Abnormal Diffusivity of Normal Appearing Brain Tissue in Multiple Sclerosis: A Diffusion-Weighted MR Imaging Study

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Objective: *To assess whether water diffusivity in normal appearing brain tissue including white and gray matter of multiple sclerosis (MS) patients shown by diffusion-weighted imaging (DWI) differs from normal individuals.*

Material and Method: *Conventional MRI and DWI were performed in 37 multiple sclerosis patients and 31 control subjects, matched for age and sex. Quantitative diffusivity values were obtained from variable locations of normal appearing white and gray matter from both hemispheres by using a standardized region of interest template.*

Results: *Mean diffusivity was higher in both normal appearing white matter (NAWM) and normal appearing gray matter (NAGM) of MS patients (mean $\pm$ SD: 85.71 x 10^{-5} $\pm$ 2.9 x 10^{-5} mm^2/s and 85.90 x 10^{-5} $\pm$ 2.45 x 10^{-5} mm^2/s) than normal control subjects (NAWM: 73.46 X 10^{-5} $\pm$ 1.77 x 10^{-5} mm^2/s and NAGM: 82.90 x 10^{-5} $\pm$ 0.91 x 10^{-5} mm^2/s) with p-value < 0.0001.*

Conclusion: *Water diffusivity was higher in all NAWM regions, deep gray matter regions, and some cortical gray matter region of MS patients than normal controls. DWI can quantify the presence and extent of MRI-undetectable pathology in the normal appearing brain tissue that were the disease burden.*

Keywords: *Diffusivity, Multiple sclerosis (MS), Diffusion-weighted imaging (DWI), Normal appearing white matter (NAWM), Normal appearing gray matter (NAGM)*

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Magnetic Resonance (MR) imaging has come to have an increasingly important role in the diagnosis and management of multiple sclerosis (MS) because it has been shown to increase diagnostic specificity and to augment the assessment of treatment effects in this clinically and pathologically heterogeneous disease[1]. However, it has limitations. These include the inability to detect subtle abnormalities in the so called normal appearing white matter (NAWM)[2].The tendency of disease extension beyond the areas of plaques has been shown on previous histological and MR spectroscopic studies[3-5]. Alteration of these normal appearing tissues is of great importance because its true pathophysiological significance is not completely understood. Neuronal and axonal damage has become a crucial issue. Recent evidence of previous studies indicates this damage can occur from the beginning of the disease and may even be occurring most rapidly in the early stages of MS[6,7]. To the extent that this damage was irreversible, it must be associated with

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irreversible neurological deficit. Process of cortical adaptation maybe able to maintain normal function and to minimize disability from axon injury in the early stages, but can no longer compensate in the later, chronic stages of the disease. Then an imaging technique that can detect greater this disease burden than that seen on conventional MR imaging would provide valuable information for both exploratory clinical trials of new drug agents and monitor therapeutic efficacy.

Diffusion-weighted MR imaging can provide quantitative information regarding tissue structures based on the molecular motion of water[8], and there has been great interest in the use of this technique for the study of stroke[9]. The apparent diffusion coefficient (ADC) provides a rotationally invariant measurement of the total diffusion of water within a tissue[10]. Diffusion-weighted MR imaging gives an opportunity to determine the structural characteristics of tissues. Cellular structures in the CNS restrict water molecular motion. Pathologic processes that modify tissue integrity, thus reducing "restricting barriers", can result in increased ADC.

The purpose of the present study was to determine if there is quantifiable diffusion differences among normal appearing brain tissue that appear qualitatively normal on conventional MR images.

Material and Method

Subjects

Thirty-seven patients with clinically defined multiple sclerosis who underwent MRI at UCSD medical center during January 2001-December 2003, were studied. The patient population consisted of 26 females and 11 males who were 16-60 years old (mean, 38.08 years). Twenty-six patients were classified clinically as having relapsing-remitting disease, and eleven patients had secondary progressive disease.

Thirty-one normal control subjects, matched for age and sex (20 female and 11 male; mean age 33 years) were recruited in the present study for comparison.

MRI protocols

Brain MRI was performed on patients and controls using a 1.5 Tesla MR system. The MR protocol included the following sequences: Sagittal and axial T1-weighted spin-echo (TR/TE = 450/15, 5-mm thick sections, 256 x 192 matrix, 24-cm field of view [FOV]); axial fast spin-echo T2-weighted (TR/TEeff = 3786/96, echo train length = 8, 5-mm thick sections with 2.5-mm spacing, 196 x 512 matrix, 24-cm FOV); axial fluid-attenuated inversion-recovery (FLAIR) images (TR/TE /TI = 9000/105/2500, 5-mm thick sections with 2.5-mm spacing, 256 x 154 matrix, 22-cm FOV); and sagittal FLAIR (TR/TE/TI = 8500/105/2500, 3-mm thick sections with 1-mm spacing, 256 x 196 matrix, 22-cm FOV). A multislice single-shot spin-echo echo-planar diffusion-weighted imaging sequence (TR/TE = 4521/110, 5-mm thick sections with 1.5-mm spacing, 128 x 96 matrix, 24-cm FOV) was also employed. The diffusion gradients were sequentially applied in the x, y and z axis directions with three different *b* values (0, 500, 1000 s/mm^2). In each region of interest (ROI), the ADCs in the x, y and z directions were calculated using the Stejskal and Tanner equation by linear fitting of the logarithm of the SI (ln SI) versus *b* value. Diffusion trace maps were computed from the isotropic diffusion image and the baseline image on a pixel-by-pixel basis. Finally, gadolinium dimeglumine (0.1 mmol/kg) was injected intravenously and axial and coronal T1-weighted images with fat suppression technique (TR/TE = 774/14, 5-mm thick sections, 256 x 144 matrix, 22-cm FOV) were obtained immediately after injection.

The region of interest selection

Different white matter and gray matter areas of brain parenchyma on ADC mapping images of DWI that show normal signal intensity on both T2-weighted and FLAIR images were chosen for placing the regions of interest (ROIs). ROIs of uniform size (40 mm^2) were manually positioned in different white matter areas (frontal, parietal, temporal, occipital and cerebellum) and smaller ROIs (20 mm^2) were positioned in cortical gray matter (frontal, temporal, parietal, occipital and cerebellum), deep gray matter (head of caudate nucleus, putamen and thalamus) in both patients and controls (Fig. 1). All ROIs were chosen by one observer who was blinded to clinical subgroup information and disability scores.

Reliability of MRI measurements

The same individual, blinded to clinical data, re-measured the ADCs of all patients and controls at least 3 weeks after the initial analysis.

Average intraclass correlation coefficient for determine intraobserver reliability were 0.78 and 0.90 for the ADC of NAWM and NAGM of patients.

Statistical methods

The authors used Mean (SD) and 95% confidence interval CI to describe ADC values of different NAWM and NAGM areas. Paired-t test was used to

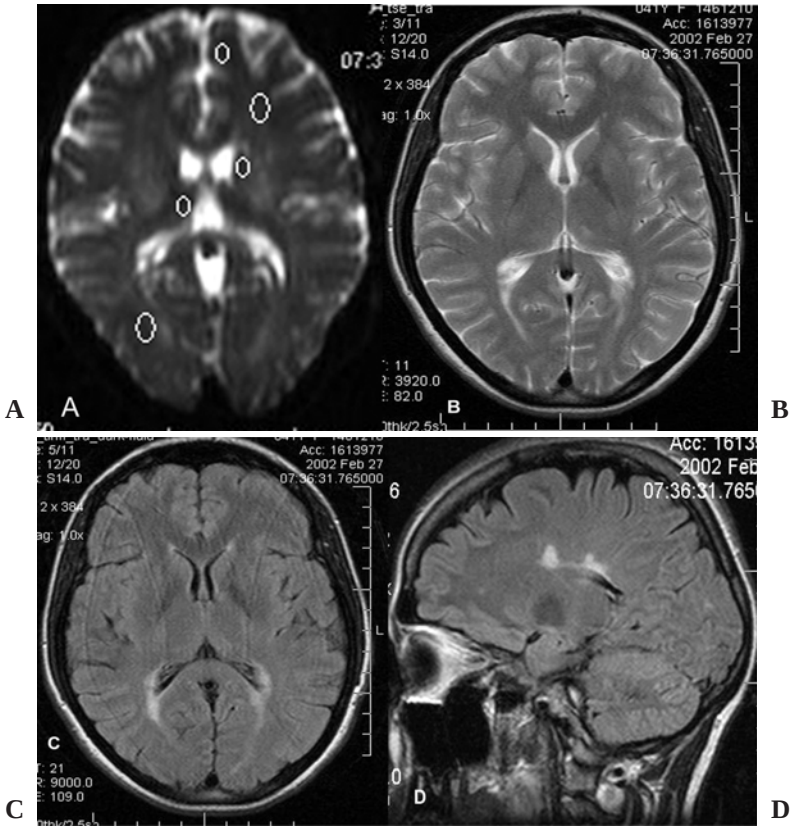

Fig. 1 A-D, Axial ADC map diffusion image (A), fast SE T2-weighted image (B), FLAIR image (C), and Sagittal FLAIR image (D), show the absence of the lesion or abnormality at the areas of placing the ROI (ellipse cursor in A) on both T2W (B) and FLAIR images (C), the presence of MS plaques at callososeptal region in the same patient (D)

compare mean ADC of different areas of NAWM and NAGM between patients and normal controls. The statistical tests were performed at a significance level of 0.05 by using STATA software package (version 8.0).

Results

The mean $\pm$ SD of ADC value was 85.71 $\pm$ 2.9 x 10^{-5} mm^2/s in the NAWM of MS patients, 73.46 $\pm$ 1.77 x 10^{-5} mm^2/s in the white matter of normal controls.

Mean difference was 12.25 with statistical significance ($p < 0.0001$). Statistical significant differences in ADC value were found in all regions of measured white matter: frontal, temporal, parietal, occipital and cerebellum (Table 1). In deep gray matter,

Table 1. Comparison of mean ADC measurement of normal appearing white matter (NAWM) between MS cases and control subjects

Location	Mean ADC (x 10^{-5} mm^2/s) (SD)		Mean difference (95% CI)	p-value
	Control subjects (n = 31)	MS cases (n = 37)		
White matter	73.46 (1.77)	85.71 (2.90)	12.25 (13.45, 14.04)	<0.0001
Frontal	74.58 (2.59)	87.01 (2.54)	12.43 (11.05, 13.82)	<0.0001
Temporal	75.03 (2.40)	87.12 (2.49)	12.09 (10.62, 13.54)	<0.0001
Parietal	73.52 (2.32)	86.87 (3.30)	13.35 (11.75, 14.94)	<0.0001
Occipital	72.73 (2.58)	85.52 (3.15)	12.79 (11.24, 14.34)	<0.0001
Cerebellum	72.73 (2.58)	80.26 (3.36)	7.53 (10.50, 11.24)	<0.0001

Table 2. Comparison of mean ADC measurement of normal appearing gray matter (NAGM) between MS cases and control subjects

Location	Mean ADC (x 10^{-5} mm^2/s) (SD)		Mean difference (95% CI)	p-value
	Control subjects (n = 31)	MS cases (n = 37)		
Gray matter	82.90 (0.91)	85.90 (2.45)	3.00 (2.08, 3.94)	<0.0001
Frontal	89.07 (1.63)	90.46 (4.93)	1.39 (-3.24, 0.46)*	0.0696*
Temporal	88.95 (1.66)	91.14 (3.80)	2.19 (0.73, 3.66)	0.0020
Parietal	88.88 (1.97)	91.41 (5.85)	2.53 (0.33, 4.73)	0.0123
Occipital	89.98 (1.61)	90.38 (4.83)	0.40 (-2.21, 1.41)*	0.3307*
Thalami	81.20 (1.88)	89.14 (5.07)	7.94 (6.02, 9.86)	<0.0001
Head of caudate nucleus	72.50 (2.23)	76.43 (5.72)	3.93 (1.75, 6.11)	0.0003
Putamen	81.01 (2.32)	84.25 (4.63)	3.24 (1.42, 5.07)	0.0004

* = not statistically significant

mean ADC of thalami- head of caudate nucleus - putamen of patients ($89.14 \pm 5.07 \times 10^{-5}$, $76.43 \pm 5.72 \times 10^{-5}$, $84.25 \pm 4.63 \times 10^{-5}$ mm^2 /s,respectively) were all higher than controls ($81.20 \pm 1.88 \times 10^{-5}$, $72.50 \pm 2.23 \times 10^{-5}$, $81.01 \pm 2.32 \times 10^{-5}$ mm^2 /s,respectively) (Table 2). However, the authors found statistical significant difference of mean ADC value between patients and controls in normal appearing cortical gray matter of only temporal and parietal lobes (Table 2). Mean ADC value of the remaining frontal and occipital lobes was not significantly different.

Discussion

The authors found that the mean ADC was significantly higher in the NAWM of patients than in controls. Several causes have been proposed for this subtle change of NAWM, including diffuse astrocytic hyperplasia, perivascular infiltration, myelin breakdown products, activation of microglia, axonal loss and patchy edema[11]. Preliminary DWI/DTI[12,13], magnetization transfer[14] and magnetic resonance spectroscopic[15] studies have suggested that the discrepancy in correlation between lesion load and clinical disability may be caused by an occult disease burden that is not visible on conventional MR imaging.

Measurement of white matter ADC in several different brain areas in the present study confirmed that NAWM is actually abnormal in MS patients, and the changes are diffuse. Several pathologic studies give additional support to the concept that MS is actually a diffuse disease process. The authors found smaller elevations of ADC within the cerebellar white matter. Clinically, pathologically and on the imaging studies, the cerebellum is involved to a lesser degree than the cerebrum.

For the last result of the present study, the authors found significantly increased mean ADC in the deep gray matter of MS patients compared to control subjects. This was more obvious than the result of increased diffusivity in the cortical gray matter, only significant in temporal and parietal lobes. This may imply that the deep gray matter was more involved than the cortical area. The most striking increase of ADC was in both thalami. Smaller increases were seen in the head of caudate nucleus and putamen. Elevated ADC values in the thalami were also reported by Fabiano and his group[16]. The present results were a little different, in that the presented data revealed slightly higher ADC values in the left thalamus, as apposed to the right thalamus in their data. The right-to-left differences were small and could be explained by slightly different patient groups. Recently, this interhemispheric asymmetry was found in normal individuals and have explained due either to a greater neuronal attenuation or to a greater number of reciprocal connections with neighboring brain regions on the side with reduced diffusivity[17]. However, the presented data confirmed that DWI could detect subtle changes in deep gray matter structures that appear normal or free of MS plaques on conventional MR imaging. Involvement of gray matter structures has been detected by other advanced imaging modalities, including positron emission tomography[18], magnetization transfer[19] and magnetic resonance spectroscopy[20]. A previous diffusion tensor study showed small changes in mean ADC in the basal ganglia and thalamus in MS patients compared to normal controls, but the differences were not statistically significant, possibly due to a small sample size[19]. The pathogenesis of gray matter change in MS patients remains unknown,

but both indirect mechanisms (diaschisis and wallerian degeneration)[18,21] and direct injury (inflammation, demyelination, neurotoxicity and iron deposition)[22,23] have been implicated. In support of direct injury, Cifelli et al[22] noted substantial neurodegeneration, neuronal metabolite depletion and macroscopic volume loss in the thalami of a patient with MS. The present study may have limitations. The ROIs for ADC measurement were not uniform in size, which could account in part for the wide variability in the standard deviations of the measurements. Finally, the authors do not have histopathological data to correlate with the presented DWI findings. However, the presented data confirmed the global change in MS patients and revealed deep gray matter involvement in MS. Further study is needed to identify specific patterns of involvement and to apply with other advanced imaging modalities in a longitudinal study to enhance monitor therapeutic efficacy and discover new drugs.

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ความผิดปกติของปริมาณการแพร่กระจายของโมเลกุลน้ำในเนื้อเยื่อสมองส่วนที่ไม่เห็นรอยโรคในผู้ป่วยมัลติเปิลสเคอโรซิส

วรินทร พุทธรักษ์, วาณีรัตน์ กาฬสีห์, วัลลภ เหล่าไพบูลย์, มาลินี เหล่าไพบูลย์, John R Hesselink

วัตถุประสงค์*: เพื่อประเมินความแตกต่างของปริมาณการแพร่กระจายของโมเลกุลน้ำในเนื้อเยื่อสมองส่วนที่ไม่เห็นรอยโรคทั้งในส่วนเนื้อขาวและเนื้อเทาของผู้ป่วยมัลติเปิลสเคอโรซิสจากกลุ่มคนเปรียบเทียบที่ปกติโดยใช้ดิฟฟิวชั่นเอ็มอาร์ไอ*

วัสดุและวิธีการ*: ทำการตรวจเอ็มอาร์ไอปกติและ ดิฟฟิวชั่น เอ็มอาร์ไอ ในผู้ป่วยมัลติเปิลสเคอโรซิสจำนวน 37 คน และกลุ่มคนเปรียบเทียบที่ปกติโดยมีเพศและอายุตรงกับผู้ป่วยจำนวน 31 คน และทำการวัดปริมาณการแพร่กระจายของโมเลกุลน้ำในตำแหน่งต่าง ๆ ของเนื้อสมองส่วนที่ไม่เห็นรอยโรค โดยใช้ cursor รูปวงรีวางในภาพดิฟฟิวชั่นเอ็มอาร์ไอ ในตำแหน่งที่ไม่เห็นความผิดปกติจากภาพเอ็มอาร์ไอปกติ ซึ่งจะออกมาเป็นตัวเลข และนำมาคำนวณออกมาเป็นค่าเฉลี่ย และค่าสัมประสิทธิ์การเบี่ยงเบน*

ผลการศึกษา*: ค่าเฉลี่ยของปริมาณการแพร่กระจายของโมเลกุลน้ำของเนื้อเยื่อสมองส่วนที่ไม่เห็นรอยโรคทั้งในเนื้อขาวและเนื้อเทาของผู้ป่วยมัลติเปิลสเคอโรซิส (ค่าเฉลี่ย $\pm$ ค่าสัมประสิทธิ์การเบี่ยงเบน: $85.71 \times 10^{-5} \pm 2.9 \times 10^{-5}$ และ $85.90 \times 10^{-5} \pm 2.45 \times 10^{-5}$ ตารางมิลลิเมตรต่อวินาที ตามลำดับ) มีค่ามากกว่าเนื้อเยื่อสมองส่วนเนื้อขาวและเนื้อเทาของกลุ่มคนเปรียบเทียบที่ปกติ ($73.46 \times 10^{-5} \pm 1.77 \times 10^{-5}$ และ $82.90 \times 10^{-5} \pm 0.91 \times 10^{-5}$ ตารางมิลลิเมตรต่อวินาที ตามลำดับ) โดยมีนัยสำคัญทางสถิติ ($p\text{-value} < 0.0001$)*

สรุป*: พบว่ามีการเพิ่มขึ้นของค่าเฉลี่ยของปริมาณการแพร่กระจายของโมเลกุลน้ำในเนื้อสมองส่วนเนื้อขาวและ เนื้อเทาที่อยู่ชิดโพรงสมองทุกตำแหน่งที่ได้ทำการวัด และในบางตำแหน่งของเนื้อสมองส่วนเนื้อเทาที่อยู่บริเวณเปลือกสมองในกลุ่มผู้ป่วยมัลติเปิลสเคอโรซิส เมื่อเปรียบเทียบกับกลุ่มคนปกติ จะเห็นได้ว่าดิฟฟิวชั่นเอ็มอาร์ไอ นั้นสามารถวัดและบอกว่ามีความผิดปกติเกิดขึ้นในเนื้อสมองส่วนที่ไม่สามารถเห็นความผิดปกติจากการตรวจด้วยเอ็มอาร์ไอวิธีปกติ ซึ่งเป็นตำแหน่งที่เชื่อว่ามีพยาธิสภาพของโรคซ่อนอยู่*