In-Vivo Proton Magnetic Resonance Spectroscopy (PMRS) changes in Extra-hepatic Portal Vein Obstruction (EHPVO) induced Hepatic Encephalopathy (HE) are Different from Chronic Liver Disease (CLD)

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Introduction-Hepatic encephalopathy (HE) has been reported in patients with extra-hepatic portal vein obstruction (EHPVO) in the absence of intrinsic liver disease (1). The main consequence of EHPVO is the development of collateral veins that results in portal-systemic shunting. The term type B hepatic encephalopathy has been proposed for this condition (2). Some of these patients with apparently normal neurological status may have abnormal cognitive functions on neuropsychological tests (NPT) and are described as having minimal hepatic encephalopathy (MHE) MHE may results from portal-systemic shunting in the absence of intrinsic liver disease (3). It is propose that HE is secondary to the direct effect of several toxins on the brain, the most important being ammonia. Due to the portal-systemic shunting, the detoxification of ammonia into urea occurs at the peripheral sites like brain and mussels, thus increases the concentration of glutamine in brain (4). Magnetic resonance imaging (MRI) studies, including MR spectroscopy, volumetric MRI, functional MRI, magnetization transfer, and diffusion-weighted imaging have improved our understanding of the pathophysiological alterations in cirrhotic patients with HE, however sparse information is available in EHPVO (5). The aim of this study was to compare the brain metabolites changes in children of EHPVO with controls, using *in-vivo* proton magnetic resonance spectroscopy (PMRS) and to look for any significant correlation between brain metabolites with neuropsychological tests (NPT).

Material and Methods-Fourteen children with EHPVO [13 males, median age = 9.3 years] were included in this study, for the purpose of comparison 12 healthy age and sex match controls were included in this study. All subject's parents gave their informed consent for imaging and battery of NPT. EHPVO was diagnosed by imaging techniques such as Doppler ultrasound, computed tomography or MRI and presence of upper gastrointestinal bleeding, reserved liver function tests with absence of jaundice (6). For measurement of ammonia, arterial blood was taken after overnight fasting, and measured by ammonia checker. Cognitive, motor, and visual functions in both the patients and controls were assessed by using Indian adaptation of Revisie Amsterdamse Kinder Intelligentie Test (RAKIT). It is adapted to the Indian child populations, includes closure, exclusion, memory span, verbal span, maze, learning names, quantity, discs and hidden figures (6). A Student's independent t-test was performed to evaluate the differences among metabolite ratios between patients and healthy controls. Bivariate analysis of correlation was performed to study the relationship between the metabolite ratios with NPT score.

Image Acquisition-Imaging was performed on a 1.5-Tesla MR scanner using standard quadrature head coil. *In-vivo* MR spectra were obtained by using a water suppressed localized single voxel spin echo (SE) sequence with TR / TE = 3000 ms / 35 ms. A voxel of $2 \times 2 \times 2$ cm^3 was located mainly in the right basal ganglion region of the brain in all the cases, containing a mixture of white and gray matter (putamen and caudate nucleus).

Data Processing-For evaluation and quantification of all individual spectra, the LC-Model software package (Version 6.0) was used for processing the PMRS data. N-acetylaspartate (NAA), choline (Cho), glutamate (Glu), glutamine/glutamate (Glx), and myoinositol (mI) ratios were calculated with respect to creatine (Cr).

Results-Conventional MRI, *in-vivo* PMRS, measurement of arterial ammonia and NPT were performed in these patients as well as controls. EHPVO patients exhibited significantly increased Glx/Cr ratio compared to controls. No statistical significant difference between patients and controls was observed in the case of NAA/Cr, Cho/Cr and mI/Cr ratios. The blood ammonia ranged in controls from 90-124 µmol/l, while in EHPVO it ranged from 113-266 µmol/l. Out of 14 patients, 3 (21%) showed impairment in neuropsychological tests. In this study we observed significant correlation between neuropsychological indices and Glx/Cr ratio. Performance on closure, mazes and discs show strong negative correlation with Glx/Cr ratio. Patients with increased ratio of Glx/Cr showed impaired NPT.

Discussion-In this study we observed *in-vivo* PMRS derived abnormal metabolite ratio, increased arterial blood ammonia with impairment in NPT in patients with EHPVO. These patients exhibited significantly increased Glx/Cr with no significant change in NAA/Cr, Cho/Cr and mI/Cr ratio. It has been reported that increased ratio of Glx/Cr with decreased ratio of mI/Cr and Cho/Cr is the hallmark of HE (7). At present sparse MR spectroscopic brain metabolite information are available in EHPVO. The significantly increased Glx/Cr ratio with no change in other metabolites ratio in patient in EHPVO is in contradiction MHE secondary to cirrhosis. These changes can be explained by portal-systemic shunting with no abnormality in liver parenchyma. It clearly indicates that mI and Cho changes may be primarily associated with liver function rather than to the presence of HE. Minguez et al reported significant increase in Glx/Cr and decrease in mI/Cr in adult patients with EHPVO (8). A few studies have reported liver dysfunction in adults with long standing EHPVO (9, 10). It appears that presence of adults in Minguez et al study may be responsible for the mI/Cr ratio discrepancy. It has been reported that cerebral edema, a characteristic features of HE, results from hyperammonemia induced swelling of astrocytes. Increased Glx/Cr ratio in these children along with increased arterial blood ammonia levels directly implicates ammonia as central to MHE in the present study. In this study, patients with normal liver functions show increased Glx/Cr ratio with abnormal NPT when compared to controls indicates that ammonia is responsible for changes in NPT in these children. In the case of EHPVO impairment of NPT like visual-motor coordination, spatial orientation is characteristics of MHE were observed. We observed strong negative correlation between few tests of NPT battery related to cognitive, visual impairment and memory function with Glx/Cr ratio further supports the view that ammonia is probably responsible for changes in neuropsychological test in children with EHPVO.

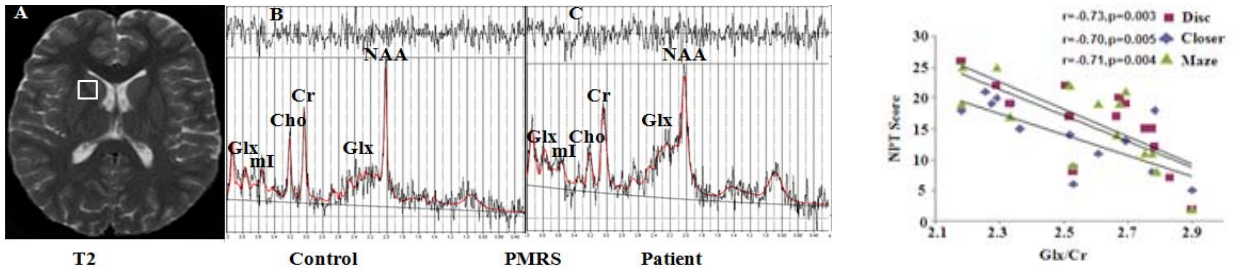

Fig.1:A. Axial T2-weighted image, B &C. LC-Model processed 1H-MRS image of control and patient. Fig.2: show correlation between NPT score and Glx/Cr ratio

Study	Metabolites (Mean±SD) NAA/Cr	Cho/Cr	Glx/Cr	mI/Cr
Controls(n=12)	1.1±0.20	0.21±0.03	2.1±0.4	0.51±0.18
Patients(n=14)	1.2±0.09	0.21±0.02	2.5±0.2	0.48±0.16
p value	0.145	0.771	0.002	0.716

Table1: Metabolite concentrations relative to those of Cr in parietal white and gray matter in patients with EHPVO compared with healthy controls.

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