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# USE OF LOCALIZED PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN CANAVAN'S DISEASE*

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**SUMMARY: Aydınlı N, Çalışkan M, Calay M, Özmen M. (Division of Pediatric Neurology, Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa - İstanbul, Turkey). Use of localized proton nuclear magnetic resonance spectroscopy in Canavan's disease. Turk J Pediatr 1998; 40: 549-557.**

**Canavan's disease is characterized by megalencephaly, leukodystrophy and early motor and mental retardation. On computerized tomography and magnetic resonance imaging, severe changes compatible with white matter disease due to demyelination is observed. It has been demonstrated that urinary N-acetylaspartate levels are increased because of a deficiency of aspartoacylase (N-acyl-L-aspartate aminohydrolase) in these patients. In this study, with the use of proton nuclear magnetic resonance spectroscopy, we were able to demonstrate elevated levels of N-acetylaspartate compared to choline and creatine in the frontal region white matter of three patients. The in vivo measurement of N-acetylaspartate, choline and creatine in the brain by magnetic resonance spectroscopy offers an additional noninvasive diagnostic test for establishing the diagnosis of Canavan's disease.** *Key words: Canavan's disease, $^{1}H$ nuclear magnetic resonance spectroscopy.*

Canavan's disease (CD) is a rare neurodegenerative disorder which belongs to the group of leukodystrophies. Neonatal, infantile and juvenile forms have been described. The autosomal recessive infantile form is the most common and usually presents with megalencephaly in the first months of life and progresses with a rapid deterioration of psychomotor and mental abilities, spasticity, blindness and occasionally deafness and convulsions[1,2]. On computed tomography (CT) and magnetic resonance imaging (MRI) severe white matter disease due to demyelination is observed[3-5]. Hagenfeldt et al.[6] first reported a patient with leukodystrophy and N-acetylaspartic aciduria due to aspartoacylase deficiency in 1987. In 1988, Matalon et al.[7] correlated aspartoacylase deficiency with CD by finding the characteristic spongy degeneration at brain biopsy of three children with increased N-acetylaspartate (NAA) levels in urine and plasma. N-acetylaspartate is synthesized from acetyl-

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CoA and aspartate by L-aspartate-N-acetyl transferase in the brain. It is metabolized to aspartate and acetate by aspartoacylase (aminoacylase II). In deficiency of aspartoacylase, NAA concentration in the brain is elevated and the substance is excreted in large amounts in the urine[2,8-13].

Two predominant mutations in aspartoacylase (E285A and Y231X) that lead to loss of enzymatic activity accounted for 97 percent of the mutant chromosomes among Ashkenazi Jewish patients, while in non-Jewish patients a wide spectrum of Canavan mutations has been identified[14].

Proton ($^1$H) nuclear magnetic resonance spectroscopy (MRS) may complement MRI in the diagnostic assessment of CD. In vivo qualitative measurements of cerebral metabolites, including NAA, choline (Ch), and creatine (Cr) levels, have been made using MRS. This method can noninvasively detect changes in patterns of metabolites and may allow an earlier and more specific determination of neurodegeneration without the use of ionizing radiation[15-21].

We report three non related patients with radiologically and biochemically proven CD and discuss the role of MRS in the diagnosis of this disorder.

## Material and Methods

All patients were diagnosed on the basis of clinical symptoms, MRI findings and urinary NAA levels.

Patient 1. He was the first child of healthy non-consanguineous parents. At birth a macrosomia was noticed. By three months of age, developmental delay was apparent and he was noted to be hypotonic. At 23 months the child showed macrocephaly [head circumference (HC)>97$^{th}$ centile] with a weight at the 10$^{th}$ centile and length at the 25$^{th}$ centile. He had visual tracking difficulties but he smiled when talked to. Light reflexes and ocular fundoscopy were normal on both sides, but there was thin pendular nystagmus. He was unable to hold up his head or sit unsupported. He had spasticity of all limbs with hyperactive reflexes.

Patient 2. He was the second child of healthy unrelated parents. Developmental delay was first noted at four-and-a-half months of age at which time he had visual tracking difficulties and the onset of apathy and passivity was observed. At the age of 19 months he had relative macrocephaly with a HC between 25-50$^{th}$ centile, weight below 3$^{rd}$ centile, and length between 10-25$^{th}$ centile. He failed to fix or follow a visual target but responded to auditory stimuli and could recognize his mother's voice. Lack of head control and emotional responses, and spontaneous movements accompanied by limb spasticity with hyperactive reflexes were noticed. The light reflexes were normal on both sides. On fundoscopy, papillae were pale. He could be fed orally.

Patient 3. She was the first child of healthy consanguineous parents. The neonatal period was marked by hyperirritability and sucking problems. Developmental delay was apparent at seven months. at 35 months her HC was at the 50$^{th}$ centile, and her weight and length were below 3$^{rd}$ centile. She also had relative macrocephaly. She did not pay attention to visual stimuli, she had horizontal nystagmus and strabismus. Light reflexes were normal on both sides. She could recognize her mother's voice. Motor activity was decreased; she exhibited opisthotonus posture. The primitive reflexes were strongly positive, she had no head control, and there was a profound tetraspasticity and a profuse increase in deep-tendon reflexes. On fundoscopy, papillae were pale bilaterally. She could be fed orally with semi-solid food.

Investigations: In all patients, MRI demonstrated diffuse symmetrical prolongation of both the T1 and T2 relaxation times in the cerebral and cerebellar white matter, causing hypointensity of the supratentorial white matter on T1-weighted and hyperintensity on T2-weighted images. These were interpreted as demyelination or dysmyelination. There was more involvement of the cerebral white matter than of the cerebellar white matter. The internal capsule, corpus callosum and deep cerebellar white matter were unaffected.

The analysis of urine organic acids by gas chromatography mass spectrometry yielded a prominent peak of NAA not recognizable in normal age-matched children (986, 1148, 622 mmol/mol creatinine, respectively; normal <50 mmol/mol creatinine).

Magnetic resonance spectroscopy protocol: MRS examinations were performed on a 1.5-T magnetic resonance scanner (General Electric, Echospeed, Milwaukee, WI, USA) using the quadrature adult head coil. The children were sedated by means of rectal administration of diazepam (0.5 mg/kg). The method used, Probe-P, is a version of PRESS (point resolved spectroscopy), which is more sensitive to the metabolites with longer T2s, such as Cr, Ch, and N-acetylaspartate. A voxel of 2x2x2 cm in the frontal region white matter was selected using graphically defined areas of interest in the axially taken images. After global and localized shimming (in the three orthogonal planes), the water suppression was optimized and thereafter the spectra of the desired area of interest were obtained with 9 minutes 42 seconds data acquisition. For the volume selective spectroscopy, a spin echo sequence with three frequencies selective 90, 180, 180 pulses was used. The TR (repetition time) and TE (echo time) were 1500 msec and 270 msec with 256x128/8 NEX. After the scan a single phased spectrum was obtained. The examination was considered unsuccessful if the patient moved during the data acquisition; it was repeated until the spectra clearly showed the peaks of metabolites. The probe spectra covered a spectral window from 4.40 ppm - to 0.40 ppm (parts-per-million) in X-axes and the intensity in Y-

axes. The three prominent resonances were identified as NAA at 2.02 ppm, Cr at 3.0 ppm, and Ch at 3.21 ppm according to the published criteria[16,17,20,22-24]. The ratios of the intensity peaks of the metabolites were calculated.

In our patients, MRS examinations were performed at the ages of 23, 19 and 35 months, respectively. In MRS studies it is important to compare spectra from patients with those from healthy subjects of similar age[20]. We did not attempt to acquire data from healthy children for this purpose; rather, the published normal spectra were used as control data[20].

## Results

The metabolite ratios obtained from the frontal region of the patients with CD are listed in Table I. When NAA peaks were compared with Cr and Ch peaks, the ratios were found to be considerably increased in favor of NAA. No significant lactate signal was observed. Table I also shows normal metabolite ratios obtained from the paraventricular region white matter of age-matched healthy children reported in the study of van der Knaap et al.[20], and from the occipital lobe white matter of a healthy 27-month-old child presented in the article of Marks et al.[25]. Representative T1-weighted image as well as localized spectra from the frontal region of patient 1 are shown in Figure 1.

## Discussion

Symmetric diffuse low signal intensity on T1-weighted images and high signal intensity on T2-weighted images are characteristic MRI findings of CD. The white matter involvement is more pronounced in the subcortical region than in the periventricular area[4,26]. Symmetric hyperintensity in the dentate nuclei and involvement of the brainstem are also described[26].

Table I: Metabolite Ratios from Patients with CD and Control Subjects

| Patients | NAA/Ch | Ch/Cr | NAA/Cr |
|---|---|---|---|
| 1 | 6 | 0.77 | 4.62 |
| 2 | 5 | 0.48 | 2.4 |
| 3 | 2.92 | 1.33 | 3.88 |
| Control subjects | | | |
| 27-month-old child* | | 0.238 | 1.20 |
| Age-matched children** | 2.2 ± 0.6 | 2.9 ± 0.7 | 3.4 ± 0.6 |

* From the article of Marks et al. (1991).
** Values are deduced from Figure 4 of van der Knaap et al. (1990).
NAA: N-acetylaspartate, Ch: choline, Cr: creatine

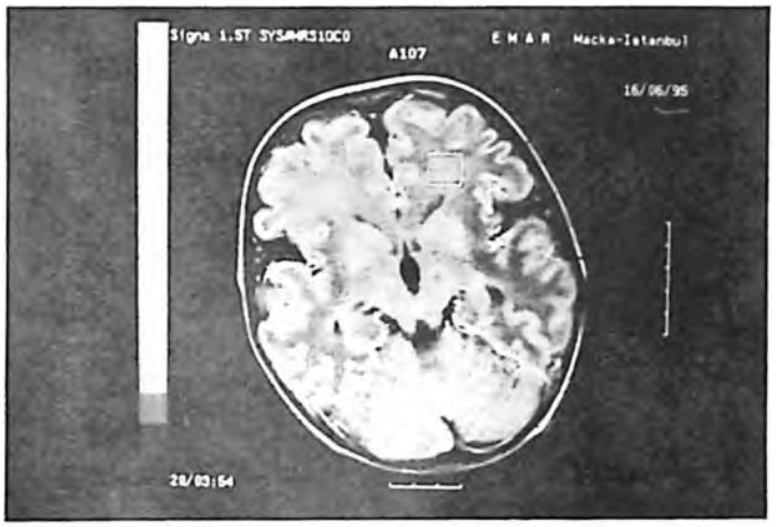


Fig. 1a

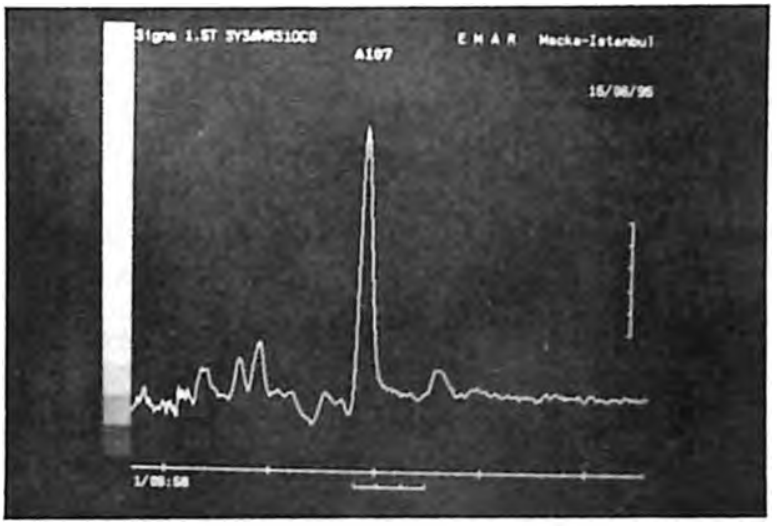


Fig. 1b

Fig. 1: T1-weighted axial image with voxel localization (a) and localized spectra from the frontal region (b) of patient. The diseased areas show signal alterations on the image. The metabolite ratios are NAA/Ch = 6, Ch/Cr = 0.77, NAA/Cr = 4.62.

The direct detection of NAA in the brain by MRS offers an additional diagnostic approach to CD. The metabolite ratios in our Probe-P studies were based on the data obtained from PRESS with a long TE sequence. Magnetic resonance spectroscopy at long echo times (135-270 msec) allows detection of four types of metabolites: Ch compounds, Cr/phosphocreatine, NAA and lactate. Because we could not correct for relaxation or saturation effects, the values of metabolite peaks reflect only relative changes and give no direct information for real metabolite concentrations. The area of interest in our study was in the frontal

region white matter, whereas in control studies the voxel was placed in different areas (paraventricular, occipital lobe white matter). It has been reported that slight differences of metabolite ratios can be seen between the regions, with the frontal regions generally showing slightly reduced values compared to those from the occipitial regions[13,17]. Different pulse sequences with long echo times (PRESS or STEAM) have been used in earlier studies[3,25]. Because only the ratios of metabolites are compared, pulse sequences seem not to be important. We agree with Rajanayagam et al.[17] that measurement of absolute concentrations rather than ratios is desirable, but to date no generally accepted method of quantification exists. As a result, a range of concentration values of the metabolites with large errors have been reported by different authors, even in the normal brain. Therefore, at present, metabolite ratios are preferable for evaluating patients.

In our study, compared to age-matched healthy children[20], NAA/Ch ratios were higher in all our patients. This finding is in accordance with results of earlier studies[23,24], in which increases in NAA and decreases in Ch resonance intensities have been reported. The high ratio of NAA to Ch may result either from an excessive amount of NAA due to a mutation of aminoacylase II resulting in a loss of function, or from the decrease in Ch which is a major component of lecithin and sphingomyelins. It has been reported that the increase of NAA in CD can be as high as several-fold over that of control[13]. One consequence of the defective catabolism of NAA is impaired myelin synthesis because NAA provides acetyl groups for de novo lipid synthesis[13]. Changes in the resonance intensity of Ch probably result mainly from increases in the steady state levels of phosphocholine and glycerol-phosphocholine. These choline-containing membrane phospholipids are released during active myelin breakdown. Thus, the resonance intensity of Ch increases in acute demyelinating lesions in humans. It is reported that chronic, slowly progressive leukodystrophies are associated with normal Ch/Cr resonance intensity ratios, presumably because the loss of myelin is so slow that significant increases of released membrane phospholipids do not accumulate[23]. In our study, Ch/Cr ratios were decreased in all cases compared to normal age-matched children. N-acetylaspartate to Cr ratio was increased in patient 1, decreased in patient 2 and within the normal range in patient 3. The increase in NAA to Cr ratio may be due either to the increase in NAA or to the decrease in Cr, which is consistent with the altered metabolic state of the brain. Tien et al.[18]. reported that a low creatine level frequently corresponded to the low-energy status of the tissue. It is also reported that total Cr concentration is relatively constant throughout the brain and tends to be relatively resistant to change[23]. Only when the total creatine pool changes as a result of a prolonged perturbation is this expected to show up as a reduction

of the MRS signal of total creatine. We suggest that the absence of a lactate signal excludes hypoxia or anaerobic glycolysis because lactate is the end product of glycolysis and accumulates when oxidative metabolism cannot meet energy requirement[23,24].

In the study of Grodd et al.[15], MRS performed in the right occipital lobe of a patient with CD revealed an increased peak from the methyl group of NAA, a reduced peak for creatine/phosphocreatine, a lack of Ch compounds, and no additional signal of lactate. In comparison with the control, the NAA signal in the child with Canavan's disease was increased by 20-25 percent. The authors explained the absence of a Ch peak with the decrease of myelin and they reported that the absence of lactate signals excluded hypoxia or anaerobic glycolysis for the reasons given above.

Grodd et al.[16] reported a seven-month-old boy with CD whose MRS revealed increased NAA content and lack of Ch-containing compounds in comparison with those values in age-matched control subject. We could not compare our results with those of the study above since the ratios of metabolites were not reported.

Marks et al.[25] demonstrated elevated levels of NAA in the occipital lobe of a 38-month-old patient with CD. In comparison with the control subject (a 27-month-old child), their patient demonstrated a 50 percent increase in NAA, a 44 percent increase in lactate, and a 51 percent decrease in Ch. The authors explained the elevation in the lactate seen in their patient as a result of greater mitochondrial dysfunction due to the increasing age of the patient. They thought that the 51 percent decrease in choline-containing compounds seen in their patient was consistent with severe demyelination. In our study, NAA/Cr and Ch/Cr ratios were found higher in all three cases compared to those of the patient reported in Marks et al.[25].

Barker et al.[3] have used MRS to quantitatively determine cerebral NAA concentrations in frontal lobe white matter of four patients with CD and in four age-matched control subjects. The most prominent features were an elevated ratio of NAA to Cr and Ch, and absolute quantification indicated that this was due to decreased Cr and Ch, as opposed to increased NAA. Cerebral NAA, measured in terms of micromoles/g weight, was not significantly different from normal despite the large increase in urinary NAA in the patients. They explained decreased Cr and Ch levels in the patients with a reduction in the number of cells per unit volume. They also noted that the reduced Ch signal could be associated with demyelination. In this study, the lactate and inositol levels were found to increase with age, indicating progressively decreasing mitochondrial oxidative activity.

Some of the discrepancies in the comparison of our results with earlier reports regarding metabolite ratios can probably be explained by differences of enzyme levels, age, and localization of the volume of interest in the cases studied. The only way to progress in the evaluation of the neurodegenerative diseases is to collect sufficient material from homogeneous populations examined with good technique and quantification of data. To do this it is necessary to have data on a sufficient number of healthy volunteers in all age classes to compare and determine normal values and confidence levels.

In conclusion, although determination of urinary NAA remains relatively simple and less expensive for establishing the diagnosis of CD, MRS provides a noninvasive method of investigating brain metabolism in infants and children. $^{1}H$ MRS in particular can be readily incorporated into integrated MRI-MRS examinations, and it is apparent that spectroscopy can provide new insights into the biochemical and pathophysiological basis of neurological disorders.

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