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ARTICLE

Diffusion tensor magnetic resonance imaging may show abnormalities in the normal-appearing cervical spinal cord from patients with multiple sclerosis

Exames de imagem por tensor de difusão podem mostrar alterações na coluna cervical aparentemente normal em pacientes com esclerose múltipla

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ABSTRACT

Objective: This study aims to evaluate "in vivo" the integrity of the normal-appearing spinal cord (NASC) in patients with multiple sclerosis (MS) compared to controls, using diffusion tensor MR imaging. **Methods:** We studied 32 patients with MS and 17 without any neurologic disorder. Fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) were calculated within regions of interest at C2 and C7 levels in the four columns of the spinal cord. **Results:** At C2, FA value was decreased in MS patients. Besides, RD value was higher in MS than in controls. At C7, MD values were increased in MS. **Conclusion:** The NASC in the right column of the cervical spinal cord showed abnormal FA, RD and MD values, which is possibly related to demyelination, since the FA abnormality was related to the RD and not to the AD.

Key words: diffusion tensor imaging, multiple sclerosis, cervical spinal cord.

RESUMO

Objetivo: Este estudo avalia "in vivo" a integridade da medula espinhal cervical aparentemente normal (MEAN) em pacientes com esclerose múltipla (EM) comparados aos controles, usando a imagem por tensor de difusão. **Métodos:** Foram selecionados 32 pacientes com EM e 17 controles. Foram calculadas fração anisotrópica (FA), difusão axial (DA), difusão radial (DR) e difusibilidade média (DM) dentro das regiões de interesse nos níveis C2 e C7 nas quatro colunas da medula espinhal. **Resultados:** Em C2, o valor de FA foi reduzido em pacientes com EM. Além disso, o valor da DR se mostrou mais elevado na EM do que nos controles. Em C7, os valores de MD foram maiores na EM. **Conclusão:** A MEAN na coluna direita da medula cervical mostrou valores alterados de FA, RD e MD, possivelmente relacionados à desmielinização, uma vez que a alteração de FA está relacionada à DR e não à DA.

Palavras-Chave: imagem por tensor de difusão, esclerose múltipla, medula cervical.

Multiple sclerosis (MS) is a common demyelinating disease that affects central nervous system at a highly variable pace. In addition to loss of myelin and oligodendrocytes, axonal injury is a prominent pathologic feature of MS[1,2]. Brain demyelinating lesions are quite common in MS, but they do not always explain the clinical symptoms, an event recognized as the clinical-radiological paradox. Some hypotheses are raised to explain this fact, including the normal-appearing white matter damage and also spinal cord lesions. The involvement of the spinal cord is a common finding in patients with MS. Magnetic resonance (MR) imaging studies have shown cord lesions in up to 90% of these patients[3]. New lesions in the spinal cord can cause disabling symptoms, because important neurological functions are conveyed within spinal cord[4]. The

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criteria proposed by McDonald et al. to diagnose MS, including the Barkhof criteria for MR imaging findings, are represented by spinal cord lesions as a hallmark of the disease. Besides, it was reported that the presence of asymptomatic spinal cord lesions place subjects with radiologically isolated syndrome at substantial risk for clinical conversion to either an acute or progressive event, a risk that is independent of brain lesions on MR imaging. Recent studies with histopathological analyses have shown that the cord damage is more extensive than the macroscopic lesions seen on conventional MR imaging[5], remitting to the concept of normal appearing spinal cord (NASC)[3]. NASC is an area of microscopic damage that has normal signal intensity on conventional MR imaging sequences. Such areas are better evaluated with advanced MR imaging techniques, such as diffusion tensor MR imaging (DTI). This technique allows the assessment of tissues at a cellular level, once the microstructural architecture preservation of the white matter tracts are evaluated by water molecular diffusion along the axon fibers[6,7].

The DTI consists of three *eigenvectors* and to each one there is a corresponding diffusion axis that reports to the direction of diffusive water motion. Each *eigenvector* has a concerning *eigenvalue* that gives the magnitude of water diffusion in that direction. DTI-derived indices can be obtained by combining the three *eigenvalues*: the fractional anisotropy (FA), which measures the directionality of water molecular diffusion; the 'axial diffusivity' (AD), which is the main *eigenvalue* (ϵ_1); the 'radial diffusivity' (RD), obtained by the average of the second (ϵ_2) and third (ϵ_3) *eigenvalues* of the DTI; and 'mean diffusivity' (MD), which represents the average among the three *eigenvalues*, reflecting the amount of water diffusion regardless of its preferential directionality. AD represents the water diffusion parallel to the axon bundle, and its increase is usually related to axonal damage, whilst the RD represents the water diffusion perpendicular to this direction, and its increase is more commonly associated with demyelination[8].

This study aims to evaluate "in vivo" the integrity of the NASC in patients with MS, using diffusion tensor MR imaging. We hypothesize that MS patients will have lower FA values in the spinal cord compared with controls. This FA reduction is probably mainly caused by RD alteration, reflecting the demyelination process.

METHODS

Population

This study included 32 patients with relapsing-remising MS (20 female, mean age 39.3 years (standard deviation=5,5), range from 18 to 57 years) and 17 healthy controls (13 female, mean age of 40 years (standard deviation=2,1), range from 21 to 69 years) without any known spinal disease or neurologic disorder. The inclusion criteria for MS patients were: standard for the clinical diagnosis of MS established by McDonald et al.[9], age between 18 and 70 years, regardless of gender, and patients in follow-up at the Department of Neurology of our University. All subjects and controls signed informed consent and the Institutional Review Board of our University Hospital approved the study.

Before MR imaging acquisition, the MS patients were clinically assessed by an experienced neurologist, who performed the Expanded Disability Status Scale (EDSS), in order to standardize their neurological condition. The average scale value was 5.2 (ranging from 2 to 7, standard deviation=1,9).

Magnetic Resonance imaging acquisition

All patients underwent MR imaging on a 1.5-T Scanner (Avanto, Siemens, Erlangen, Germany), using an eight-channel phased-array headmatrix coil attached to a neck-matrix coil in order to have higher signal-to-noise ratio at the cervical region. The conventional MR imaging protocol included the following: sagittal STIR images (repetition time [TR]: 4170 milliseconds; inversion time [TI]: 150 milliseconds; echo time [TE]: 87 milliseconds; field of view [FOV]: 250 mm; matrix: 256×320; with a 10% gap; slice thickness 3 mm with 30% of interval), axial T2* gradient echo (TR: 606 milliseconds; TE: 18 milliseconds; FOV: 200 mm; matrix: 192×320; 30 slices with 3 mm thickness and 30% gap) and sagittal T1 (TR: 500 milliseconds; TE: 9 milliseconds; FOV: 220 mm; matrix: 320×240; 12 slices with 3 mm thickness and 30% gap) after administration of contrast medium (dimeglumine gadolinium, 0.1 mmol/kg; Schering AG, Berlin, Germany). In addition, post-gadolinium injection, diffusion-weighted single-shot echo-planar imaging was acquired with bipolar diffusion gradients applied along twenty non-collinear directions in the sagittal plan (b0=0 and b=800 s/mm^2, TR: 2800 milliseconds; TE: 88 milliseconds; FOV: 260 mm; matrix: 128×128; 16 slices with 3 mm thickness and 10% gap; 1 average) for fifteen minutes. Motion correction artifacts were removed using an internal program from the manufacturer.

Diffusion tensor magnetic resonance imaging post-processing

Four regions of interest (ROIs) were drawn based on the anatomic landmarks of the reformatted axial b0 image at C2 and C7 level in the NASC, using VB15 (Siemens, Erlangen, Germany). In the workstation, we used the software NEURO 3D and DTI Evaluation (version 1.0, Siemens, Erlangen, Germany) for reconstruction. The ROIs were located around the central canal of the spinal cord, in the anterior and posterior columns (ROI size: 6 voxels (20.42 mm^3) and in the left and right lateral columns (ROI size: 4 voxels (13.62 mm^3), as shown in Figure, based on previous published data[4]. Lesions locations were evaluated by an experienced neuroradiologist, and mapped across the whole spinal cord. ROIs that included at least one lesion at any of these columns at the level of C2 or C7 detected on conventional MR imaging were excluded from the analysis. The total number of ROIs considered for analysis at each level and each column was described in Table.

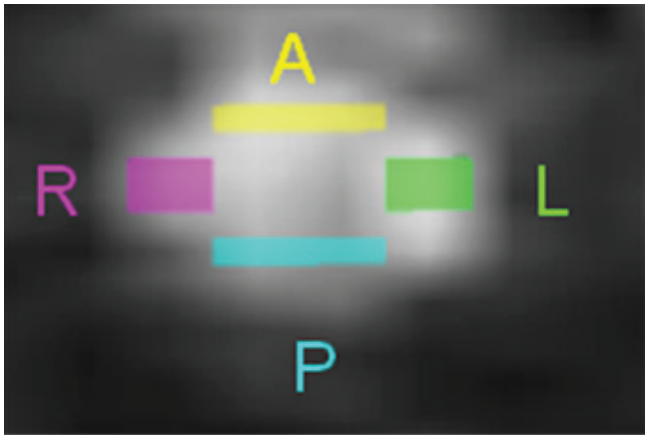
FA, RD, AD and MD values were automatically generated from each ROI, and the average of each parameter was calculated for each column and for each spinal cord level for MS patients and controls.

Statistical analysis

All statistical calculations were performed with the Statistical Package for the Social Sciences version 17.0 (SPSS, Chicago, IL, USA). A p-value of less than or equal to 0.05 was considered statistically significant. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the values. All variables were normally distributed and two-tailed paired Student's t-test was used for the comparison of DTI parameters for each region separately. The average value of each DTI parameter at C2 and C7 cervical spinal cord level of MS patients was compared with controls.

To investigate the association between the FA abnormalities and the RD and AD values in MS patients, we applied Pearson correlation.

Pearson test was also used to evaluate the correlation between altered DTI parameters in MS patients and the clinical data available using EDSS.



A: anterior; R: right lateral; P: posterior; L: left lateral.

Figure. The regions of interest placement in the axial reformatted diffusion tensor magnetic resonance imaging images for each cervical spinal cord column at C2 and C7 levels.

RESULTS

Table reflects the lesions encountered in the C2 and C7 levels considering the lateral, anterior and posterior columns.

The comparison between MS and controls showed statistical significance in DTI parameters only in the right column. At C2 level, the FA value was decreased in MS patients (0.59±0.20 *versus* 0.75±0.14, p=0.01). In addition, RD value was higher in MS than in controls (0.71×10^{-3} ±0.57 mm^2/s *versus* 0.50×10^{-3} ±0.29 mm^2/s, p=0.03). At C7 level, MD values were increased in MS patients (1.19×10^{-3} ±0.57 mm^2/s *versus* 1.00×10^{-3} ±0.20 mm^2/s, p=0.03).

The Pearson correlation performed in the right column at the C2 level demonstrated inverse strong correlation between FA and RD values (r=-0.630, p=0.000).

The clinical correlation performed in altered DTI data and EDSS average values using Pearson coefficient showed no significant correlation: FA and EDSS (r=-0.062, p=0.747), and RD and EDSS (r=-0.023, p=0.905).

Table. Comparison between diffusion tensor magnetic resonance imaging parameters in multiple sclerosis and controls in the four columns of the spinal cord at C2 and C7 levels.

C2	MS *versus* CONTROLS									
	Right					Left				
		FA	AD	RD	MD		FA	AD	RD	MD
MS - Mean±SD	n=23	0.59±0.20	2.19±0.45	0.71±0.57	1.36±0.45	n=28	0.65±0.23	2.29±0.37	0.75±0.57	1.29±0.45
CONT - Mean±SD	n=17	0.75±0.14	2.43±0.98	0.50±0.29	1.08±0.36	n=17	0.72±0.18	2.25±0.58	0.62±0.50	1.16±0.50
p-value		0.01*	0.44	0.03*	0.14		0.48	0.85	0.59	0.57
C2	Anterior					Posterior				
		FA	AD	RD	MD		FA	AD	RD	MD
MS - Mean±SD	n=30	0.58±0.06	2.15±0.43	0.76±0.24	1.24±0.31	n=26	0.64±0.23	2.32±0.26	0.78±0.57	1.32±0.45
CONT - Mean±SD	n=17	0.63±0.08	1.99±0.38	0.67±0.22	1.20±0.29	n=17	0.76±0.12	2.25±0.59	0.54±0.39	1.13±0.41
p-value		0.08	0.32	0.34	0.34		0.14	0.7	0.24	0.32
C7	Right					Left				
		FA	AD	RD	MD		FA	AD	RD	MD
MS -Mean±SD	n=29	0.51± 0.18	2.01±0.61	0.79±0.58	1.19±0.57	n=32	0.62±0.13	2.04±0.39	0.68±0.28	1.13±0.29
CONT - Mean±SD	n=17	0.71±0.12	2.05±0.31	0.67±0.52	1.00±0.20	n=17	0.63±0.09	2.03±0.37	0.79±0.51	1.21±0.38
p-value		0.09	0.14	0.58	0.03*		0.48	0.85	0.59	0.57
C7	Anterior					Posterior				
		FA	AD	RD	MD		FA	AD	RD	MD
MS -Mean±SD	n=32	0.57±0.11	2.15±0.43	0.76 ±0.24	1.24 ±0.31	n=31	0.52±0.11	2.40±0.52	1.05±0.41	1.49±0.43
CONT - Mean±SD	n=17	0.58±0.12	2.06±0.67	0.80 ±0.56	1.22 ±0.57	n=17	0.57±0.15	2.53±0.47	0.99±0.44	1.51±0.42
p-value		0.93	0.94	0.54	0.61		0.35	0.52	0.23	0.95

SD: standard deviation; FA: fractional anisotropy; AD: axial diffusivity; RD: radial diffusivity; MD: mean diffusitvity.

DISCUSSION

This study aimed to assess "in vivo" the NASC damage in the cervical spinal cord of patients with MS, using DTI. Our results demonstrated that DTI parameters were mainly affected in the right column. At the C2 level, the FA value was decreased whereas the RD value was increased in MS patients. The FA and RD measurements at this level were inversely correlated in the Pearson analysis. Also, in the C7 level, the MD value was increased in this same column.

Freund et al.[4] evaluated spinal cord lesions, using DTI parameters, of 14 MS patients throughout first, third and sixth months after a clinical relapse. They concluded that DTI is a possible tool for assessing the prognosis of spinal cord damage, notably the RD, as it predicted clinical outcome after a relapse and reflected the dynamic pathological changes, and thus influences recovery. Apart from that, Kang et al.[10] showed FA changes distal to the site of a lesion, in the area of possible Wallerian degeneration. The reduced FA values we found in the C2 level is probably related to distal lesions, as already described, but the inversely correlation between FA and RD may reflect concomitance of demyelination in the histopathological damage in that area. The increased MD values of this same column in the C7 level, also corroborates for the idea of distal lesions promoting damage in the NASC.

In this series we found that the right side was predominantly damaged. Supporting this idea, a study using functional MR imaging including 23 patients with primary progressive MS demonstrated different activity at specific regions of the spinal cord. A higher occurrence of functional MR activity in the anterior section of the right side of the spinal cord at the level of the C6-7[11] was verified. They showed tactile-associated cervical spinal cord over-activation, possibly owing to injured interneurons, which was related to lower FA values in such column. This study corroborates our results of decreased FA values in the right column of the spinal cord, and further studies are necessary to suggest the occurrence of some functional compensation mechanism in our group. This main activation in the right side may be correlated to the hemispherical predominance of the group. Right-sided and left-sided patients should be further compared in order to verify its pattern of lesions and also if there is any side predominance of functional reorganization. The pattern of MS lesions in the spinal cord is not predictable and the studies simply describe their location and their effect in distal parts of the spinal cord. They focus in the repercussion of the lesions in the NASC, which is characterized by the DTI parameters, remaining the conclusion stable throughout the papers, despite the level lesions. Because of this, our findings mostly localized in the C2 level may be more representative in the C7 level in other studies.

There are some limitations of this study. The DTI has several technical limitations, mainly related to low signal-to-noise ratio and movement artifacts associated to breath and pulsation. Some technical improvement may be obtained using the kurtosis diffusion sequence (DKI), as it uses a different statistical process, including not only a number reflecting the parameters in a single voxel, but its variation in this same voxel. In addition, the tensor model may tend to underestimate or overestimate FA values in areas where fibers cross. Also, the ROI method of analysis is operator dependent and could be subject of bias. Finally, some of the clinical data including treatment type, disease duration and number of relapses, were not available and, therefore, could not be controlled for the statistical analysis.

In conclusion, the results of our series suggest that DTI has a potential role for the assessment of the spinal cord of MS patients. The inverse correlation between FA and RD in the NASC of the right column of the cervical spinal cord points toward demyelination as an additional pathological process, and not only Wallerian degeneration. Further studies are necessary to establish a better correlation between molecular biology and neuroimaging findings, as well as to define the role of DTI as a clinical and prognostic marker in MS patients.

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