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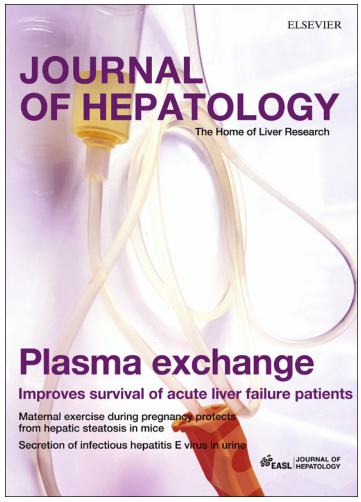

Minimal hepatic encephalopathy in children with chronic liver disease: prevalence, pathogenesis and magnetic resonance-based diagnosis

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Minimal hepatic encephalopathy in children with chronic liver disease: prevalence, pathogenesis and magnetic resonance-based diagnosis

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Key words: Minimal hepatic encephalopathy, ^{1}H Magnetic resonance spectroscopy, diffusion tensor imaging, hyperammonemia, neuropsychological tests, chronic liver disease, children

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List of Abbreviations: MHE, minimal hepatic encephalopathy; ^{1}HMRS, 1H magnetic resonance spectroscopy; DTI, diffusion tensor imaging; BA, blood ammonia; MRI, magnetic resonance imaging; CLD, chronic liver disease; NPT, neuropsychological tests; CPS, Child Pugh score; MELD, Model for End-Stage Liver Disease; PELD, pediatric end-stage liver disease; IL6 , interleukin 6; TNFα, tumor necrosis factor α; MD, mean diffusivity; RAKIT, revised Amsterdamse kinder Intelligentie test; NCT, number connection test; FCT, figure connection test; PC, picture completion; DS, digit symbol; PA, picture arrangement; OA, object assembly; BD, block design; CN, caudate nuclei; ALIC/PLIC, anterior /posterior limb internal capsule; P, putamen; GP, globus-pallidus; T, thalami; FWM/OWM, frontal and occipital white matter; G, genu ; CG, cingulate gyrus; S, splenium; FA, fractional anisotropy; T1SI, T1 signal intensity; ROIs, region-of-interest(s); Glx, glutamine/ glutamate; MI, myoinositol; Cho, choline; NAA, N-acetylaspartate; ROC, receiver operating curve; AUC, area under curve; AILD, autoimmune liver disease; OHE, overt hepatic encephalopathy, BCAA, branched chain amino acid; AAA, aromatic amino acid; DWI, diffusion weighted imaging

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Author contribution

Anshu Srivastava- obtained funding, study concept and design, study supervision, analysis and interpretation of data, drafting of the manuscript, final approval of the version to be published

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Saurabh Chaturvedi- acquisition of data, analysis and interpretation of data, drafting of the manuscript, final approval of the version to be published

RK Gupta - obtained funding, study concept and design, critical revision of the manuscript for important intellectual content, final approval of the version to be published

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Amrita Mathias- acquisition of data, technical support, critical revision of the manuscript for important intellectual content, final approval of the version to be published

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Sunil Jain - acquisition of data, study supervision, critical revision of the manuscript for important intellectual content, final approval of the version to be published

CM Pandey -statistical analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval of the version to be published

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Running title: MR based diagnosis of MHE in children

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Abstract

Background and aims: Data on minimal hepatic encephalopathy (MHE) in children is scanty. We evaluated children with chronic liver disease (CLD) to determine prevalence of MHE, its correlation with changes in brain metabolites by magnetic resonance spectroscopy (^{1}HMRS), diffusion tensor imaging (DTI) derived metrics, blood ammonia (BA) and inflammatory cytokines and the accuracy of MR based investigations for diagnosis.

Methods: 67(38 boys; age 13[7-18] years) CLD and 37 healthy children were evaluated with neuropsychological tests (NPT), BA, interleukin-6 [IL6], tumor necrosis factor alpha [TNFα], magnetic resonance imaging (MRI),^{1}HMRS and DTI.

Results: 34(50.7%) children with CLD had MHE on NPT. MHE subjects had higher BA (30.5[6-74] vs14[6-66]µmol/L; p=0.02), IL-6 (8.3[4.7-28.7] vs. 7.6 [4.7-20.7] pg/ml; p=0.4), TNFα (17.8[7.8-65.5] vs. 12.8[7.5-35] pg/ml; p=0.06) and higher glutamine (2.6[2.1-3.3] vs. 2.4[2.0-3.1]; p=0.02), lower choline (0.20[0.14-0.25] vs. 0.22[0.17-0.28]; p=0.1) and myoinositol (0.25[0.14-0.41] vs. 0.29 [0.21-0.66]; p=0.2) on ^{1}HMRS than No-MHE. Mean diffusivity (MD) on DTI was significantly higher in 6/11 brain areas in patients with MHE vs. No-MHE. Brain glutamine had a significant positive correlation with BA, IL-6, TNFα and MD of various brain regions. NPT showed a negative correlation with BA, IL6, TNFα, glutamine and MD. Frontal white matter MD had a sensitivity and specificity of 73.5% and 100 % for diagnosis of MHE.

Conclusions: 50% children with CLD have MHE. There is a significant positive correlation between markers of hyperammonemia, inflammation and brain edema and these correlate negatively with NPT scores. MD on DTI is a reliable tool for diagnosis of MHE.

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Lay summary

50% children with chronic liver disease perform poorly on neuropsychological testing and have minimal hepatic encephalopathy (MHE). These children have raised blood ammonia, inflammatory cytokines and mild cerebral edema on diffusion tensor imaging as compared to children without MHE. The higher the ammonia, inflammatory cytokines and cerebral edema the poorer is the performance on neuropsychological assessment. Estimation of mean diffusivity on diffusion tensor imaging is an objective and reliable method for diagnosis of MHE.

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Introduction

Minimal hepatic encephalopathy (MHE) represents the mildest form of hepatic encephalopathy (HE) seen in patients with liver dysfunction and /or portosystemic shunting [1]. MHE is characterized by subtle motor and cognitive deficits, affecting attention, speed of information processing, motor abilities and co-ordination [1, 2]. The prevalence of MHE in adults with chronic liver disease (CLD) ranges from 30-84% [2, 3]. This variation is attributed to difference in severity of liver disease, extent of porto-systemic shunting, and cut-offs for abnormal neuropsychological tests (NPT) for diagnosis of MHE [2]. The single study in 30 CLD children found MHE in 57% cases [4].

MHE patients have no recognizable clinical symptoms of HE and thus cannot be diagnosed on standard neurological examination. Neuropsychological testing using a battery of tests is the current gold standard for diagnosis in adults [2]. However, NPT have their own limitation of being affected by patient's age, education, anxiety, and interest in participation [5, 6]. These factors assume greater significance while testing children, especially the younger ages. Various advanced MRI (magnetic resonance imaging) methods (spectroscopy [^{1}HMRS], diffusion tensor imaging [DTI], magnetization transfer ratio and voxel based imaging), have been used for evaluation and characterization of MHE [7, 8, 9]. ^{1}HMRS and DTI have been shown to be safe, non-invasive and reliable for evaluating MHE in children with extrahepatic portovenous obstruction (EHPVO) [10].

The diagnosis of MHE assumes importance because of multiple reasons. It impairs the daily functioning and quality of life [11], increases risk of developing overt HE and reduces survival in adults with cirrhosis [12, 13]. In children, MHE leads to poor performance in educational and vocational areas [14]. And most important, it can be treated effectively and easily [2, 11]. Thus, there is a need to study MHE in children with CLD and to validate non-invasive objective tests which can assist in its diagnosis.

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The objectives of our study were a) to determine the prevalence of MHE in children with CLD, b) to evaluate the correlation of MHE with changes on brain metabolites on ^{1}HMRS, DTI derived metrics, blood ammonia (BA) and inflammatory cytokines and c) to determine the potential of ^{1}HMRS and DTI derived metrics for diagnosis of MHE as an objective tool compared to NPT.

Material and methods

Stable children with CLD diagnosed on the basis of liver biopsy, and/or clinical, imaging and endoscopic findings (≥ grade II esophageal varices) were prospectively enrolled from the in-patients ward. A questionnaire regarding demography and history was filled and physical examination including detailed neurological evaluation was done to exclude presence of any illness that could affect the NPT. The West Haven criteria were used to differentiate between grade 0 and 1HE [1, 15]. A complete hemogram, liver function tests, serum creatinine, electrolytes and abdominal ultrasound were done in all patients. Child-Turcotte-Pugh (CTP), PELD and MELD score were calculated to assess severity of liver disease. The etiology of CLD was determined as per standard diagnostic criteria and patients with no known etiology were labeled as cryptogenic [16].

Exclusion criteria–CLD children with overt HE or history of overt HE, gastrointestinal hemorrhage or antibiotic use during the past 6 weeks, porto-systemic shunt surgery, significant systemic co-morbid illness, any neurological/psychiatric problems, patients on psychoactive drugs , colour blindness or cataract or child not willing to cooperate with the tests were excluded. Subjects with Wilson's disease were also excluded.

Healthy Controls: 55 healthy age and gender matched children (36 boys, 14[6-18] years) were enrolled as controls and subjected to a detailed history and complete physical and neurological examination prior to enrollment to ascertain that the subject qualifies to be enrolled as per the exclusion/inclusion criteria.

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All CLD subjects and 37 healthy controls were evaluated with NPT, MRI, ^{1}HMRS, DTI, BA, and cytokine (interleukin-6[IL6], tumor necrosis factor α [TNF α]) estimation. In addition, 18 healthy controls underwent NPT alone. Sampling for BA and cytokines was done before the MRI.

Neuropsychological Assessment

NPT were done in all subjects using a specially designed test battery for children. Younger children (age ≤12y) were subjected to the Revised Amsterdamse Kinder Intelligentie test (RAKIT) and older ones (age >12y) were subjected to a battery of 8 tests including number connection tests (NCT 1 and 2), figure connection test (FCT) and picture completion (PC), digit-symbol (DS), picture arrangement(PA), object assembly(OA) and block design(BD) test as part of Wechsler Adult Intelligence Scale (WAIS-P, adopted for Indian population) [10, 17]. A NP test score value of < 2SD from normal was considered abnormal [18]. MHE was diagnosed in patients with ≥ 2 abnormal NP tests [17].

Blood ammonia and cytokine estimation

BA was measured by the Micro diffusion method by the Pocket chem. BA, Japan Kit Ammonia test kit II^{TM} (Arkray factory Inc, Japan) in the sample collected after overnight fasting [19]. The continuous measurement range of BA by this is 7-286 micromol/L; normal BA being <35 micromol/L. Cytokines (IL-6 and TNF-α) were quantitatively measured by enzyme-linked immunosorbent assay (ELISA) (BD opt EIA, San Diego, CA, USA) in duplicate. ELISA was performed as per manufacturer's guideline and value of TNF- **α** and IL-6 in samples were determined by a standard curve.

Magnetic Resonance Imaging (MRI)

All children were subjected to MRI under sedation after psychometric assessment and proper pre-procedure counseling. Two experienced radiologists blinded to the results of the NPT evaluated the MRI. Imaging was done on a 3.0-Tesla Signa MR system (General Electric

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Healthcare Technologies, Milwaukee, WI) using a 12 channel head coil. The details are provided elsewhere [20].

Diffusion Tensor Imaging (DTI)

DTI data was acquired using a single-shot echo-planar dual SE sequence with ramp sampling [21]. The DTI acquisition, processing, and data analysis was done as described earlier [7, 10]. Regions of interest(s) were placed on bilateral caudate nuclei (CN), bilateral internal capsules (posterior and anterior limb, ALIC/PLIC), putamen (P), globus-pallidus (GP), thalami (T), frontal and occipital white matter (FWM, OWM), genu (G), cingulate gyrus (CG), and splenium (S) in patients and healthy controls to quantify fractional anisotropy (FA) and mean diffusivity (MD) (Fig. 1).

Magnetic resonance spectroscopy (*^{1}HMRS*)

The process of ^{1}HMRS acquisition and quantification of brain metabolites (Glx, glutamine/ glutamate; MI, myoinositol; Cho, choline; NAA, N-acetylaspartate) was done as described elsewhere [10, 22]. The total time taken for the MR imaging was ~20 -25 minutes.

T1Signal Intensity (T1SI)- Elliptical region-of-interest(s)(ROIs) of 6×6 pixels on the right and left globus-pallidus (GP) on T1-weighted images at the level of 3rd ventricle to quantify SI using Image J, a JAVA based software [10].

*Ethics approval***:** The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Parents/legal guardians who decided to enroll their children (both CLD patients as well as controls) were explained the study details, procedure of MRI and all potential risks and benefits. Written informed consent from the parent was taken for all participants.

*Statistical analysis***:** Results are reported as median (range) and proportions. Mann Whitney U test was used to compare the continuous and Chi square test for discrete variables between groups. Pearson correlation coefficient was used to look for correlation between NPT and

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DTI derived metrics, metabolite ratios, BA, and cytokines. Receiver operating curves (ROCs) were drawn to calculate the area under curve. The cut-off value was selected depending on (Sp + Se) max. All analysis was done using Statistical Package for Social Sciences (SPSS version 17.0, SPSS Inc, Chicago, USA). A p value <0.05 was considered significant.

Results:

Of the 247 CLD children evaluated, 67(13[7-18] years, 38 boys) were enrolled (fig 2). Autoimmune liver disease (AILD, 28; 41.8%) was the most common etiology followed by Budd Chiari Syndrome (18; 26.9%), biliary cirrhosis (9; 13.4%), cryptogenic (9; 13.4%) and hepatitis B (3; 4.5%).

NPT and prevalence of MHE: CLD patients performed worse as compared to healthy controls on NPT (Fig 3a and 3b). For the NPT in RAKIT and the tests in modified WAIS-P, a higher score suggests better performance. But for the NCT and FCT a lower score indicates better performance. Based on the NPT cut-off values, 34 CLD children (50.7%) had MHE.

Comparison of CLD patients with controls

The blood ammonia (21[6-74] vs. 11[6-38] µmol/L; p=0.001), IL6 (8.1[4.7-28.7] vs. 4.7[4.7-10.3] pg/ml; p<0.001) and TNF-α (16[7.5-65.5] vs. 7.7[7.7-21.8] pg/ml; p=0.002) were higher in CLD subjects than controls. At ^{1}HMRS, CLD subjects had higher Glx (2.41[2.02-3.29] vs. 2.07[1.48-2.6]; p<0.001), lower choline (0.21[0.14-0.28]vs. 0.24[0.20-0.39]; p<0.001) and MI (0.28[0.14-0.66] vs. 0.48[0.26-0.62]; p<0.001) and similar NAA (1.23[1.03-1.99] vs. 1.3[1.01-1.56]; p=0.1) than controls. CLD patients had higher T1S1 in the globus pallidus (1057.0[696-3843.8] vs. 825.2 [662.1- 1578.9]; p<0.001) and higher MD on DTI in all brain regions evaluated than healthy controls.

Comparison of CLD patients with MHE and No-MHE

There was no difference in the age (13[7-18] vs. 13[7-18] years; p=0.9), gender (23 /34 boys vs. 15/33 boys, p= 0.08) and etiology of CLD between MHE and No-MHE groups. More

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patients in Child class B/C had MHE in comparison to class A (55% [22/41] vs. 45% [11/25]; p= 0.6) but it was not significant. The PELD score (n=22, 11[5-32] vs. 9.5[1-21]; p=1) and MELD score (n=45, 14.5[7-27] vs. 14[7-34]; p=0.8) was not significantly different between MHE and No-MHE patients. The weight Z score (-0.99[-2.3 to +2.7] vs. -1.21[-2.5 to +0.4]; p=0.6) and height Z score (-1.6[-4.0 to +1.2] vs. -1.7[-4.1 to +1.5]; p=0.9) was similar in patients with MHE vs. no-MHE and so was the serum albumin (3.2[1.5-4.8] vs. 3.4 [1.9-4.6] g/dL; p=0.6).

MHE prevalence was similar in AILD and non-AILD cases (11/28 vs. 23/39; p=0.1). However, in AILD patients, MHE was more common (8/16 vs. 3/12; p=0.2) in those evaluated at diagnosis (n=16) than in follow-up on therapy (n=12, treatment duration of 22[1-72] months)**.**

Of the 67 cases, 58 (86.6%) were in follow-up for 25 [1-54] months, 9 (13.4%) being lost to follow-up. Overt HE (OHE) developed in 7/58(12%) cases; 3 of them died and 4 recovered. The number of cases with OHE is small (MHE-[3/29] vs. No-MHE [4/29]) with no significant difference between the groups. However, OHE developed earlier (at 1, 3 and 6 months) in MHE as compared to No-MHE group (6, 14, 19 and 30 months). Patients (n-7) who developed OHE, had a significantly higher Childs (OHE [n-7, 10[8-12] vs. no-OHE [n-51, 7[5-12]; p=0.005) and MELD score (OHE [n-6, 20[16-27] vs. no-OHE [n-33, 13[7-32]; p=0.02) in comparison to those who did not (n=51). Only 1 child with OHE was <12years, the PELD score was higher (21 vs. 11; p=0.4). OHE was less common in patients who received specific etiology based therapy than those who did not (3/19 vs. 4/39; p=0.6).

MHE subjects had higher BA (30.5[6-74] vs.14[6-66]µmol/L; p=0.02) and inflammatory cytokines { IL-6 (8.3[4.7-28.7] vs. 7.6 [4.7-20.7] pg/ml; p=0.4), TNFα (17.8[7.8-65.5] vs. 12.8[7.5-35]pg/ml; p=0.06)} than those with No-MHE . ^{1}HMRS showed higher glutamine (2.6[2.1-3.3] vs. 2.4[2.0-3.1]; p=0.02), lower choline (0.20[0.14-0.25] vs.0.22[0.17-0.28];

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p=0.1) and myoinositol (0.25[0.14-0.41] vs. 0.29 [0.21-0.66]; p=0.2) on ^{1}HMRS than No-MHE (figure 4). T1 signal intensity was similar in MHE and No-MHE (1060.9[800.3-1701.5] vs.1049.0 [696.0-3843.6]; p=0.9) patients.

DTI showed significantly higher MD in MHE patients in comparison to No-MHE in 6/11 brain areas i.e. frontal white matter, caudate nucleus, anterior and posterior limb of internal capsule, globus pallidus and occipital white matter as shown in Table 1. Fractional anistotropy values were similar in the two groups.

Correlation between inflammatory cytokines, blood ammonia and metabolites at ^{1}HMRS.

Brain glutamine had a significant positive correlation with ammonia (r= 0.36, p=0.01), IL-6 (r=0.40, p=0.01) and TNF α (r=0.35, p=0.01). There was a negative correlation between brain choline and inflammatory cytokines (IL-6 [r= - 0.29, p=0.005] and TNF α [r= -0.32, p=0.001]) but not significant with BA (r= -0.18, p =0.06). MI also showed a negative correlation with IL6 (r= -0.23, p=0.03), TNF α [r= -0.23, p=0.03) and BA (r= -0.19, p=0.05).

Correlation between NPT and BA, inflammatory cytokines and metabolites at ^{1}HMRS

A negative correlation was seen between the NPT scores and ammonia, brain glutamine, IL6 and TNFα as shown in Table 2. This suggests that CLD patients with higher blood ammonia, brain glutamine and inflammatory cytokines perform poorer on NPT. MI and choline showed a positive correlation with the NPT, lower the values poorer the performance.

Correlation between Neuropsychological tests (NPT) and mean diffusivity(MD)

A negative correlation was seen between MD of different brain regions and the NPT scores (Table 3). This suggests that patients who performed poorly on NPT and had MHE had higher MD signifying presence of brain edema. Frontal white matter, caudate nucleus, anterior and posterior limb of internal capsule, globus pallidus, putamen and occipital white matter showed correlation with most of the NPT in younger and older children.

Correlation between mean diffusivity (MD) and brain metabolites at ^{1}HMRS

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The MD value of FWM,OWM, ALIC, PLIC, genu, CG, CN, GP and thalamus showed a significant positive correlation with brain glutamine and negative correlation with myoinositol and choline (significant in all regions except PLIC).

Discrimination between MHE and No-MHE

Variables which were significantly different between MHE and No-MHE patients included Glx on ^{1}HMRS, mean diffusivity of frontal white matter, occipital white matter, anterior and posterior limb of internal capsule, caudate nucleus & globus pallidus and blood ammonia. Inflammatory cytokines (TNFα and IL6), choline and MI were different but not significant. On drawing ROC curves as shown in figure 5, the MD value of various brain regions (AUC of 0.73 to 0.89) performed better than metabolites on ^{1}HMRS, BA and cytokines. The FWM-MD was the best discriminator (AUC 0.89); a value of 0.805 had a sensitivity of 73.5% and specificity of 100 % for diagnosis of MHE. The best cut-off for MD in the other regions and their sensitivity and specificity is as follows- ALIC (0.775, 76.5%, 100%), OWM (0.795, 76.5%, 82%), PLIC (0.765, 76.5%, 91%), CN (0.795, 79.4%, 79%), GP (0.79, 59%, 76%). In comparison, Glx (AUC 0.67; a value of 2.36 had 76.5% sensitivity and 57.6% specificity) performed poorly.

Discussion

In this study of 67 CLD children, 34 (50.7%) had MHE on NPT. MHE patients had significantly higher BA, brain Glx and MD in various brain regions than patients with No-MHE. The inflammatory cytokines (TNFα and IL-6) were higher while choline and myoinositol were lower on ^{1}HMRS in MHE than No-MHE, but the difference was not significant. T1SI in globus pallidus and fractional anisotropy (FA) values of various brain regions on DTI were similar in MHE and No-MHE groups. There was a positive correlation between BA and brain Glx and both of these showed a positive correlation with the inflammatory cytokines (IL6 and TNFα). The NPT scores showed a negative correlation with

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inflammatory cytokines, markers of hyperammonemia (BA, brain Glx) and MD of various brain regions. Brain choline and myoinositol showed a negative correlation with inflammatory cytokines. MD of various brain regions had a sensitivity of 74-79% and specificity of 79-100% for diagnosis of MHE.

The prevalence of MHE in adults with cirrhosis varies between 30-84% [2]. The single pediatric study found MHE in 57% (17/30) CLD children [4] which is similar to our figure of 50% in 67 children. This is higher than the 32% MHE prevalence in EHPVO [10]. In adults, MHE patients have higher Child's/MELD score than those with No-MHE [23, 24, 25]. However, in our study, the MELD/PELD scores were not significantly different between MHE and No-MHE. This could be due to smaller number of cases and has been found in few adult studies [8, 26].

^{1}HMRS showed higher Glx and lower MI and choline in children with MHE than No-MHE. This is the classical signature pattern of CLD [4, 26, 27]. The increased BA due to liver dysfunction and portovenous shunting in cirrhosis is detoxified by the astrocytes and converted into glutamine. Myoinositol, the osmolyte in astrocytes is reduced as a compensatory effort at regulation of astrocyte osmolality [26]. MI has been shown to have a significant correlation with the plasma BCAA/AAA (branched chain amino acids/aromatic amino acids) ratio in children with MHE [27]. The ratio of BCAA/AAA reduces in CLD patients [28] and is causally linked to HE by increasing the brain uptake of AAA and altering neurotransmission [29]. The reduced choline reflects liver dysfunction and differentiates MHE in cirrhosis from that due to isolated portosystemic shunting in EHPVO (normal choline) [10, 30].

Diffusion weighted imaging helps in evaluation of intra and extra-cellular brain water content, as mean diffusivity (MD) is an index of water movement across cell membrane and fractional anisotropy (FA) reflects white matter microstructural integrity [31]. We found an

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increase in MD in MHE patients in comparison to No-MHE in 6/11 brain regions with no changes in FA which is similar to the observation of Kale et al [7] and suggests presence of interstitial edema. Other workers have also shown raised MD in MHE [4, 8]. The number of brain areas with higher MD and the actual MD values correlate with increasing severity of HE [7].

Both BA and the inflammatory cytokines (IL6 and TNFα) were increased in patients with MHE vs. No-MHE vs. controls. Higher levels of endotoxin, TNFα, IL6 and IL18 have been shown in adult cirrhotics with MHE [26, 32] and no data is available in children. Our observation supports the hypothesis that hyperammonemia and inflammation act together in the pathogenesis of MHE [33]. Raised proinflammatory cytokines are attributed to liver dysfunction or endotoxemia [33]. Improvement of NPT after lactulose therapy concomitant with reduction in BA, brain Glx, TNFα, IL6 and endotoxin further supports this hypothesis [26].

We found a positive correlation among inflammatory markers (IL6, TNFα), hyperammonemia (BA and brain Glx) and MD of several brain areas suggesting their contribution to brain edema in MHE. The negative correlation between NPT and MD suggests that the brain edema is causally related to the deranged neurocognition in MHE. This is further supported by the improvement in NPT along with reduction of cerebral edema (reduced MD) after treatment of MHE with Lactulose in adults [7, 34].

The signal intensity in GP was higher in CLD than controls but not different between MHE and No-MHE and showed no correlation with BA, Glx, or cytokines in our study. This increased signal intensity is attributed to manganese deposition [35]. Lack of correlation between the TIS1 and ^{1}HMRS ratio or NPT scores has also been reported by Singhal et al [36]. This suggests that the increased T1S1, is a marker of liver disease/ portosystemic shunting [7, 10], but it does not reflect the severity of HE.

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Amongst the various parameters deranged in MHE, the MD values especially of the FWM, had the best diagnostic capability (fig 4) with an AUC of 0.84-0.89. It performed better than markers of hyperammonemia (BA and Glx) and inflammation (IL6, TNFα). This is explained by the fact that the MD values reflect changes in brain water content, secondary to both hyperammonemia and inflammation.

Although MHE has a global effect on the brain but the susceptibility to the effects of hyperammonemia and severity of changes may vary from one brain region to another [37]. In our study the FWM-MD showed the best discrimination between MHE and No-MHE. Sugimoto et al also showed that the FWM-MD had a sensitivity and specificity of 90% to diagnose MHE in adults [8]. Frontal lobe ^{1}HMRS metabolites mICh ratio had the best diagnostic accuracy followed by the occipital lobe ratio [36]. The frontal lobe is closely related to working memory and cognition, the functions most affected in MHE [38].

We found that CLD patients with No-MHE also have changes in brain metabolites on ^{1}HMRS and increased MD which is significantly different from controls but of a lesser degree than MHE patients. This suggests that MR based methods are more sensitive than NPT and can detect changes at an earlier stage. These observations are in line with the literature [4, 7, 39].

The MR based evaluation scores over NPT in terms of sensitivity, objectivity, ease of repetition, lack of "learning effect" and not requiring patient cooperation. In addition, very young children cannot be evaluated by NPT. The median age of our CLD children was 13 (7-18) years and the other paediatric studies on MHE have also evaluated children >6years with NPT[4, 14, 27]. However, the cost and accessibility is a limitation of MR based investigations.

The diagnosis of MHE assumes importance as it predicts occurrence of overt HE and is a marker of poor survival in adults [12, 13]. Both severity of liver disease (CPS/MELD) and

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the PHES score predict poor survival in adults with CLD [13, 18, 24]. Further, Patedar et al showed that MHE predicts poorer survival and higher overt HE even after controlling for MELD score [25], thereby, suggesting that MHE is a valuable marker of prognosis. In our study, only the CPS/MELD predicted overt HE in children. The inability of MHE to predict overt HE could either be due to the smaller number of cases or because children received specific etiology based treatment which can modify the disease course. This is supported by the lower rate of overt HE in patients with treatable etiology of CLD than those without treatable etiology and lower MHE rates in AILD patients on therapy than at diagnosis. Larger studies are required to validate it in children.

Presence of permanent brain changes (reduced brain tissue density) which persist after liver transplantation in adult cirrhotics with history of overt HE also argue for the utility of diagnosis of MHE and offering "neuroprotection" to these patients [40].

In order to establish the utility of diagnosing MHE in children, we need to provide objective evidence that MHE affects the school performance and that prompt therapy can revert this. This aspect was not addressed and may be considered a limitation of this study.

We also need to evaluate the response to therapy in our CLD patients with MHE. There are no studies on treatment of MHE in children. Although literature shows improvement in cognitive tests and quality of life with treatment of MHE in adults [11, 41], but still there is no agreement about optimal strategy and duration of therapy [42].

In conclusion, we have shown that 50% CLD children have MHE. Both hyperammonemia and inflammation lead to interstitial cerebral edema as evidence by increased MD and are a cause of the poor performance of these children on NPT. There is a significant positive correlation between markers of hyperammonemia markers, inflammation and brain edema and these are negatively correlated with the NPT scores. FWM-MD is the best predictor of MHE.

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References

[1] Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy-definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002;35:716–21.

[2] Dhiman RK, Saraswat VA, Sharma BK, Sarin SK, Chawla YK, Butterworth R, et al. Minimal Hepatic encephalopathy: consensus statement of a working party of the Indian National Association for study of the liver. J Gastroenterol Hepatol 2010:25;1029-41.

[3] Agrawal S, Umapathy S, Dhiman RK. Minimal hepatic encephalopathy impairs quality of life. J Clin Exp Hepatol. 2015;5(Suppl 1):S42-8.

[4] Razek AAKA, Abdalla A, Ezzat A, Megahed A, Barakat T. Minimal hepatic encephalopathy in children with liver cirrhosis: diffusion-weighted MR imaging and proton MR spectroscopyof the brain. Neuroradiology 2014;56:885–891.

[5] Randolph C, Hilsabeck R, Kato A, Kharbanda P, Li YY, Mapelli D, et al. Neuropsychological assessment of hepatic encephalopathy: ISHEN practice guidelines. Liver Int 2009;29:629–635.

[6] Stewart CA, Smith GE. Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol 2007;4:677–85.

[7] Kale RA, Gupta RK, Saraswat VA, Hasan KM, Trivedi R, Mishra AM, et al. Demonstration of Interstitial Cerebral Edema With Diffusion Tensor MR Imaging in Type C Hepatic Encephalopathy. Hepatology 2006;43:698-706.

[8] Sugimoto R, Iwasa M, Maeda M, Urawa N, Tanaka H, Fujita N, et al. Value of the apparent diffusion coefficient for quantification of low-grade hepatic encephalopathy. Am J Gastroenterol 2008;103:1413–1420.

ACCEPTED MANUSCRIPT

[9] Kumar R, Gupta R, Elderkin-Thompson V, Huda A, Sayre J, Kirsch C, et al. Voxel-based diffusion tensor magnetic resonance imaging evaluation of low-grade hepatic encephalopathy. J Magn Reson Imaging 2008;27:1061–1068.

[10] Yadav SK, Srivastava A, Srivastava A, Thomas MA, Agarwal J, Pandey CM, et al. Encephalopathy assessment in children with extra-hepatic portal vein obstruction with MR, psychometry and critical flicker frequency. J Hepatol 2010;216:683-91.

[11] Prasad S, Dhiman RK, Duseja A, Chawla YK, Sharma A, Agarwal R. Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy. Hepatology. 2007;45:549-59.

[12] Romero-Gomez M, Boza F, Garcia-Valdecasas MS, García E, Aguilar-Reina J. Subclinical hepatic encephalopathy predicts the development of overt hepatic encephalopathy. Am J Gastroenterol 2001;96:2718–23.

[13] Ampuero J, Simón M, Montoliú C, Jover R, Serra MÁ, Córdoba J, et al. Minimal hepatic encephalopathy and critical flicker frequency are associated with survival of patients with cirrhosis. Gastroenterology 2015;149:1483-9.

[14] Mack CL, Zelko FA, Lokar J, Superina R, Alonso EM, Blei AT, et al. Surgically restoring portal blood flow to the liver in children with primary extrahepatic portal vein thrombosis improves fluid neurocognitive ability. Pediatrics. 2006;117:e405-12.dx.doi.org/10.1542/peds.2005-1177

[15] Poordad FF. Review article: the burden of hepatic encephalopathy. Alimentary Pharmacol Ther 2007;25 (supp1):3-9.

[16] Jagadisan B, Srivastava A, Yachha SK, Poddar U. Acute on chronic liver disease in children from the developing world: recognition and prognosis. J Pediatr Gastroenterol Nutr. 2012 ;54:77-82.

ACCEPTED MANUSCRIPT

[17] Das A, Dhiman RK, Saraswat VA, Naik SR. Prevalence and natural history of subclinical hepatic encephalopathy in cirrhosis. J Gastroenterol Hepatol 2001;16:531–5.

[18] Amodio P, Del Piccolo F, Marchetti P, Angeli P, Lemmolo R, Caregaro L et al. Clinical features and survival of cirrhotic patients with subclinical cognitive alterations detected by the number connection test and computerized psychometric tests. Hepatol 1999;29;1662-67.

[19] Murawaki Y, Tanimoto K, Hirayama C, Ikuta Y, Watabe N. A simple and rapid microdiffusion method for blood ammonia using a reflectance meter and a reagent plate, and its clinical evaluation for liver diseases. Clin Chim Acta. 1984;144:195-202.

[20] Gupta RK, Yadav SK, Saraswat VA, Rangan M, Srivastava A, Yadav A, et al. Thiamine deficiency related microstructural brain changes in acute and acute-on-chronic liver failure of non-alcoholic etiology. Clin Nutr 2012;31:422-8.

[21] Le Bihan D. editor. Diffusion and perfusion Magnetic Resonance Imaging: applications to functional MRI. New York. Raven Press Ltd; 1995.

[22] Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LC Model. NMR Biomed 2001;14:260-264.

[23] Hartmann IJ, Groeneweg M, Quero JC, Beijeman SJ, de Man RA, Hop WC, et al. The prognostic significance of subclinical hepatic encephalopathy. Am J Gastroenterol. 2000;95:2029-34.

[24] Dhiman RK, Kurmi R, Thumburu KK, Venkataramarao SH, Agarwal R, Duseja A, et al. Diagnosis and Prognostic Significance of Minimal Hepatic Encephalopathy in Patients with Cirrhosis of Liver. Dig Dis Sci 2010;55:2381–2390.

ACCEPTED MANUSCRIPT

[25] Patidar KR, Thacker LR, Wade JB, Sterling RK, Sanyal AJ, Siddiqui MS, et al. Covert hepatic encephalopathy is independently associated with poor survival and increased risk of hospitalization. Am J Gastroenterol 2014;109:1757–1763.

[26] Jain L, Sharma BC, Srivastava S, Puri SK, Sharma P, Sarin S. Serum endotoxin, inflammatory mediators, and magnetic resonance spectroscopy before and after treatment in patients with minimal hepatic encephalopathy. J Gastroenterol Hepatol 2013;28:1187–1193.

[27] Foerster B.R, Conklin LS, Petrou M, Barker PB, Schwarz KB. Minimal Hepatic Encephalopathy in Children: Evaluation with Proton MR Spectroscopy AJNR Am J Neuroradiol 2009;30:1610–13.

[28] Campollo O, Sprengers D, McIntyre N. The BCAA/AAA ratio of plasma amino acids in three different groups of cirrhotics. Rev Invest Clin. 1992;44:513-8.

[29] Dejong CH, van de Poll MC, Soeters PB, Jalan R, Olde Damink SW. Aromatic amino acid metabolism during liver failure. J Nutr. 2007;137(6 Suppl 1):1579S-1585S.

[30] Mínguez B, García-Pagán JC, Bosch J, Turnes J, Alonso J, Rovira A, et al. Noncirrhotic portal vein thrombosis exhibits neuropsychological and MR changes consistent with minimal hepatic encephalopathy. Hepatology 2006 ;43:707-14.

[31] Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed. 1995;8:333-44.

[32] Montoliu C, Piedrafita B, Serra MA, del Olmo JA, Urios A, Rodrigo JM, et al. IL-6 and IL-18 in blood may discriminate cirrhotic patients with and without minimal hepatic encephalopathy. J Clin Gastroenterol. 2009;43:272-9.

[33] Shawcross DL, Wright G, Olde Damink SW, Jalan R. Role of ammonia and inflammation in minimal hepatic encephalopathy. Metab Brain Dis. 2007;22:125-38.

ACCEPTED MANUSCRIPT

[34] Rai R, Ahuja CK, Agrawal S, Kalra N, Duseja A, Khandelwal N, et al. Reversal of Low-Grade Cerebral Edema After Lactulose/Rifaximin Therapy in Patients with Cirrhosis and Minimal Hepatic Encephalopathy. Clin Transl Gastroenterol. 2015 Sep 17; 6:e111 doi: 10.1038/ctg.2015.38.

[35] Binesh N, Huda A, Bugbee M, Gupta RK, Rasgon N, Kumar A, et al. Adding another spectral dimension to 1H MR Spectroscopy of hepatic encephalopathy. J Magn Reson Imaging 2005;21:398-405.

[36] Singhal A, Nagarajan R, Hinkin C, Kumar R, Sayre J, Elderkin-Thompson V, et al. Two-dimensional MR spectroscopy of minimal hepatic encephalopathy and neuropsychological correlates in vivo. J Magn Reson Imaging 2010;32:35–43.

[37] Taylor-Robinson SD, Sargentoni J, Marcus CD, Morgan MY, Bryant DJ. Regional variations in cerebral proton spectroscopy in patients with chronic hepatic encephalopathy. Metab Brain Dis 1994;9:347–59.

[38] Funahashi S. Prefrontal cortex and working memory processes. Neuroscience 2006;139:251–261.

[39] Laubenberger J, Haussinger D, Bayer S, Gufler H, Hening J, Langer M. Proton magnetic resonance spectroscopic studies of the brain in symptomatic and asymptomatic patients with liver cirrhosis. Gastroenterology 1997;112:1610-1616.

[40] **Guevara M, Baccaro ME**, Gómez-Ansón B, Frisoni G, Testa C, Torre A, et al. Cerebral magnetic resonance imaging reveals marked abnormalities of brain tissue density in patients with cirrhosis without overt hepatic encephalopathy. J Hepatology 2011;55:564–573.

[41] Sidhu SS, Goyal O, Mishra BP, Sood A, Chhina RS, Soni RK. Rifaximin improves psychometric performance and health-related quality of life in patients with minimal hepatic encephalopathy (The RIME Trial). Am J Gastroenterol 2011;106:307–316.

ACCEPTED MANUSCRIPT

[42] Henderson P K., Herrera J L. Should We Treat Minimal/ Covert Hepatic Encephalopathy, and with What? Clin Liver Dis 2015;19:487–495.

"Authors names in bold designate shared co-first authorship"

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Legends to figures

Figure 1 Details of magnetic resonance imaging. (A) Axial gray scale fractional anisotropy (FA) map showing region of interest placement for DTI measures (1 ACG, 2 FWM, 3 Genu, 4 CN, 5 ALIC, 6 GP, 7 Putamen, 8 PLIC, 9 Splenium, 10 OWM). **(B)** and **(C)** proton spectra from a control and a patient with minimal hepatic encephalopathy obtained from a voxel of 2x2x2 cm^3 placed on left basal ganglion which included grey and white matter (not shown in figure)

Figure 2 Flowchart showing overview of patient enrolment.

Figure 3 Bar plots depicting the neuropsychological test scores in controls and CLD patients with MHE and No-MHE. (A) In children ≤ 12years **(B)** In children >12 years

Figure 4 Box plots showing metabolite values on ^{1}HMR spectroscopy in controls and CLD patients with MHE and No-MHE. (A) Glutamine, **(B)** Choline, **(C)** Myoinositol

Figure 5 Receiver operator characteristic (ROC) curves for variables differentiating between CLD patients with MHE and No-MHE. (A) Mean diffusivity (MD) of various brain areas, **(B)** Metabolites on ^{1}HMRS, **(C)** Ammonia, IL6 and TNFα, **(D)** Table showing value of area under curve (AUC) with 95% CI and p value

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Table 1: Mean diffusivity of various areas in controls and CLD patients with MHE and No-MHE

Brain area	Controls (n=37)	No-MHE (n=33)	MHE (n=34)	p value MHE vs. No-MHE
Genu	0.73[0.59-0.90]*^	0.87[0.80-0.95]	0.87[0.81-0.93]	0.8
Frontal white matter	0.74[0.65-0.87] *	0.75[0.70-0.79]	0.88[0.70-0.92]	<0.001
Cingulate gyrus	0.86[0.68-1.0] *^	1.0[0.9-1.2]	1[0.9-1.15]	0.5
Caudate nucleus	0.73[0.64-0.85]*^	0.78[0.65-0.81]	0.82[0.70-0.89]	<0.001
Anterior limb internal capsule	0.71[0.53-0.79] *	0.72[0.70-0.77]	0.81[0.71-0.90]	<0.001
Posterior limb internal capsule	0.71[0.64-0.80] *	0.72[0.70-0.78]	0.78[0.66-0.86]	<0.001
Globus pallidus	0.71[0.56-0.77]*^	0.78[0.62-0.81]	0.80[0.71-0.88]	0.009
Thalamus	0.74[0.68-0.84]*#	0.79[0.72-0.87]	0.79[0.72-0.82]	0.8
Splenium	0.73[0.56-0.89]*^	0.88[0.80-0.96]	0.85[0.79-0.93]	0.1
Occipital white matter	0.70[0.60-0.81]*^	0.75[0.63-0.81]	0.83[0.64-0.88]	<0.001

All values as median with range, * p value <0.001 between MHE and controls, ^ p value of <0.001 between No-MHE and control, # p value of <0.03 between No-MHE and control.

MHE: minimal hepatic encephalopathy.

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Table 2: Correlation of the NPT with ammonia, cytokines and brain glutamine and choline at ^{1}HMR Spectroscopy

NPT	Ammonia	IL 6	TNFα	Choline	MI	Glx
Closure	-0.189	-0.121	-0.362**	0.223	0.27*	-0.019
Exclusion	-0.366**	-0.137	-0.222	0.246	0.27*	-0.182
Memory span	-0.412**	-0.247	-0.348**	0.363**	0.31*	-0.225
Verbal meaning	-0.179	-0.073	-0.147	0.041	0.24	-0.066
Mazes	-0.371**	-0.341**	-0.442**	0.430**	0.41**	-0.359**
Learning names	-0.299*	-0.071	-0.347**	0.350**	0.35**	-0.074
Quantity	-0.346**	-0.020	-0.129	0.160	0.34**	-0.259*
Discs	-0.191	-0.326*	-0.405**	0.150	0.31*	-0.070
Hidden figure	-0.382**	-0.293*	-0.386**	0.261*	0.45**	-0.116
Number connection1	0.326*	0.353*	0.301	-0.403**	-0.47**	0.563**
Number connection 2	0.155	0.270	0.186	-0.261	-0.37*	0.430**
Figure connection test	0.168	0.558**	0.513**	-0.560**	-0.46**	0.589**
Picture completion	-0.225	-0.125	-0.102	0.337*	0.32*	-0.477**
Digit symbol	-0.147	-0.321	-0.325*	0.381*	0.17	-0.483**
Block Design	-0.156	-0.244	-0.201	0.227	0.22	-0.339*
Picture arrangement	-0.002	-0.243	-0.174	0.356*	0.29	-0.525**
Object Assembly	-0.258	-0.235	-0.231	0.233	0.14	-0.290

** significant at 0.01 level, * significant at 0.05 level, NPT: neuropsychological test, IL6: interleukin 6, TNF α: tumor necrosis factor α, MI: myoinositol, Glx: glutamine

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Table 3: Correlation of NPT with mean diffusivity on Diffusion tensor imaging

NPT	FWM	CN	ALIC	PLIC	GP	Put	OWM
Closure	-0.51#	-0.45#	-0.51#	-0.49#	-0.39#	0.34#	-0.34#
Exclusion	-0.48#	-0.44#	-0.42#	-0.43#	-0.36#	0.39#	-0.43#
Memory span	-0.41#	-0.21	-0.46#	-0.44#	-0.38#	0.48#	-0.36#
Verbal Meaning	-0.36#	-0.32*	-0.36#	-0.36#	-0.44#	0.28*	-0.22
Mazes	-0.66#	-0.55#	-0.696#	-0.59#	-0.60#	0.41#	-0.51#
Learning names	-0.49#	-0.34#	-0.47#	-0.52#	-0.33#	0.34#	-0.40#
Quantity	-0.49#	-0.41#	-0.51#	-0.45#	-0.63#	0.37#	-0.37#
Discs	-0.39#	-0.19	-0.32*	-0.24	-0.14	0.295*	-0.28*
Hidden Figure	-0.58#	-0.46#	-0.59#	-0.41#	-0.43#	0.50#	-0.47#
No Connection1	0.62#	0.57#	0.44#	0.29	0.54#	-0.06	0.38*
No Connection 2	0.42#	0.33*	0.32*	0.33*	0.33*	-0.09	0.42#
Figure connection test	0.50#	0.51#	0.47#	0.52#	0.35*	-0.06	0.46#
Picture Completion	-0.49#	-0.35*	-0.36*	-0.35*	-0.35*	0.003	-0.499#
Digit Symbol	-0.49#	-0.36*	-0.299	-0.33*	-0.296	0.06	-0.54**
Block Design	-0.44#	-0.35*	-0.25	-0.32*	-0.37*	0.16	-0.499#
Picture Arrangement	-0.41#	-0.55#	-0.43#	-0.45#	-0.29	-0.02	-0.46#
Object Assembly	-.39#	-0.32*	-0.29	-0.38*	-0.26	0.24	-0.51#

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significant at 0.01 level, * significant at 0.05 level, FWM: frontal white matter, CN: caudate nucleus, ALIC: anterior limb of internal capsule, PLIC: posterior limb of internal capsule, GP: globus pallidus, Put: putamen, OWM: occipital white matter

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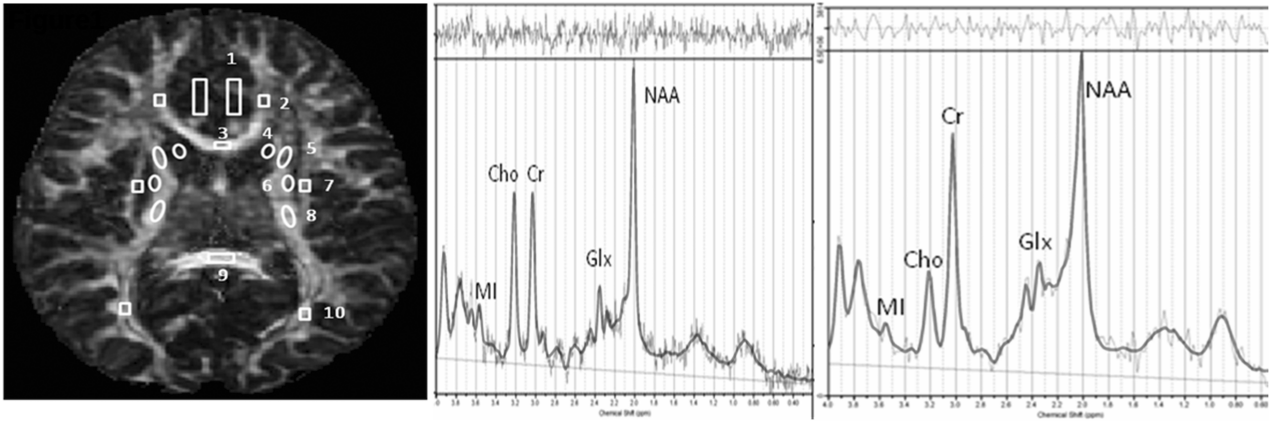


(A) (B) (C)

Figure 2

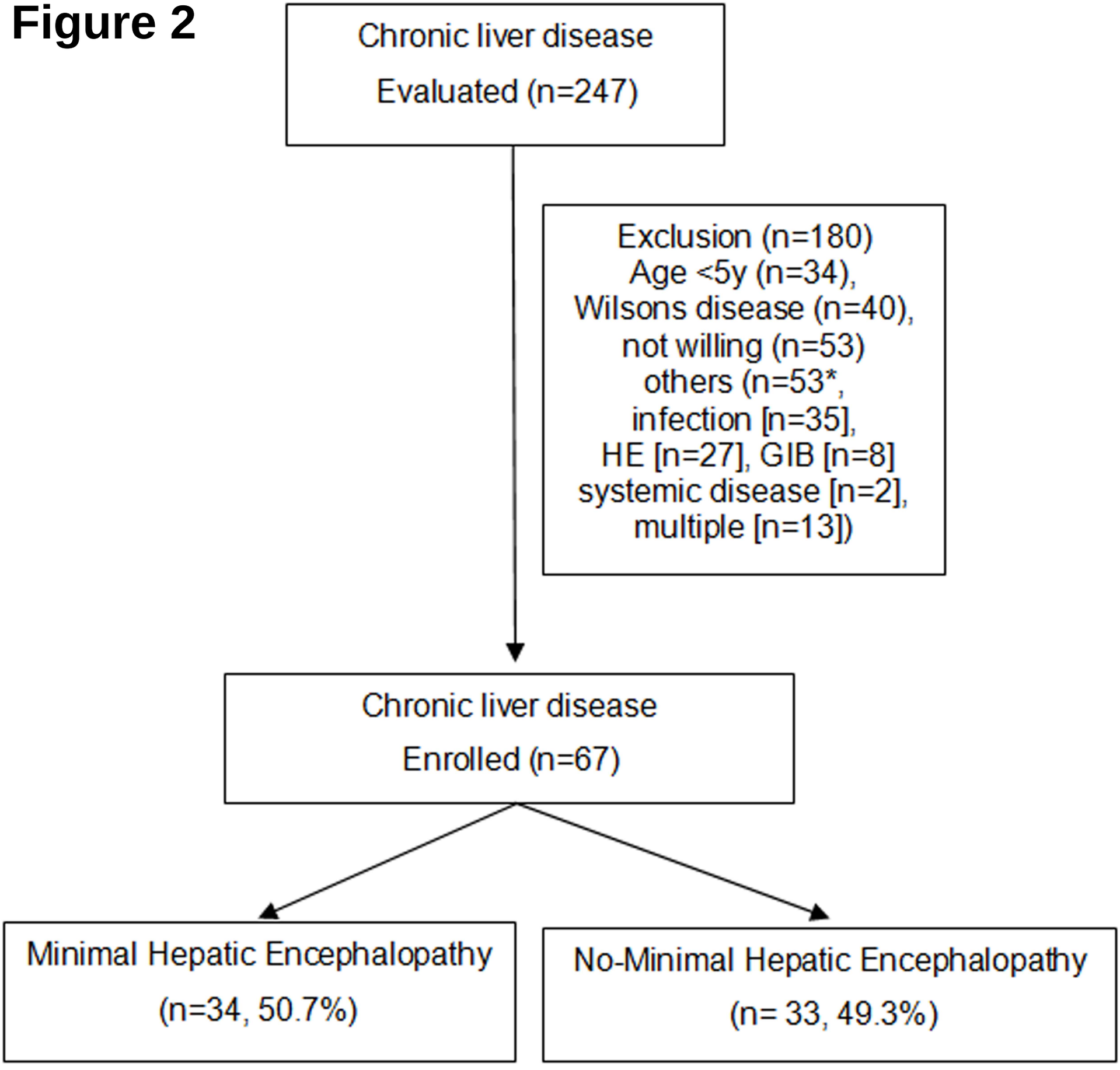
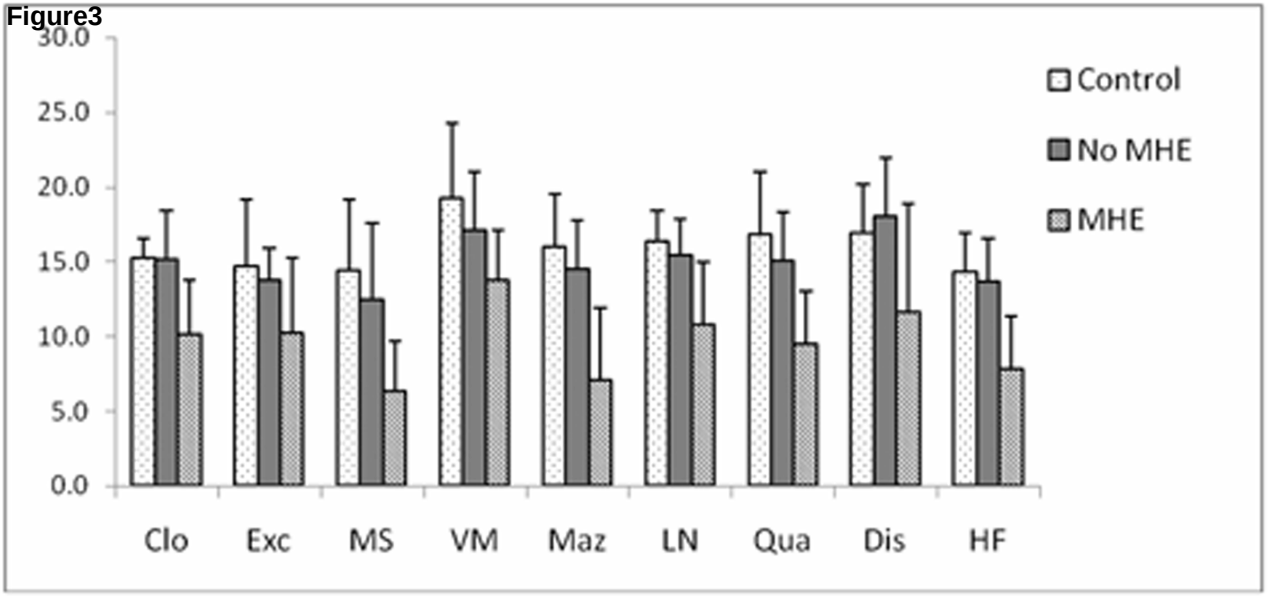

Chronic liver disease
Evaluated (n=247)
Exclusion (n=180)
Age <5y (n=34),
Wilsons disease (n=40),
not willing (n=53)
others (n=53*,
infection [n=35],
HE [n=27], GIB [n=8]
systemic disease [n=2],
multiple [n=13])
Chronic liver disease
Enrolled (n=67)
Minimal Hepatic Encephalopathy
(n=34, 50.7%)
No-Minimal Hepatic Encephalopathy
(n= 33, 49.3%)

Figure3



(A) Clo: Closure, Exc: Exclusion, MS: memory span, VM: verbal meaning, Maz: mazes, LN: Learning names, Qua: quantity, Dis: discs, HF: hidden figure

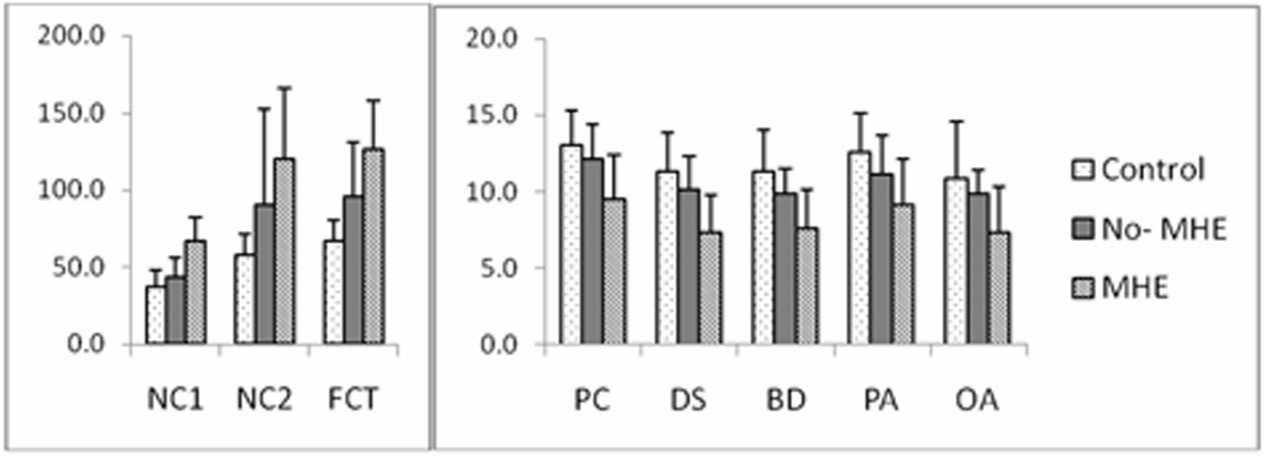


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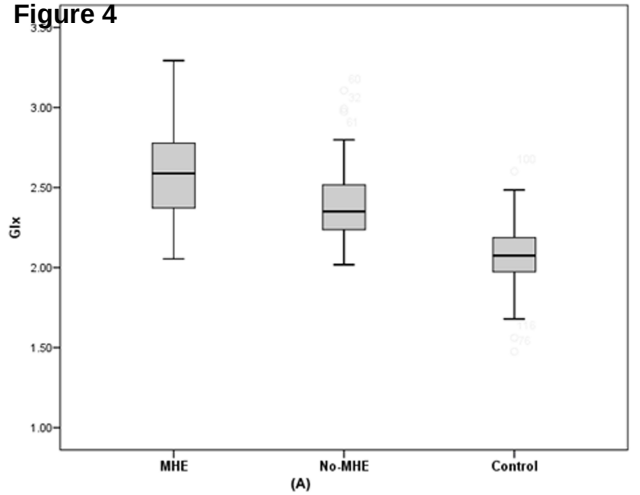
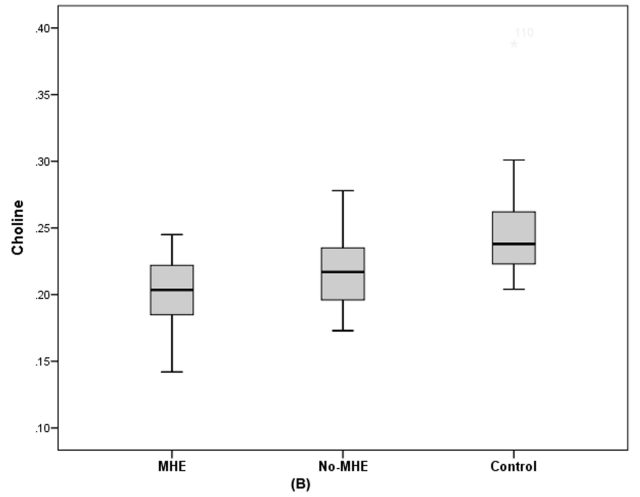
Figure 4


Glx
3.00
2.50
2.00
1.50
1.00
MHE
No-MHE
Control

(A)


Choline
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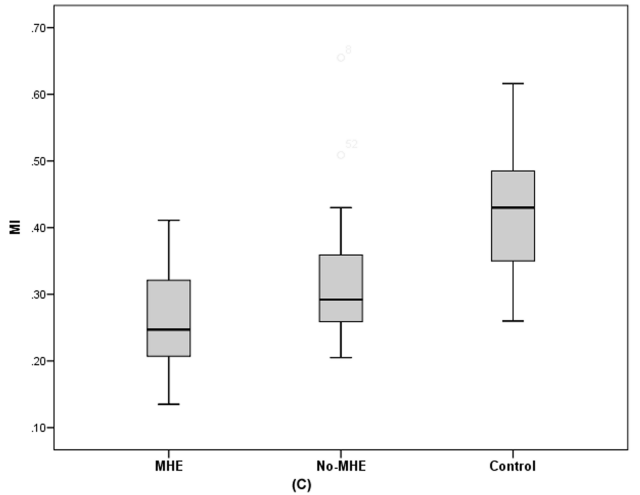
.35
.30
.25
.20
.15
.10
MHE
No-MHE
Control

(B)


MI
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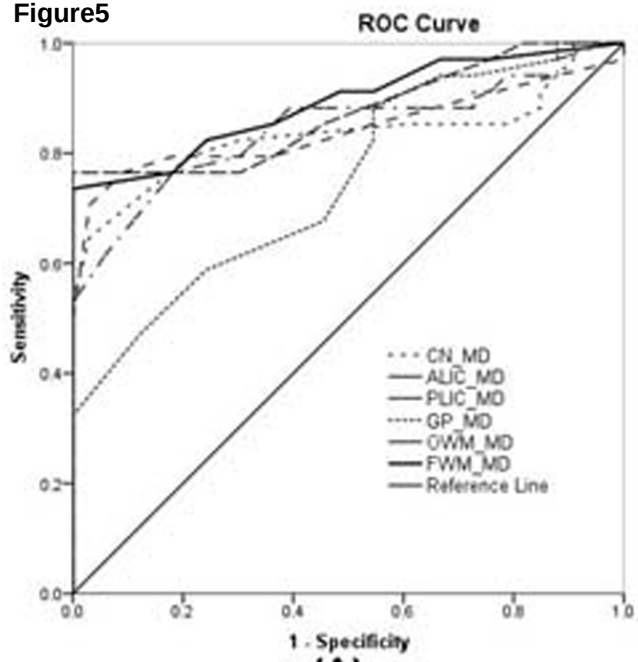
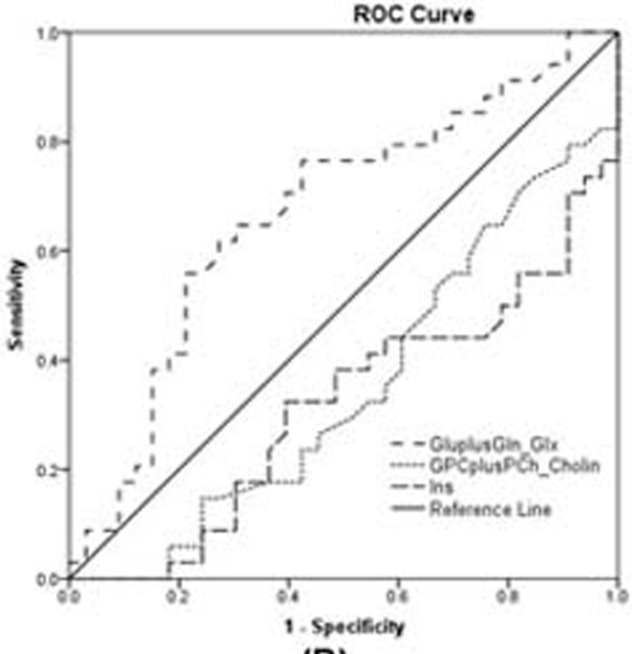
.60
.50
.40
.30
.20
.10
MHE
No-MHE
Control

(C)

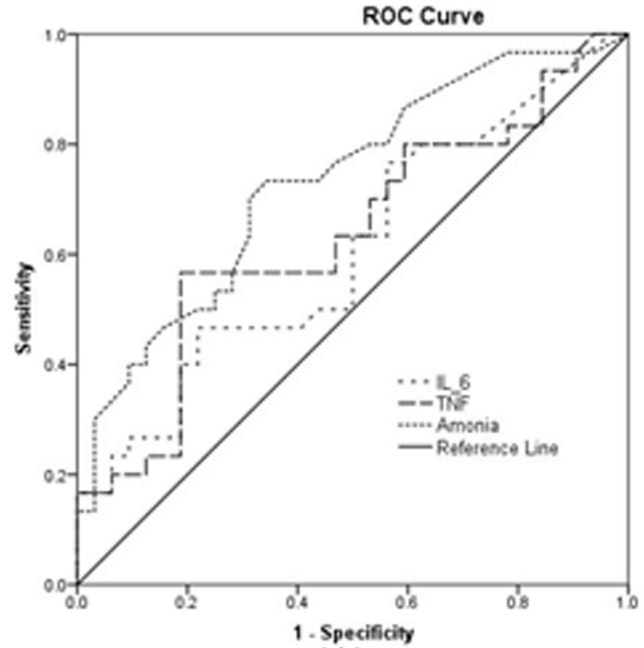
Figure5



(A)



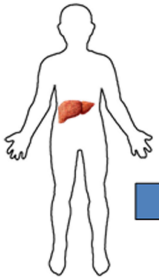
(B)



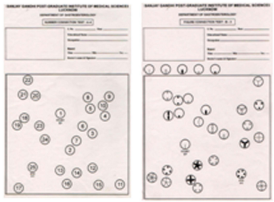
(C)

Variable	AUC (95% CI); p value	Variable	AUC (95% CI); p value
FWM-MD	0.89(0.81-0.97);<0.001	Glx	0.67(0.52-0.79); 0.003
ALIC-MD	0.87(0.77-0.96); <0.001	Cho	0.35(0.22-0.49); 0.05
OWM-MD	0.85(0.74-0.95); <0.001	MI	0.31(0.17-0.44); 0.009
PLIC-MD	0.84(0.73-0.95); <0.001	BA	0.73(0.61-0.86); 0.002
CN-MD	0.83(0.73-0.95);<0.001	IL6	0.61(0.46-0.75);0.14
GP-MD	0.73(0.60-0.85); 0.002	TNF-alpha	0.63(0.49-0.77); 0.06

***Graphical Abstract**

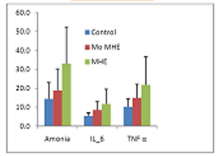


Child with chronic liver disease



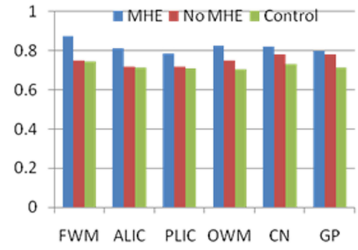
Abnormal Psychometric tests s/o Minimal hepatic encephalopathy

Blood



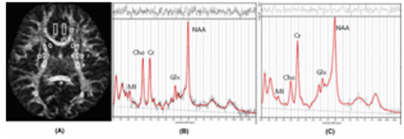
Hyperammonemia & Endotoxemia

Mild cerebral edema



Increased mean diffusivity On diffusion tensor imaging

Increased Glutamine Reduced choline & Myoinositol



Brain (^{1}HMRSpectroscopy)