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Metabolite Profile of Cerebrospinal Fluid in Patients With Spina Bifida: A Proton Magnetic Resonance Spectroscopy Study

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Study Design. The present study was carried out to assess the metabolic differences between cerebrospinal fluid samples of patients with spina bifida and age-matched control individuals.

Objectives. To study the metabolite profile of cerebrospinal fluid of patients with spina bifida using proton magnetic resonance spectroscopy, compare the levels of metabolites with controls, establish correlation of underlying neuronal dysfunction with metabolic changes in patients with spina bifida, and evaluate the potential use of this technique as an additional tool for diagnostic assessment.

Summary of Background Data. Combination of embryopathy, stretching, ischemia, compression, and trauma is responsible for cord dysfunction in spina bifida. Changes in neuronal metabolism leads to changes in the local milieu of cerebrospinal fluid in the cord. Change in metabolite profile of cerebrospinal fluid in spina bifida in terms of increase in products of anaerobic metabolism, nerve membrane integrity, and nerve ischemia has not yet been studied.

Methods. Cerebrospinal fluid obtained from patients and control individuals were characterized using various one- and two-dimensional proton magnetic resonance spectroscopy techniques. Concentration of various metabolites was calculated using the area under the nuclear magnetic resonance peak.

Results. Statistically significantly higher levels of lactate, choline, glycerophosphocholine, acetate, and alanine in the cerebrospinal fluid of patients with spina bifida was observed compared with control individuals.

Conclusions. Significantly higher levels of metabolites were observed in patients with spina bifida, representing a state of nerve ischemia, anaerobic metabolism, and disruption of neuronal membrane.

Key words: spina bifida, anaerobic metabolism, lactate, choline, glycerophosphocholine, proton nuclear magnetic resonance spectroscopy, tethered cord. **Spine 2005;30:E68–E72**

Spina bifida is the most common central nervous system birth defect and traditionally defined by characteristic development abnormalities of the vertebrae and spinal cord. More recently, associated profound changes in the cerebrum, brain stem, and peripheral nerves have been observed. The spectrum of anomaly includes meningomyeloceles (MMC), lipomeningocele, lipomeningomyeloceles, and the spina bifida occulta group (including diastematomyelia, tight filum terminale, fatty filum terminale, atrophic MMC, spinal lipoma, myelocystocele, and anterior MMC).

The present study addresses the degree of underlying neural dysfunction in these patients. The pathophysiology of spinal dysfunction may be attributed to abnormal function of the spinal cord and roots during embryogenesis[1] or a local mass effect from the fatty tissue/bony spur.[1] Reigel *et al*[2] have postulated that the stretching of cord during pelvic flexion, cervical flexion, and growth, development, and change in curvature of vertebral column results in repetitive ischemia in a setting of tethering of cord and roots. General neurologic examination, nerve conduction studies, somatosensory evoked potential, electrocystometrogram, neuroradiological studies (*e.g.*, ultrasonography of spine, computed tomography scan, magnetic resonance imaging, magnetic resonance myelography) have been used to assess the spinal cord dysfunction.[3,4] The inadequacy of these methods in providing a clear assessment of the underlying spinal dysfunction, especially in identifying the reversible/preventable cause of these dysfunctions, have also been reported.[5–11]

Cerebrospinal fluid (CSF) is considered the window of the central nervous system.[12–15] Spinal dysfunction directly affects neuronal metabolism and changes the local milieu of CSF in the spinal column.[16–18] Many researchers have studied metabolites, neurotransmitters, and free radical levels of CSF to understand the neuronal dysfunction in several systemic[17–23] and local disorders[24–33] affecting the spinal cord. The proton magnetic resonance spectroscopy (^{1}H MRS) of body fluids, including CSF, enables the quantification of water-soluble components, including amino acids, tricarboxylic acid intermediates, lactate, and glucose.[34,35] Many markers of neuronal damage (*e.g.*, neurofilament, glial fibrillary acid protein, S-100 protein, neuron-specific enolase, creatine, citrate, and inositol),[25,27] nerve ischemia (*e.g.*, superoxide, dismutase, alanine, phenylalanine, tyrosine, lysine, and branched chain amino acids),[30,31] products of anaerobic metabolism (*e.g.*, lactate, acetate, and ethanol),[24–29] and

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Table 1. Patient Profile of Meningomyeloceles Group

No.	Age (Days)	Sex	Lower Limb Weakness	Bladder Involvement	Bowel Involvement	Lesion Characteristics
1	8	M	++	+	+	Lumbar MMC with low-lying cord at L4 and myelomalacia at L1
2	30	F	++	−	−	Lumbar MMC with dorsolumbar syrinx (D5-L1)
3	24	M	++	−	−	Thoracolumbar MMC with tethering of cord to hamartomatous mass
4	40	M	++	+	+	Lumbosacral lipo MMC with low-lying tethered cord S2
5	36	F	++	+	+	Lumbosacral MMC with diastematomyelia L4+ tethered cord
6	10	M	++	+	+	Lumbosacral lipo MMC with low-lying tethered cord L5

MMC = meningomyeloceles.

cell membrane damage (*e.g.*, choline and products of lipid peroxidation)[36,37] have been identified.

To the knowledge of the authors, there are no systematic data available in the literature on the metabolite profile of CSF in patients with spina bifida. In the present pilot study, the authors evaluated the metabolite levels in CSF by ^{1}H MRS in patients with spina bifida and compared them with the levels of metabolites in CSF of age-matched control individuals.

■ Materials and Methods

Patients. Sixteen infants and children with spina bifida presenting to the Department of Pediatric Surgery of the authors' institute from January 2000 to December 2002 were considered for the study. Six were cases of meningomyeloceles, and 10 were operated cases of spina bifida with retethering of cord or second lesion causing worsening of neurologic symptoms. Age, sex, nature of the defect, findings of ultrasonography or magnetic resonance imaging for spine and degree of neurologic deficit, and intraoperative findings were recorded.

Controls. Ten age-matched children served as a control group. These were the patients who underwent lumbar puncture at the pediatric medicine department of the authors' institute for suspected meningitis, but cytology and biochemical examination were negative. Children (both control and patients) with accidental bleeding during the collection of CSF were excluded.

Processing of Cerebrospinal Fluid. CSF samples were collected in clean glass vials, immediately frozen in liquid nitrogen, transported in liquid nitrogen containers, and stored at −35 C. Nuclear magnetic resonance (NMR) spectral acquisition was carried out within 2 days of sample collection. Before NMR experiments, the samples were thawed, and 60 μL of deuterium oxide (D_2O) (Aldrich Chemical Co., Milwaukee, WI) containing 0.5mmol/L of sodium 3-(trimethylsilyl-2,2,3,3-*H*)-1-propionate (TSP) (Merck Biosciences, Darmstadt, Germany) was added to 540 μL of native CSF sample. Solution was transferred to a 5-mm NMR tube, and experiments were performed immediately (~10 minutes). Since the samples were subjected to NMR analysis immediately, the authors do not expect any alterations of metabolite levels with this protocol.

To verify the effect of storage on the CSF samples at −70 C and −35 C, the authors divided three samples in two parts. One part was stored at −70 C and the other at −35 C. NMR experiments were carried out following the procedure mentioned above. No change in the concentration of metabolites was observed for samples stored at these two temperatures. The changes observed in the concentration of the metabolites were within the experimental error of ± 5%. Levine *et al*[38] carried out NMR experiments on CSF samples exposed to room temperature for 72 hours. They found no effect of exposure on CSF to room temperature on most of the metabolites, including alanine, acetate, and glucose.[38] In the present study, the samples were not exposed to room temperature, except for carrying out NMR experiments at 25 C for a period of 10 minutes.

Spectroscopy. Proton spectrums were acquired at a frequency of 400.13 MHz using DRX-400 (Bruker AG, Fällanden, Switzerland) NMR spectrometer equipped with broadband inverse probe. Typical parameters were used for one-dimensional proton NMR experiments using a single pulse sequence with the following parameters: pulse width, 90°; number of data points, 32 K; spectral width, 5000 Hz; number of scans, 32 to 48; and relaxation delay, 14 seconds. A 0.3 Hz line broadening was applied before Fourier transformation. Intensity of various metabolites resonances was measured with reference to TSP resonance.

Quantification of Metabolites. Quantitative data were obtained by calculating metabolite concentrations (mmol/L) from the area of their corresponding resonance(s) with respect to the area of the TSP resonance after correction for the number of protons.[39] Investigators (US and NRJ) who were unaware of the diagnosis performed the characterization and quantification of the spectrums.

Statistical Analysis. The data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL). Concentration of the various metabolites in the CSF samples of patients with spina bifida and control individuals were expressed as mean values ± standard deviation. One-way analysis of variance and *post hoc* least statistical difference were used to assess the differences between the groups. Values of $P < 0.05$ were considered significant.

■ Results

Tables 1 and 2 present the patient profile data, whereas the expanded regions of a typical NMR spectrum of CSF from a control and a patient with MMC are shown in Figure 1, A and B and Figure 2, A and B. Peaks corresponding to lactate, alanine, acetate, creatine/phosphocreatine, choline, glycerophosphocholine (GPC), and glucose were assigned in the spectrum using two-dimensional NMR methods. The detailed assign-

Table 2. Patient Profile of Retethering Group

No.	Age (Mo)	Sex	Lower Limb Weakness	Bladder Involvement	Bowel Involvement	Initial Lesion	Postop Characteristics (MRI + Intraop Findings)
1	16	M	++	−	−	Sacral lipo MMC	Retethering to residual lipoma + low lying cord
2	18	M	+++	+	+	LS MMC	Retethering to scar tissue
3	20.5	F	++	−	−	Lumbar MMC	Retethering to scar tissue + hydromyelia (L2–L3)
4	24	F	++	−	−	Thoracic MMC	Retethering to scar
5	22	M	+++	+	+	LS MMC	Retethering to thoracic spur (diastematomyelia)
6	12.5	M	++	−	−	Thoracic MMC	Retethering to scar + syrinx (T11-L1)
7	26	F	+++	−	−	LS MMC	Retethering to scar + low-lying cord (S1)
8	36	F	++	−	−	Thoracolumbar MMC	Retethering to scar + kyphosis
9	30	M	+++	+	+	LS Lipo MMC	Retethering to residual lipoma + scar
10	34	M	++	−	−	LS MMC	Retethering to scar + hydromyelia (L1–L2)

MRI = magnetic resonance imaging; LS = lumbrosacral.

ments of the resonances will be published elsewhere. The concentration of various metabolites were determined and compared between the groups. Concentrations of CSF metabolites in control, MMC, and retethering groups are shown in Table 3.

Meningomyelocele Group

In the MMC group, there were six patients with a mean age of 0.84 ± 0.2 months and a male/female ratio of 4:2. Resonances corresponding to GPC and Cho were seen clearly in the spectrum of CSF of this group (Figure 2B), whereas these were not detected in the control group. In the MMC group, significantly higher concentrations were found for lactate ($P < 0.001$), acetate ($P < 0.001$), Cho ($P = 0.002$), alanine ($P < 0.001$), and GPC ($P = 0.002$) compared with control individuals. The concentrations of creatine and glucose were not statistically different from the control group.

Retethering Group

Ten patients were studied in the retethering group. Mean age was 23.9 ± 7.6 months (range, 12.5–36 months) with a male/female ratio of 6:4. Statistically significant higher levels for lactate ($P = 0.022$), acetate ($P = 0.003$), Cho ($P = 0.033$), alanine ($P = 0.022$), and GPC ($P = 0.003$) were observed compared with control individuals. The concentrations of Cr and Glc were not statistically different from control individuals.

■ Discussion

A combination of embryopathy, stretching, ischemia, compression, and surgical trauma is reported to be responsible for the cord dysfunction in variety of expressions of the pathology of spina bifida.[40–43] However, no systematic investigation has been reported as to whether substances involved in energy metabolism that are present in CSF are different in patients with spina bifida. To the best of the authors' knowledge, the present study represents the first preliminary investigation where ^{1}H NMR spectroscopy was used to determine the concentration of various metabolites in the CSF of patients with spina bifida and control individuals. Several interesting observations emerged from the present study.

The concentration of lactate and alanine were higher in patients with MMC and retethered cord. Studies using cell culture media revealed that astrocytes release metabolites such as citrate, glutamine, lactate, alanine, and succinate, which are absorbed by neurons and, to some extent, released to the CSF, and alterations in their concentration reflect altered uptake or production.[44–46] It is known that alanine and lactate are converted into pyruvate, which is used through trichloroacetic acid cycle. The increase in the concentration of these metabolites might suggest that tethering of cord results in metabolic derangements in the form of impaired oxidative and glucose metabolism. Increase in lactate was documented to be related to the severity of neuronal dysfunction and the degree of impairment in oxidative metabolism.[2,3] Similar increase was reported in the CSF from fetal lambs[36] subjected to controlled hypoxia and in the CSF of cat spinal cord following trauma.[47] Garseth *et al*[24] demonstrated a reduction in lactate and alanine in patients with lumbar disc herniation.

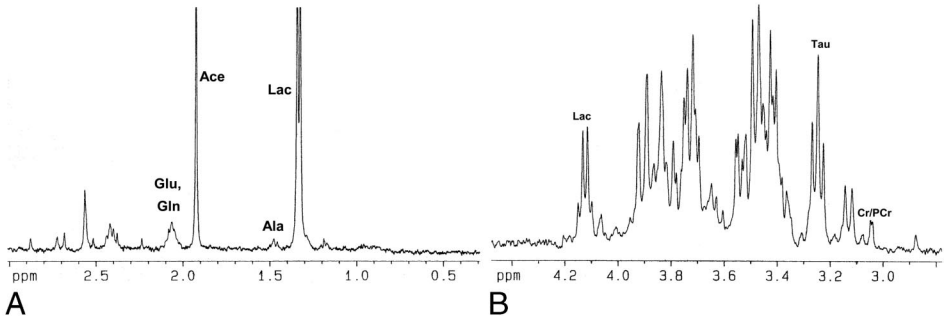

Figure 1. **A,** expanded region (0.2–3.0 ppm) of the proton NMR spectrum of a representative CSF sample from control individual at 298 K. The sample contains 10% of D_2O and 0.5 mmol/L of TSP. **B,** expanded region (2.8–4.5 ppm) of the proton NMR spectrum of a representative CSF sample from control individual at 298 K. The sample contains 10% of D_2O and 0.5 mmol/L of TSP.

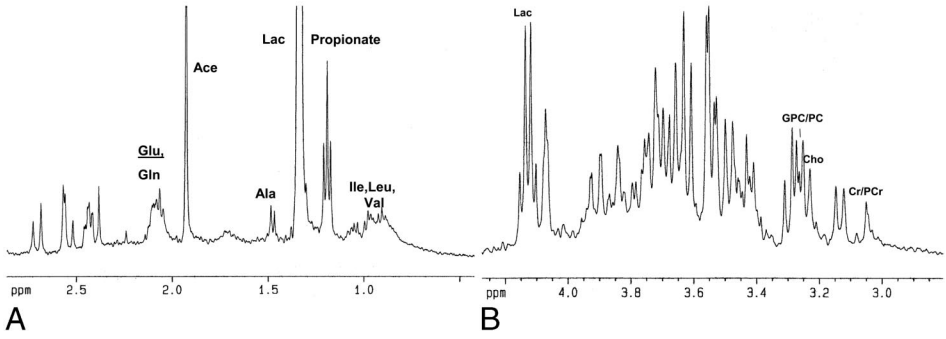

Figure 2. **A,** expanded region (0.5–2.8 ppm) of the proton NMR spectrum of a representative CSF sample from a patient with MMC at 298 K. The sample contains 10% of D_2O and 0.5 mmol/L of TSP. **B,** expanded region (2.8–4.25 ppm) of the proton NMR spectrum of a representative CSF sample from a patient with MMC at 298 K. The sample contains 10% of D_2O and 0.5 mmol/L of TSP.

Caution should be exercised in interpreting the concentration of metabolites such as lactate that might alter due to degradation on exposure to room temperature during processing[38] or anaerobic metabolic activity. However, no change in the concentration was reported for alanine, acetate, or glucose.[38] In the present study, CSF samples were snap frozen in liquid nitrogen and stored at −35 C and were not exposed to room temperature; therefore, the differences observed in the concentrations of metabolites among MMC, retethering, and control groups are attributed to changes in metabolism due to pathology. The concentrations of choline and GPC were higher in the MMC and retethered groups, whereas these were not observed in the control groups. This may be due to several factors, such as stretching and compression, that might disrupt the neuronal integrity of cord, leading to dysfunction in the setting of spina bifida and tethering of cord. Similar increase was reported in the CSF of fetal lambs following stretching, compression, and surgical trauma, which destroy the neuronal cell integrity.[36]

No significant difference in the creatine and phosphocreatine resonances in control and spina bifida groups were observed in the present study. Decrease in the tissue creatine kinase expression in the cord following traumatic injury has been reported previously.[48] The alterations in the cord with spina bifida might not be significant enough to change the concentration of these metabolites. Koshorek *et al*[32] demonstrated significant alteration in the acetate levels in CSF of patients with lumbar disc herniation, similar to the authors' findings of high levels of acetate in the MMC and retethering groups. Glucose in CSF is a reflection of blood glucose level. Although the authors did not correlate the blood sugar status with that in CSF, the comparable levels of CSF glucose among control individuals as well as the spina bifida groups suggest that there was no element of central nervous system infection or other systemic hypermetabolic states to confound the results.

■ Conclusion

The present results indicate that the CSF of patients with spina bifida show significant metabolic differences compared with control individuals. These include products of anaerobic metabolism, intermediates of the trichloroacetic acid cycle, neuronal cell membrane integrity, and nerve injury. Proton NMR spectroscopy measurements of metabolites in CSF is promising in improving both our understanding of the pathophysiology of neuronal dysfunction in patients of spina bifida and as a tool for providing additional diagnostic information.

Further longitudinal studies with larger sample size are required to establish markers for reversible neuronal dysfunction that, once clearly identified, can help in the prediction of prognosis of these patients with some objectivity. This may serve as a basis for development of noninvasive *in vivo* magnetic resonance spectroscopic

Table 3. Concentration of Metabolites in Different Groups of Spina Bifida and Control Individuals

	Control (n = 10)	MMC (n = 6)	Retethering (n = 10)
Mean age (mo ± SD)	7.3 ± 2.2	0.84 ± 0.2	23.9 ± 2.4
Sex	M = 5, F = 5	M = 4, F = 2	M = 6, F = 2
Metabolites		Concentration*	
Lactate	2.09 ± 0.54	26.23 ± 20.86	10.85 ± 3.91
Alanine	0.05 ± 0.05	1.23 ± 0.54	0.31 ± 0.18
Acetate	0.26 ± 0.32	1.25 ± 1.09	0.99 ± 0.37
Creatine, creatine phosphate	0.06 ± 0.14	0.16 ± 0.06	0.06 ± 0.02
Choline	ND	0.17 ± 0.07	0.14 ± 0.18
Glycerophosphocholine	ND	0.18 ± 0.07	0.16 ± 0.21
Glucose	2.46 ± 0.76	2.17 ± 0.61	1.94 ± 0.77

SD = standard deviation; MMC = meningomyeloceles; ND = not detectable.
* mmol/L ± SD.

analysis of CSF metabolites in spina bifida and other spinal pathologies.

■ Key Points

- The metabolic differences between CSF samples of patients with spina bifida and age-matched control individuals using ^{1}H MRS were assessed.
- Significantly higher concentrations of several metabolites, such as lactate, alanine, GPC, and choline were observed in patients with spina bifida, indicating a state of nerve ischemia, anaerobic metabolism, and disruption of neuronal membrane.
- The present results suggest that the ^{1}H MRS technique may be useful as an additional tool for diagnosis in patients with spina bifida.

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