

Tracing immature myelin in acute disseminated encephalomyelitis

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Inherent abnormalities of myelin have been suggested in the pathogenesis of multiple sclerosis (MS). We investigated myelin in acute disseminated encephalomyelitis (ADEM) patients by magnetic resonance spectroscopy (MRS) and cerebrospinal fluid (CSF) analysis for citrulline, a marker of immature myelin. A citrulline peak was observed in the normal appearing white matter of 7/15 patients and of 1/10 age-matched neurological controls ($p=0.08$). CSF citrulline was elevated in 4/6 patients. Alterations in the composition of myelin might predispose to or follow acute inflammatory disorders of the central nervous system.

Key words: myelin, acute disseminated encephalomyelitis, citrulline, magnetic resonance spectroscopy.

Acute disseminated encephalomyelitis (ADEM) results from a transient autoimmune response involving molecular mimicry between exogenous and neural antigens, or non-specific activation of autoreactive T cells against myelin. The pathogenesis of this autoimmune reaction and the basis for individual susceptibility are unclear. A genetic predisposition for autoimmune processes is unlikely: ADEM occurs in previously healthy children with no systemic immunological problems, and relapses or familial cases are uncommon. Inherent properties of myelin predisposing to damage might contribute to the development of ADEM: for instance, acute multiple sclerosis (MS) has been associated with the presence of immature myelin. Immature myelin is characterized by higher content of short-chain fatty acids and citrulline^{1,2}. In this study, we investigated the presence of immature myelin in ADEM patients by magnetic resonance spectroscopy (MRS) and cerebrospinal fluid (CSF) analysis for citrulline.

Material and Methods

Fifteen patients who had been diagnosed and treated for ADEM in Hacettepe University, Department of Pediatric Neurology, were studied 2-73 months after the acute attack. ADEM was defined by clinical and magnetic resonance imaging (MRI) features of diffuse/multifocal neurological findings of acute or subacute onset, associated with diffuse or multiple areas of increased signal intensity on T2-weighted (W) images involving the white matter or central gray matter. All MR images were evaluated by a neuroradiologist to verify the diagnosis, and ADEM was confirmed by the absence of further attacks or new lesions on MRI during at least six months' follow-up. MRS was done after recovery from the acute disorder, together with the follow-up MRI, when patients were under no treatment.

Control cases were patients investigated for the clinical diagnoses of headache (n=7), Bell's palsy (n=2), or familial congenital

deafness (n=1), who had normal neurological examination according to a pediatric neurologist, and a normal MRI. The study was approved by the Hospital Ethics Committee.

Magnetic resonance imaging was done on a 3T unit (Allegra, Siemens, Erlangen, Germany) using a standard quadrature head coil. Initially, axial T2-W fast spin echo (FSE) (TR/TE: 4000/100 ms, matrix: 448x88) imaging with 20 slices (5 mm thickness, 0.5 mm interslice gap) was performed to exclude any other diagnoses or significant findings. Following on-site evaluation of the conventional T2-W FSE imaging, a dedicated neuroradiologist performed ¹H MRS using T2-W FSE transverse images already obtained and fast T2-W scout images on coronal and sagittal planes, each consisting of five slices. Normal appearing white matter (NAWM) of the left parietal region was examined by single voxel spin echo spectroscopy (svs) in all subjects (TR: 3000, TE: 30 ms, voxel size: 15x15x15 mm³, 96 scan averages). Previous MRI examinations of ADEM patients were reviewed to ensure the NAWM was unaffected during the acute episode. When a lesion was observed in an ADEM patient, its svs imaging with the same parameters described for NAWM was also performed and compared with the unaffected contralateral parenchyma. Following data acquisition, resonance peaks of N-acetyl aspartate (NAA), choline (cho), and creatine (cr) were identified. Absolute concentrations of

NAA, cho and cr were calculated using phantom replacement method described elsewhere³. The concentration ratios of metabolites to creatine were computed by a physicist. The citrulline peak was defined at 3.15 ppm between cho and cr peaks as described by Silberstein et al.⁴. The presence of the citrulline peak was searched by augmenting the zone between the cho and cr peaks on an automated postprocessing software provided by the manufacturer on an off-line workstation (Leonardo Systems, Siemens, Erlangen, Germany). When a differentiation between a peak and noise was equivocal, it was accepted as 'peak negative'.

When available, CSF obtained from ADEM patients at the time of diagnosis and stored at -20°C up to six months was analyzed for citrulline by high pressure liquid chromatography (HPLC) using homoserine as internal standard (Duzen Laboratories, Ankara) and interpreted using Mayo Clinic reference values for CSF. Levels exceeding the upper limit by at least two-fold were considered elevated in this study.

Results

There were 15 ADEM patients: 15 had MRI studies done and six had CSF. ADEM patients' age and sex distribution (n=15, age at imaging 2.5-17, mean 11.1 years, female/male: 7/8) was similar to the control group (n=10, age at imaging 2-15, mean 10.3 years, female/male: 5/5) (unpaired Student t test, p=0.631; and Fisher's exact test, p=0.593) (Table I).

Table I. Features and Results of ADEM Cases

Case	Age	Sex	At MRS				
			Age	Months after ADEM	Neurological findings	Citrulline HAWM	Peak lesion
1	2	M	2	2	Normal	NA	-
2	4	M	4.5	8	Normal	-	NA
3	9	M	10	9	Mild behavioral disturbances	-	+
4	10	M	11	13	Normal	+	-
5	11	F	11	3	Paraparesis	-	-
6	11	M	12	13	Normal	-	NA
7	9	F	11	30	Normal	-	NA
8	5	M	11	73	Left hemiparesis	-	-
9	13	F	13.5	9	Normal	+	NA
10	13	F	13	2	Normal	+	NA
11	13	F	16	30	Normal	+	+
12	15	M	17	19	Left hemiparesis, left visual field neglect	-	-
13	13.5	F	17	42	Normal	+	NA
14	5	M	9	50	Normal	+	NA
15	5	F	7	22	Cranial nerve palsy, attention deficit	+	NA

MRS: Magnetic resonance spectroscopy. NAWM: Normal-appearing white matter. NA: Not applicable.

Six ADEM patients had three svs imagings: NAWM, lesion site, and its contralateral homologous region. One patient with no definable NAWM because of widespread residual T2 hyperintense abnormalities was examined in the affected areas only, and eight patients with no lesion and control subjects (n=10) had only one svs imaging.

Mean NAA/cr ratio was 1.59 ± 0.33 in the NAWM of the patients and 1.62 ± 0.30 in controls; mean cho/cr ratios were 0.76 ± 0.14 and 0.80 ± 0.13 , respectively. There were no significant differences between the NAWM of ADEM and control cases (independent sample t-test: $p=0.812$ for NAA/cr and $p=0.588$ for cho/cr).

In the ADEM group, lesions with volume loss or T2 hyperintensity but no edema or mass effect had lower mean NAA/cr than the NAWM (mean: 1.07 ± 0.32 for lesions and 1.59 ± 0.33 for NAWM; independent sample t-test, $p=0.011$), but mean cho/cr ratio was not different (mean: 0.82 ± 0.25 for lesions and 0.76 ± 0.14 for NAWM; independent sample t-test, $p=0.634$). The NAA/cr ratio did not correlate with the time elapsed after the clinical disease.

A citrulline peak was observed in the NAWM of 7/15 (48%) (Fig.1A,B; Fig.3C,D) and in the lesion areas of 2/7 (28.6%) (Fig. 2A,B; Fig.3A,B) ADEM patients ($p=0.08$ and $p=0.30$, respectively, compared to control, Fisher's exact test) (Table I). In the control group, one case (10%), a 10-year-old girl with headache, had a citrulline peak.

Cerebrospinal fluid samples were available for amino acid analysis in six ADEM cases (3 boys and 3 girls, aged 2-15 years). Citrulline was normal in 2/6 and elevated two- to three-fold in 4/6 cases.

Discussion

In this study, citrulline was demonstrated in the cerebral white matter more frequently in children with a history of ADEM than in control children, although the difference did not reach statistical significance. The presence of citrulline in white matter is unlikely to result from a physiological immaturity of myelin because patients and control cases were age-matched and none were below two years of age⁵. Similarly, a finding secondary

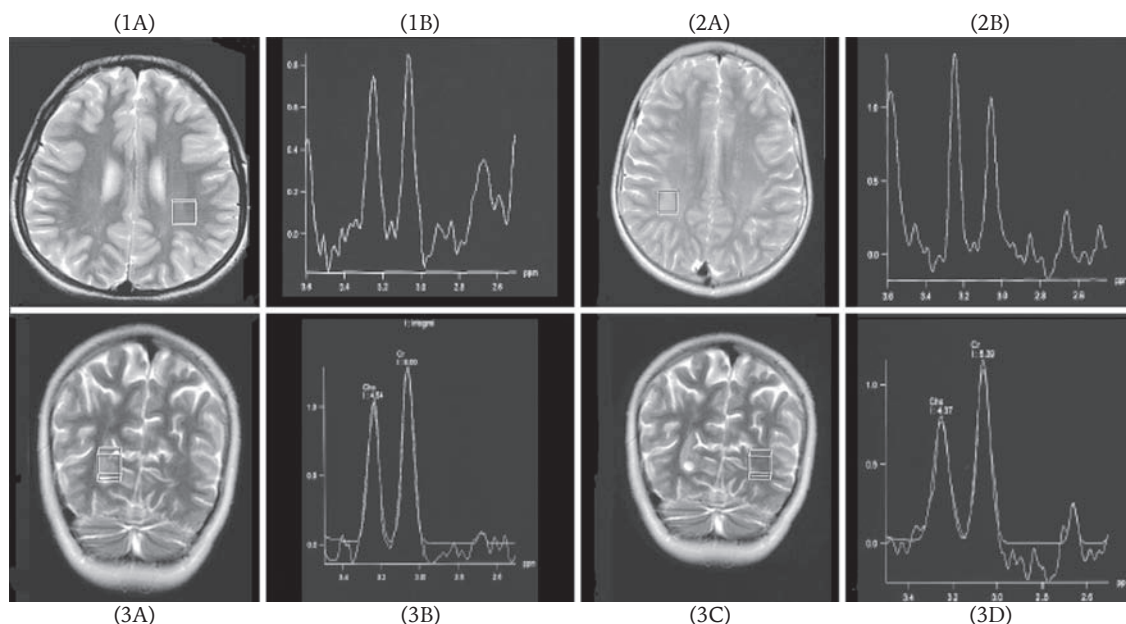


Fig. 1. Case 10. A svs (TR/TE: 3000/30 ms, voxel size: 15 mm³) imaging obtained from left parietal normal appearing white matter (A) shows a citrulline peak between choline and creatine peaks in a focused segment of spectrum (B).

Fig. 2. Case 3: right parietal old lesion (A) shows the citrulline peak (B).

Fig. 3. Case 5: (A) and (C) show reference images of svs imagings of right occipital lesion and contralateral normal appearing left occipital parenchyma, while (B) and (D) show corresponding augmented view of the spectra. The citrulline peak was absent in the lesion and normal appearing tissue.

to inflammation or remyelination is improbable because citrulline was observed often in the NAWM and less frequently in lesions; in addition, numerous studies on regenerating myelin have never associated it with citrulline. The “normality” of the NAWM is questionable. The findings of NAWM might be complicated by Wallerian degeneration of axons traversing the lesions visible on MRI^{6,7}. On the other hand, in contrast with MS where the so-called ‘NAWM’ may show subtle abnormalities on magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI), the NAWM of ADEM patients does not demonstrate such changes on MTI and DTI⁸⁻¹⁰. The few studies using MRS in ADEM show that the NAWM may have altered metabolism and its NAA levels might remain low for varying periods during recovery^{11,12}. We tried to eliminate a residual lesion in the NAWM by reviewing previous MR examinations of the patients and performing MRS from the areas unaffected in those studies. The quantitative part of our MRS analysis showed the NAWM of ADEM patients had a NAA/cr ratio similar to control cases while affected regions had variable NAA/cr ratios, indicating the NAWM, even if affected previously, had recovered according to the currently available techniques. Recently, experimental allergic encephalomyelitis, an autoimmune disorder mimicking ADEM, was reported as associated with citrullinated central nervous system proteins¹³.

The small size of the citrulline peak observed on MRS precluding quantification, we measured citrulline in the CSF and found elevated levels in 4/6 samples. This level may vary according to the phase of the disease; special conditions regarding the site, time, or degree of inflammation may be needed for this amino acid to enter the CSF.

If not secondary, the presence of citrulline in the white matter of ADEM patients might be a primary or constitutional feature. Properties of developmentally immature myelin have been implicated in the pathogenesis of demyelinating disorders. Immature myelin contains the least cationic charge isomer of myelin basic protein, MBP-C8, where citrulline replaces arginine residues. This component, normally found in all children up to two years of age, declines to adult proportions around four years, but was found in high amounts in MS patients⁵. This has been related to an enzymatic defect modifying MBP: increased enzymatic

conversion of arginine to citrulline prior to demyelination in spontaneously demyelinating transgenic mice supports this view¹⁴. The less cationic MBP may fail to polymerize and bundle actin, and to bind to lipid bilayer membrane, rendering myelin more prone to damage¹⁵. Immature myelin might also elicit an altered autoimmune response because of its modified antigenic structure. For instance, MS patients have a higher number of MBP-C8-responding T-lymphocyte lines¹⁶.

The quantitative part of our MRS studies is in agreement with previous case reports^{6,11}. The low NAA/Cr in our patients’ lesions with residual T2 hyperintensity and volume loss probably indicates irreversibly decreased neuronal-axonal density. Although a transient neuronal-axonal dysfunction may cause reversibly low NAA signal, irreversible losses are associated with permanently low NAA signal¹¹. We think the latter is more applicable to our patients because our studies were done after long intervals from the acute episode. The long intervals without new MRI lesions also allowed exclusion of MS to a great extent, in conjunction with the clinical presentation and higher incidence in childhood supporting ADEM.

Magnetic resonance imaging lesions of ADEM disappear in the majority of patients by 12 months. Periventricular lesions are the latest to disappear¹⁷. Periventricular myelin is frequently observed as T2 hyperintense areas on MR imaging until the second decade: whether late recovery is due to late maturation can be investigated by MRS, as this technique appears to be useful in assessing myelin composition.

To our knowledge, this is the first study of myelin in ADEM. The relatively small size of our series constitutes a limitation of our study and may have caused the lack of statistical significance. ADEM is a rare disorder and large series are difficult to collect: its sporadic and unpredictable occurrence hampers the design of longitudinal studies starting before the attacks, and recurrent cases may not be representative. Larger, multicentric studies with repeated examinations may clarify the findings observed in the current study.

Investigation of pediatric MS cases with this technique might also provide information about the pathogenesis and differential diagnosis of demyelinating disorders.

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