Study of Serial DTT, Brain Morphometry, Clinical, Serum Cytokines, Cognition changes in Children with Acute Liver Failure

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Introduction: Acute liver failure is associated with significant morbidity and mortality. Raised intracranial tension due to cerebral edema and increased cerebral blood flow and subsequent herniation is a major cause of morbidity and mortality in these patients[1]. Both cytotoxic and vasogenic cerebral edema is seen in ALF patients with the former being more predominant[2]. A higher prevalence of the "systemic inflammatory response syndrome" (SIRS) in adult ALF patients has been reported[3]. SIRS is a response to the presence of proinflammatory cytokines, which have been shown to significantly raised in ALF patients[4,5]. A positive correlation between the two main mediators i.e. proinflammatory cytokines and blood ammonia/brain glutamine has also been shown in adults with ALF on MRS[6]. DTI studies have shown presence of cerebral edema with incomplete normalization signifying continued presence of cerebral edema despite apparent clinical recovery over a 3week period. This suggests that the recovery of cerebral edema lags behind the apparent clinical recovery in ALF patients[7]. This prospective serial study was done to look for reversibility of changes in MRI, MRS, serum proinflammatory cytokines, thiamine, and neurocognitive functions in these children over time.

Materials and methods: A total of 11ALF patients (5boys, mean age 7.8±2.7years) and 8healthy controls (6boys, mean age 8.8±2.5year) were evaluated. Of the 11ALF cases, 1died and 8/10 cases were re-evaluated after discharge from hospital (Ist follow up) and 6/8 cases were further followed up (IInd follow up). 10/11 had hepatic encephalopathy at admission (HE grade I-3, II-1, III-4, IV-2) and the mean jaundice to HE interval was 10.6±12.1days. Routine serology and Serum TNF-α & IL-6 were quantified using ELISA kits at different time points in patients and controls. Each subject completed a NPT battery designed especially for children to detect abnormalities in neuropsychological functions by using Revised Amsterdamse Kinder Intelligentie test[8].

***Data acquisition*:** All the patients underwent conventional MRI and DTI on a 3T MRI, using 8 channel head coil. The diffusion tensor encoding used was a vender supplied DTI scheme with 10 uniformly distributed directions. DTI was performed in the axial plane. The diffusion weighting b-factor was set to 1000 s/mm^2 field of view (FOV) =240×240 mm^2, slice thickness=3mm, interslice gap=0 and number of slices=46. Spectrum was obtained with and without water suppression. Localized single voxel point resolved spectroscopy (PRESS) with TE/TR=35ms/3000ms and number of averages=64. After global shimming, voxel shimming was performed, and a full width at half maximum of 4-6 Hz was achieved in all cases. The quantification of all individual 1H-MR spectra, the LCModel software package was used[9].

***Statistical analysis*:** For comparison between two groups we used Chi square test with Yates correction or Fisher's exact test for categorical variables and student "t" test (paired and unpaired) for quantitative variables.

Results: There was significant improvement in all parameters of liver functions at Ist follow up. At the IInd follow up, there was complete normalization of the liver functions. TNF-α and IL-6 were significantly higher in ALF at diagnosis and in Ist and IInd follow up as compared to controls (fig.1). Significant reduction in the TNF-α (40.1±8.9vs29.3±8.8pg/ml,p=0.01) and IL-6 (29.2±14.4 vs 17.1±5.3pg/ml;p=0.04) at Ist follow up as compared to at diagnosis. The patients had significantly lower thiamine levels at diagnosis in comparison to controls (55.2±6.7 vs 81.8±10.2nmol/L;p=0.01). It showed significant improvement with values similar to controls at Ist and IInd follow up (fig.2). Significantly higher Glutamine/glutamate [(Glx) 23.2±3.4 vs 15.3±2.7;p=<0.000] and lower choline [1.9±0.36 vs 2.6±0.6; p=0.005] than controls at diagnosis. There was no difference in N acetyl aspartate (NAA) and myoinositol (MI) values between controls and ALF cases and follow up. At Ist follow-up, i.e 48.4±29.2days, the Glx value [16.5±3.7;p=0.5] was similar but the choline [2.0±0.4;p=0.04] was still significantly lower than controls. Significantly decreased MD values in patient group were observed in 6 regions i.e posterior limb of internal calsule (PLIC), putamen (P), splenium (S), thalamus (T), occipital white matter (OWM), Spectroscopic voxel (SV) in ALF patients at diagnosis (fig.3). A significant reduction in the right (0.26±0.06mm^3;p<0.001) and left (0.22±0.05mm^3;p<0.001) MB volume in ALF patients at diagnosis as compared to controls. It showed significant increase in Ist follow-up (left 0.35± 0.03 mm^3 and right 0.37± 0.02 mm^3) but still was significantly lower than the controls. At first follow up, the ALF subjects performed poorly in 8 of the 9 administered tests as shown in fig. 2. In the IInd follow up, the scores showed improvement in all the administered tests as compared to that in Ist follow up. In 7 tests, to that of controls.

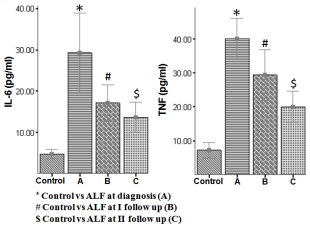

Figure 1. Bar showing the level of IL-6 and TNF-α in the serum of ALF patients at diagnosis (A), at I follow up (B), at II follow up (C) and in controls.[*, #, $] Showed significant level level <0.05.

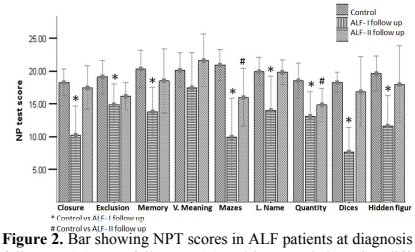

Figure 2. Bar showing NPT scores in ALF patients at diagnosis (A), at I follow up (B), at II follow up (C) and in controls by using different test batteries.[*, #, $] Showed significant level <0.05

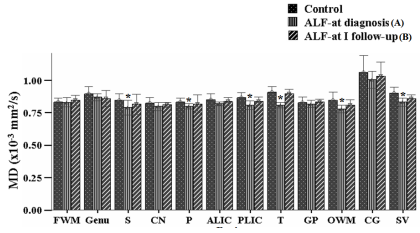

Figure 3. Bar diagram of MD values quantified from different white and gray matter regions of ALF patients at diagnosis (A), at I follow up (B), at II follow up (C) and controls. *Showed significant level level <0.05

Discussion: In this study of children with ALF, we have shown that there is cerebral edema (CE) with raised brain glutamine and blood proinflammatory cytokines (TNF-α and IL-6) at diagnosis. The MBs are significantly smaller in size without microstructural brain changes with low blood thiamine levels. The grossly deranged liver functions and low choline on MRS substantiate presence of liver dysfunction. In follow up, at 6weeks, despite absence of overt HE, there is impairment of cognitive functions as evidenced by abnormal NPT. The CE almost completely recovered with only 1/12 evaluated brain areas showing increased CS with normal glutamine. The TNF-α and IL-6 were markedly increased in ALF cases at diagnosis, consistent with the previous studies[3]. Inflammation is secondary to ammonia for the cerebral changes in ALF[5]. The much higher cytokines at diagnosis and persistent increase even after normalization of CE, supports the hypothesis that the diseased liver is the source of these cytokines and cerebral production may be contributing during the acute stage of CE. The persistence of elevated level of TNF-α and IL-6 during the study, despite normalization of liver functions are difficult to explain. It may be attributed to the process of liver regeneration as cytokines like IL-6, is important for replication of differentiated hepatocytes as well as hematopoietic cells in the liver. The reduced thiamine levels are due to ALF and consequent rapid loss of liver cell mass which lead to reduction in MBs volume. Biochemical evidence of thiamine deficiency in ALF has been observed in the past[10]. There was significant impairment on neuropsychologic testing at Ist followup despite absence of overt HE which resolve at IInd followup. This may be explained by two factors: elevated proinflammatory cytokines and MBs volume reduction. Cytokines can stimulate vagal afferents which affect brainstem, limbic and hypothalamic structures to produce illness behavior characterized by depressed mood and cognitive impairment[11]. MBs are known to play an important role in memory and cognitive functions[12] as they are the major relay nuclei with limbic and extralimbic connections. MBs were still showing loss of volume as compared to controls at Ist follow up which may have resulted in poor NP scores at Ist follow up. In addition thiamine supplementation may lead to a faster recovery of the subtle neurocognitive abnormality in ALF on followup. We conclude that children with ALF have CE and brain glutamine recovers first, followed by normalization of neuropsychological tests and liver functions. The choline and MBs take a longer time to normalize than the glutamine and CE. Raised cytokines and loss of MBs volume contribute to the neurocognitive abnormality seen in first follow-up. TNF-α and IL-6 show significant yet incomplete and gradual reduction with recovery.

References:1)Acharya et al. *Hepatology*1996;23:1448-55;**2**)Blei. *Neurochem Int*.2005;47:71-7;**3**)Rolando et al. *Hepatology*2000;32:734-9;**4**)Jalan et al. *Transplantation*2003;75:2034-9;**5**)Nagaki et al. *J infect Dis*2000;182:1103-8;**6**)Gupta et al. *Metab Brain Dis*2010;25:355-61;**7**)Rai et al. *J Magn Reson Imaging*.2008;28:334-41;**8**)Khire et al. *1st ed. India: Jnana Prabodhini's Samshodhan Sanstha*;1992;**9**)Saksena et al. *J Gastroenterol Hepatol*2008;23:e111-9; **10)**Labadarios et al. *Int J Vitam Nutr Res*1977;47:17-2;**11)** Dantzer et al. *Auton neursci*2000;85:60-5;**12)**vann et al. *Nat Rev neurosci*2004;1:35-4