Potential of *In Vitro* ^{1}H NMR Spectroscopy of Cerebrospinal Fluid in the Evaluation of Patients with Tethered Cord Syndrome

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INTRODUCTION

Tethering of spinal cord occurs in myelomeningocele (MMC), lipo MMC, intra spinal lesions etc. which may lead to intermittent, but chronic repetitive ischemia with resultant spinal cord dysfunction. Spinal cord dysfunction directly affects neuronal metabolism and changes the local milieu of cerebrospinal fluid (CSF) in the spinal column. Concentration of several metabolites were found to be higher in patients with spina bifida compared to controls (1). In the present study, the metabolite concentrations in CSF of patients of spina bifida with tethered cord syndrome prior to (pre-op MMC) and after surgery (post-op MMC) were determined using *in vitro* proton magnetic resonance spectroscopy (^{1}H-MRS) and compared with controls to establish a correlation of underlying neuronal dysfunction with metabolic changes in spina bifida.

PATIENTS AND METHODS

Six infants with MMC (n=6) presented to the Department of Pediatric Surgery considered for the study. The same patients underwent excision, laminectomy and detethering ± excision of second lesion by the same surgeon and constituted the post-operative (post-op MMC) group. Ten age matched children served as control group. These were the patients who underwent lumbar puncture for suspected meningitis but cytology and biochemical examination were negative for the same. The CSF of patients was collected by lumber puncture and immediately frozen in liquid nitrogen. Prior to NMR experiments samples were thawed and 60µl of D_2O containing 0.5mmol/L TSP (Sodium –3-trimethyl-silyl propionate – 2,2,3,3,-H4) was added to 540 µl of native CSF sample for NMR experiments. TSP served as a chemical shift reference (δ = 0.0) and concentration standard. Typical parameters used for one dimensional (1D) proton NMR experiments were: pulse width 90°, number of data points 32 K, spectral width 5000 Hz, number of scans 48 and relaxation delay of 14 s. Two dimensional (2D) total correlation spectroscopy (TOCSY) of the sample were carried out using standard software package. One-way analysis of variance (ANOVA) was used to assess the differences between the groups. Values of $p < 0.05$ were considered significant.

RESULTS

Assignment of various metabolite resonances was carried out using 2D TOCSY. Concentrations of various CSF metabolites in controls, patients with MMC and post operated cases of MMC are shown in Fig. 1a and b. In MMC (pre-op) group, significantly higher concentrations were observed for Lac ($p<0.01$), Ace ($p<0.01$), Cho ($p=0.01$), Ala ($p<0.01$) and GPC ($p=0.01$) metabolites compared to controls. Following surgery (post-op MMC), the values of these metabolites were significantly reduced and were comparable to controls.

DISCUSSION

The present study demonstrates several significant differences in the CSF metabolite levels in the patients with MMC, prior to and post surgery using NMR. In pre-op MMC, the concentration of Lac, the end product of anaerobic metabolism and Ala were found in excess compared to controls indicating an increase in anaerobic metabolism at the local site of cord pathology. Braughler et al. also noted an increase in the levels of Lac and Ala in the CSF of rat spinal cord following trauma (2). Cho and GPC levels, possible indicators of cell membrane damage, were also found to be raised significantly in pre-op MMC group. The concentration of these metabolites were significantly reduced following surgery and were similar in post-op MMC and controls indicating considerable recovery of the neuronal metabolism as well as neuro-vascular physiology. Our data suggest that in vitro ^{1}H MRS measurements of metabolites in CSF is a promising tool in improving our understanding of the pathophysiology of neuronal dysfunction in patients of spina bifida, for providing additional diagnostic information and outcome of surgery.

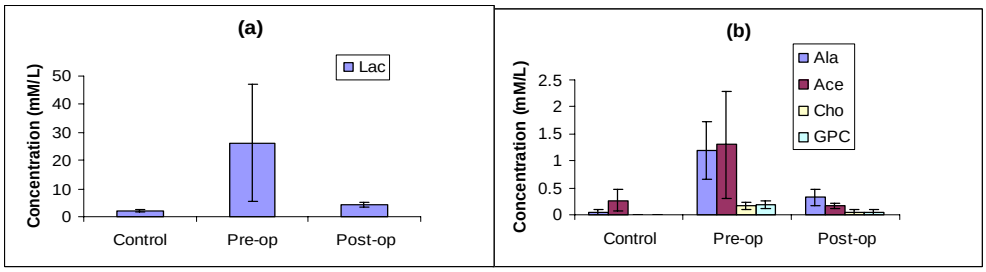

Fig.1 (a&b) Comparison of the concentration of metabolites in CSF of controls and patients with MMC and after surgery (post-op MMC).

REFERENCES

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