anders et al 2013**[27] , who also found increased Glu in both acute lesions and in NAWM of MS patients , indicating that elevated levels of Glu are involved in the progression of MS even in this non-lesional MS subtype. Therefore, there is a potential for Glu to be used as a marker of the severity of MS even in patients where no white matter lesions are found [28] **.**

In our study we correlated the EDSS score as a measuere of the disability with the clinical data of all patients ,we found significant positive correlation between the number of relapse and the score . These findings coincide with **Minneboo et al, 2004)**[29] , who emphasized the role of number of relapse in long-term prognosis for patients . Moreover, investigations in the past few decades have revealed that many clinical are predictive of long-term outcomes, including sex, higher age and pyramidal symptoms at disease onset, shorter time to second clinical attack, and number of relapses in the early disease phase[31] **.**

In our study, we studied the clinical disability of our patient, using EDSS score; also we analyzed the metabolic levels of MS plaques and NAWM of 23 MS patients and 10 controls. In the current study, we found a significant correlation between NAA and clinical score using EDSS score in accordance with findings reported by **Adalsteinsson et al , 2003**[30] **.** MRS seems to be a potential surrogate marker of disease progression **.** In contrast to our findings, recently, **Rigotti et al 2011**[31] **,** who studied whole brain NAA (WBNAA) in 43 patients with RRMS, found that no correlation between WBNAA and clinical disability using EDSS score.

CONCLUSION

Magnetic Resonance Spectroscopy is a tool that may play an important part in studies on natural history of the disease and the assessment of immunosuppressive or immunomodulatory treatment in clinical trials . It provides important data on axonal degeneration which is the subject of this study. Total T2-weighted lesion load , although more sensitive to changes with time than brain *N*-acetylaspartate, may be less relevant to understanding the progression of disability.

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