Magnetic Resonance Brain Imaging, Manganese, and Ammonia Changes in Chronic Liver Disease: Reversibility post-Liver Transplantation

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ABSTRACT

Background: In patients with chronic liver disease, the presence of hepatocellular failure and/or portosystemic shunting lead to structural and functional brain changes. These changes are due to an increase in substances that are efficiently metabolized by the liver under normal circumstances. These abnormalities can be detected by MRI and magnetic resonance spectroscopy (MRS).

Objective: This study aims to evaluate changes in MR brain imaging, manganese, and ammonia levels in patients with chronic liver disease. Additionally, to assess the reversibility of these changes post-living donor liver transplantation.

Methods: The study included 10 adult male patients with CLD who underwent LDLT and 10 age and sex-matched healthy control subjects. All patients were subjected to clinical examination, routine labs, MR and MRS examinations of the brain. Follow-up MRI and MRS brain examination, as well as ammonia and manganese levels, were performed 6 to 9 months post-liver transplantation.

Results: The CHILD score ranged from 7 to 13 with a mean of 10.4±1.8, whereas the MELD score ranged from 12 to 22 with a mean of 17.5±3.4. Ammonia levels pre-transplantation (mean 60±7.8) decreased significantly post-transplantation (mean 45.8±7.0) (P<0.001). Also, manganese levels pre-transplantation (mean 2.2±0.16 decreased significantly post-transplantation (mean 1.18±0.09) (P<0.001). Pallidal T1 hyperintense signal was detected in 8 out of 10 patients and regressed in 6 of them post-transplant. Eight patients demonstrated hyperintense whiter matter lesions (WMLs) in FLIAR WI that regressed in all patients after LT (P= 0.011). MRS findings demonstrated lower Cho/Cr and MI/Cr, and higher Glx/Cr compared to controls (P<0.05). The abnormal brain metabolites returned to normal levels post-transplant. Blood levels of ammonia and manganese significantly decreased post-transplant compared to their pre-transplant levels (P<0.01).

Conclusion: MRI and MRS findings can be used to detect metabolic brain abnormalities in chronic liver disease patients with a correlation with ammonia and manganese levels. Moreover, they can be used to monitor the patients post-transplant.

KEYWORDS: Chronic liver disease; Liver cirrhosis; Magnetic resonance imaging; Magnetic resonance spectroscopy; Ammonia; Manganese; Liver transplantation

INTRODUCTION

In patients with chronic liver disease (CLD) cerebral insults, resulting from hepatocellular failure and/or portosystemic shunting lead to structural and functional brain abnormalities. These abnormalities are due to increase in substances that under normal circumstances, are efficiently metabolized by the liver. These abnormalities can be detected by MRI and MR spectroscopy (MRS) [1].

Manganese is an essential trace metal required for normal central nervous system function, which is toxic when in excess amounts in serum. Manganese (Mn) neurotoxicity has been demonstrated

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Table 1: Comparing MRS findings in CLD pre-LT and healthy control group.

Variables	CLD	Control	P-Value
CHO/Cr (Median)	0.84	1.13	0.002
MI/Cr (Median)	0.176	0.98	0.002
GLX/Cr (Median)	0.739	0.53	0.02

in patients with chronic liver/biliary failure where an inability to excrete manganese via the biliary system causes increased serum levels. Manganese has been described to deposit selectively in the basal ganglia where blood flux is high and to induce focal neurotoxicity leading to hyperintense globus pallidus on T1 weighted MRI [2,3].

In patients with CLD, hyperammonemia leads to low-grade brain edema due to uptake of ammonia into astrocytes thus causing cellular swelling which can be detected in FLAIR sequence as diffuse high-intensity white matter lesions (WMLs) [4].

MRS is a noninvasive analytic method often paired with MRI in neuroradiology practices. MRS can provide information on brain metabolites such as Choline (Cho), Creatine, N-acetyl aspartate (NAA), Myo-inositol (MI), as well as glutamine and glutamate (Glx). In patients with cirrhosis, the increased ammonia will lead to osmolar adaptation in astrocytes that is reflected in MRS findings [5].

This study aims to evaluate changes in MR brain imaging, manganese and ammonia levels in patients with chronic liver disease (CLD). Additionally, to assess the reversibility of these changes post living donor liver transplantation (LDLT).

MATERIALS AND METHODS

This study was performed in a specialized liver transplantation center after approval of the ethical committee board. A written informed consent form was obtained from all study participants.

The study included 10 adult male patients with CLD who underwent LDLT. Their mean age of 54±6 years. Ten age and sex-matched healthy control subjects were recruited for this study.

Patients with contraindications to MRI like cardiac pace makers and metallic implants were excluded from the study.

All patients were subjected to history taking and clinical examination, routine labs, the severity of liver failure was graded using Child-Pugh's classification and MELD score.

MRI and MRS examinations were performed using 1.5 Tesla MRI superconducting scanner (Intera; Philips Medical Systems Best, the Netherlands). MRI examinations were performed before liver transplantation and repeated 6 to 9 months post-transplant.

MRI examinations included:

- Axial T1WI to determine the signal intensity of the globus pallidus which was graded visually according to a 3-point grading scale (0 = no signals, +1= minimal, and +2= marked) [6].
- Axial FLAIR to detect white matter changes.
- MR spectroscopy: MRS was performed using single voxel technique at short TE and intermediate TE. Voxel was placed at the parieto-occipital white matter. Brain metabolites Cho, MI, and Glx were evaluated and compared at different time intervals to detect brain metabolite changes, normalization and reversibility post-transplant.

Table 2: Comparing MRS findings in CLD post-LT and control group.

Variables	After LDLT	Control Group	P-Value
CHO (Median)	1.13	1.13	0.581
MI (Median)	0.896	0.98	0.624
GLX (Median)	0.435	0.53	0.270

Fasting blood ammonia and serum manganese were measured on the same day of MRI examination and correlated with MRI findings.

For testing for Ammonia: one mL blood sample was collected from each patient from a stasis-free vein. The samples were placed immediately on ice and centrifuged and sent to the lab as plasma EDTA Frozen. The analysis was performed within the same day of venipuncture. Sample analysis was performed with the enzymatic method using Cobas 6000 device. The reference range for males ranged from 16 to 60 µmol/L and for females from 11 to 51 µmol/L.

For testing for Manganese (Mn): Two mL blood sample was collected from each patient. The samples were allowed to clot and centrifuged at room temperature. Serum was refrigerated at 2-8°C. Sample analysis was performed ICP Mass Spectrometry (ICP-MS). The reference range was 0.5-1.3 µg/L.

Statistical Analysis

All patient data were tabulated using Excel 2010. Data were processed using SPSS version 20 for Windows 2010. All qualitative data were analyzed using the chi-square test as appropriate. The chi-square test was used to calculate Pearson's chi-square and its P-value when both table variables were quantitative. Paired t-test or Wilcoxon rank tests were used to compare related samples according to normality tests. Differences for which $P>0.05$ were not considered to be insignificant, differences for which $P<0.05$ were statistically significant and differences for which $P<0.001$ were highly significant.

RESULTS

Liver cirrhosis (LC) was due to HCV in 7 patients, HBV in 2 patients and cryptogenic in 1 patient.

The CHILD score ranged from 7 to 13 with a mean of 10.4±1.8, whereas the MELD score ranged from 12 to 22 with a mean 17.5±3.4.

Ammonia levels pre-transplantation (mean 60±7.8) decreased significantly post-transplantation (mean 45.8±7) ($P<0.001$).

Also, manganese (Mn) levels pre-transplantation (mean 2.2±0.16 decreased significantly post-transplantation (mean 1.18±0.09) ($P<0.001$).

Hyperintense T1 signals were detected in 8 patients (grade 1 in 2 patients and grade 2 in 6 patients), WMLs in T2 FLAIR images were evident in 8 patients, these WMLs manifested as periventricular sheets, foci or both.

Manganese levels were significantly positively correlated to the degree of hyperintense T1WI signals ($P<0.05$).

Hyperintense T1WI signals regressed post-liver transplantation in 6 patients (from grade 2 to grade 0 in three patients, from grade 2 to grade 1 in three patients). The WMLs in FLAIR T2WI also significantly regressed post-liver transplantation in all patients ($P=0.011$) (Fig 1 & Fig 2).

Cho/Cr and MI/Cr were significantly lower in CLD patients (pre- transplantation) than control group ($P=0.002$) while GLX/Cr is significantly higher in CLD (pre-transplantation) than control group ($P=0.02$) (Table 1)

Table 3: Comparing MRS finding pre & post LDLT.

Variables	Pre-Transplant	Post-Transplant	P-value
CHO [Median (IQR)]	0.84 (0.148)	1.13 (0.390)	0.005
MI [Median (IQR)]	0.176 (0.161)	0.89 (0.660)	0.005
GLX [Median (IQR)]	0.74 (0.271)	0.435 (0.209)	0.005

and (Fig 3 & Fig 4).

No statistically significant difference was detected between CLD patients (post-transplantation) and control group in all the measured metabolites indicating that they returned to normal values (Table 2).

There was significant increase of Cho/Cr and MI/Cr level post-liver transplantation (P=0.005). Whereas, Glx/Cr level significantly decreased post-liver transplantation (P=0.005) (Table 3).

DISCUSSION

In the current study, we evaluated changes in MR and MRS brain findings in patient with CLD and correlate these changes to the serum manganese and ammonia levels. Additionally, we assessed the reversibility of these changes post living donor liver transplantation (LDLT).

Most of the previous studies in this subject were done in orthotopic LT and only few of them correlated the radiological brain changes with the biological changes.

Manganese neurotoxicity has been demonstrated in patients with chronic liver/biliary failure because of the inability to excrete manganese via the biliary system. Manganese has been described to deposit selectively in the basal ganglia. Many clinical studies found a strong correlation between the increased pallidus signal in T1WI and blood concentration of Mn [7-10].

Eight out of 10 cirrhotic patients in our study showed bilateral pallidal hyperintense T1 signal (grade 1 in 2 patients and grade 2 in 6 patients). Manganese levels were elevated in all the patients with CLD (pre-LT). We have found a significant positive correlation between the degree of hyperintense signal in T1WI and blood manganese levels in CLD patients (P=0.019).

Regression of the hyperintense T1WI signal was detected in 6 patients (from grade 2 to grade 0 in three patients, from grade 2 to grade 1 in three patients). Manganese levels have significantly decreased post-transplantation compared to their pre-transplant levels and have returned to its normal reference range (P<0.001).

These post-transplantation findings are in agreement with several previous studies but with different normalization periods. Pujol et al., (1993) noted the disappearance of the hyperintense T1 signals in 21 patients, 10 to 20 months after liver transplantation [11]. Similarly, Naegele et al., (2000) reported complete recovery of the abnormal pallidal index (PI) values (a measure of T1 hyperintensity) between 10–20 months post-transplantation in 21 patients who successfully received liver transplant [12]. Additionally, Long et al., (2009) recorded progressive reduction of the abnormal T1 signals, 5 months post LT together with normalization of serum manganese levels in the 3 liver transplant recipients included in their study [13]. Lazeyras et al., (2002) found that in 14 patients 4 months after orthotopic liver transplantation, pallidal hyperintensity remained abnormal in 5/8 of cases and blood manganese levels remained abnormal in 4/6 patients with preoperative HE and 5/6 of those without HE [14].

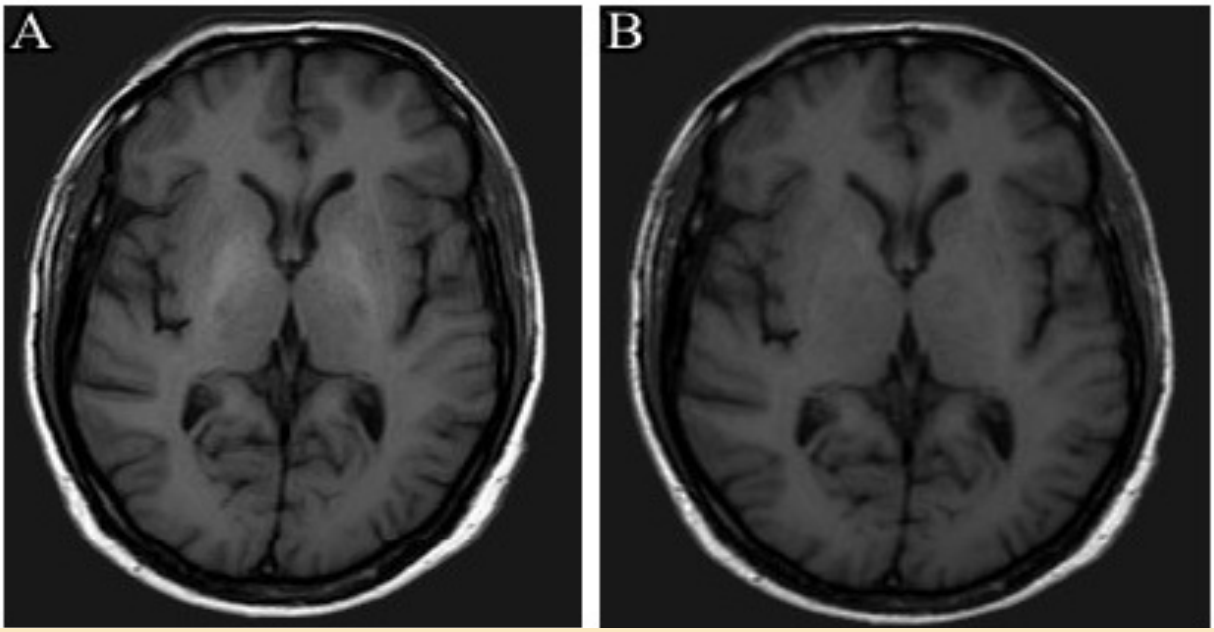

Figure 1: Axial T1-WI MRI (A) pre-transplantation showing high signal intensity (grade 2) in globus pallidus and (B) post-transplantation showing loss of signal intensity (grade 0).

In the current study, white matter lesions (WMLs) were detected in T2 FALIR WI in 8 patients. These WMLs have manifested as periventricular sheets, foci of WMLs or both. The T2 FLAIR WMLs have significantly regressed post-liver transplantation in all 8 patients (P=0.011). T2 FLAIR WMLs in patients with CLD have been also described in previous studies [15-17].

This was in accordance with the findings of the previous studies. Rovira et al., (2002) found bilateral, symmetric high FLAIR signal intensity along with the hemispheric white matter in or around the corticospinal tract in 23/24 cirrhotic patients. Gradual resoultion of the signal abnormalities were detected in the

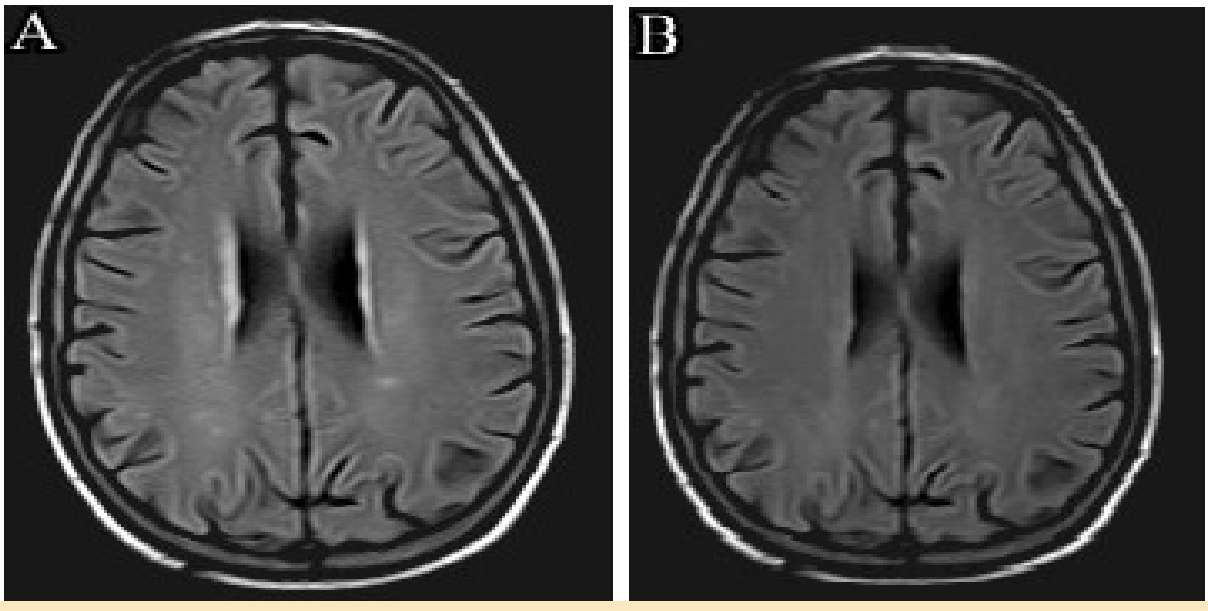

Figure 2: Axial T2-weighted fast FLAIR images (A) pre-transplantation showing peri-ventricular sheets and some foci of WMLs. (B) post- transplantation showing complete disappearance of the WMLs.

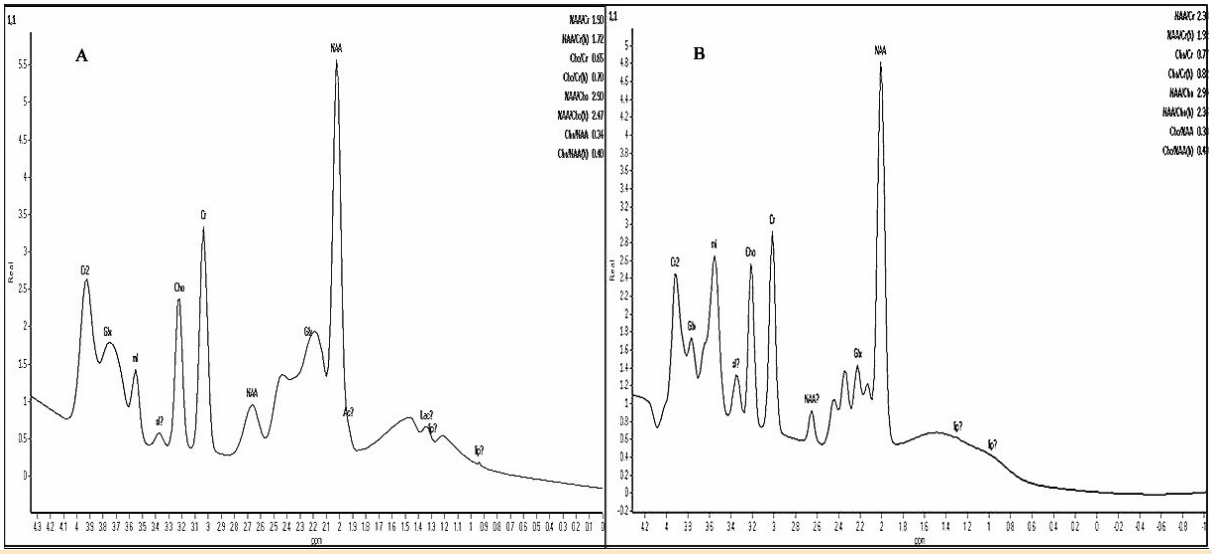

Figure 3: MRS (**A**) pre-transplantation showing low Cho/Cr and MI/Cr peaks and high Glx/Cr peak, (**B**) post-transplantation showing increased Cho/Cr and MI/Cr peaks and decreased Glx/Cr peak.

11 patients after liver transplantation [18]. Similarly, Cordoba et al., (2003) found high FLAIR signal intensity along the corticospinal tract in 20 patients with CLD with subsequent signal normalization 6 months after liver transplantation [19]. Rovira et al., (2007) conducted a study on 27 patients with cirrhosis. Baseline MRI identified focal T2-weighted lesions in 70% of the patients. They observed a significant reduction in lesions volume 6 to 14 months after liver transplantation [20].

The presence of abnormal hyperintense T2 signal along the hemispheric white matter in or around the corticospinal tract is explained by the presence of mild brain edema due to hyperammonemia in cirrhotic patients [4].

We also found high ammonia levels in 6/10 patients with significant decrease and normalization post transplantation (P<0.001). In patients with liver cirrhosis with high ammonia load, the mechanism of osmolar adaptation leads to changes in the brain metabolites that can be detected by MRS [1]. This spectral

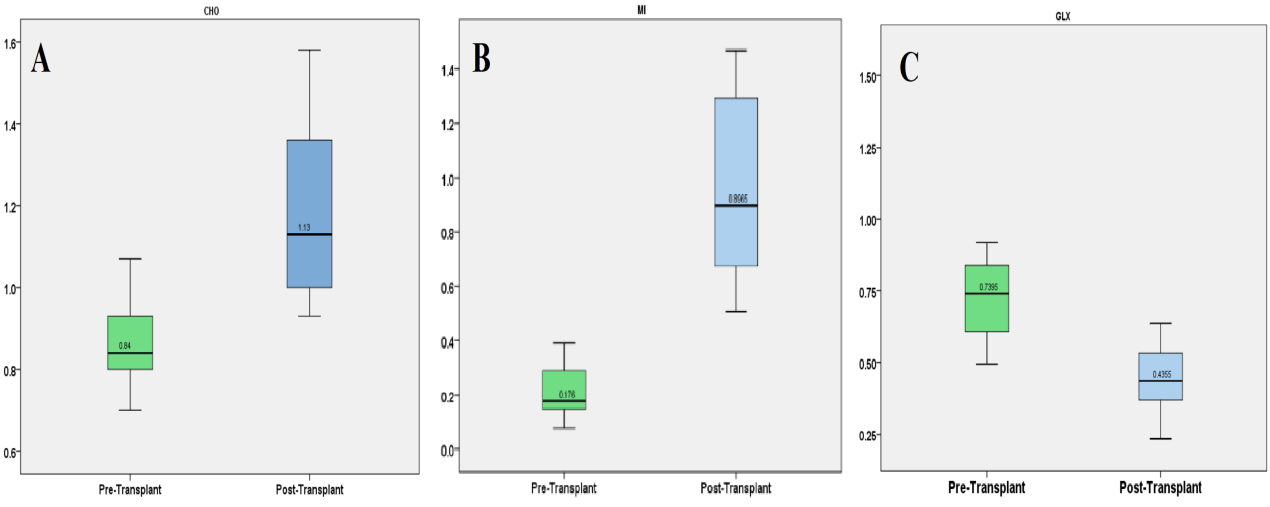

Figure 4: Comparing the metabolites Cho/Cr, MI/Cr and Glx/Cr as measured by MRS pre and post transplantation revealed: (**A**) Cho/Cr levels significantly increased post liver transplantation (P=0.005), (**B**) MI/Cr levels significantly increased post liver transplantation (P=0.005), and (**C**) Glx/Cr levels significantly decreased post liver transplantation (P=0.005).

pattern changes likely to correspond to the effects of ammonia metabolism in the brain. Once ammonia enters the brain, astrocytes use it to convert Glu to Gln by glutamine synthetase activity. So, Glutamine increases within the astrocytes as a result of ammonia detoxification through the action of glutamine synthetase. An excess of Gln disrupts cellular homeostasis and activates an osmotic compensatory mechanism through which water enters the cell, while other cellular osmolytes, such as MI, leave the cell [21,22].

In the current study, CLD patients demonstrated high Glx/Cr ratio together with a low Cho/Cr ratio and a low MI/Cr ratio compared to healthy controls ($P<0.05$). This was similar to the findings of the previous studies which reported a decrease in MI/Cr and Cho/Cr ratios accompanied by an increase in Glx/Cr ratio in CLD patients [21,23,24].

In the current study, we have compared the metabolites Cho/Cr, MI/Cr and Glx/Cr pre and 6 to 9 months post transplantation, we have found that Cho/Cr levels together with MI/Cr levels have significantly increased post-transplantation whereas, Glx/Cr levels have significantly decreased post-transplantation ($P=0.005$).

We have compared the Cho/Cr, MI/Cr and Glx/Cr ratio post-transplantation to that of the control group. We have found no statistically significant difference between the two groups indicating that they returned to normal values. Similar results were reported in the previous studies yet with variable normalization periods. Lazeyras et al., (2002) found that MRS changes normalized in all 14 patients included in their study 4 months after OLT [14]. Naegele et al., (2000) conducted a study on 21 patients and found that the Cho peak normalizes earlier than the other peaks at 1–2 months. The glutamine/glutamate peak normalizes at 2-3 months. The MI level normalizes slower than the other peaks and may take 3–7 months to reach normal values [12]. However, Long et al; 2009 found that the ratios of Cho/Cr, mI/Cr, and Glx/Cr returned to normal within 3 months in 3/3 patients after transplantation [13].

We have also found a negative correlation between ammonia levels and MI/Cr ratio ($P=0.028$) as well as a positive correlation between Manganese levels Glx/Cr ratio ($P=0.03$). These findings were in agreement with the findings of Long et al., (2009) who found a positive correlation between blood ammonia levels and Glx/Cr ratio in 50 cirrhotic patients. Yet, they have not found any correlation between manganese levels and MRS metabolites [13].

In a study done by Foerster et al., (2009) on 12 children ranging in age from 9 to 19 with clinically suspected minimal hepatic encephalopathy. They have found that ammonia levels were negatively correlated with Cho and MI levels and positively correlated with Glx [26]. Zhan et al., (2010) found that level ammonia had a significant negative correlation with Cho/Cr and MI/Cr in 52 cirrhotic patients [21].

In conclusion, MRI and MRS could be used to accurately assess the morphological and metabolic abnormalities in the brain in patients with CLD. In addition, it can be used to monitor therapeutic efficacy by showing recovery of a normal spectral pattern within 6 to 9 months after liver transplantation. In addition to blood ammonia, estimation of serum manganese could be useful in the evaluation patients with CLD as well as monitor changes after post-liver transplantation.

CONFLICTS OF INTEREST: None declared.

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