Differentiating Cirrhosis from Healthy Controls using T1 Signal Intensity Rather than Normalized Signal Intensity Ratio

Abstract:

Background: T1 shortening on brain MRI is a marker of Manganese deposition among those with liver failure and portal hypertension. This study evaluates a fully automated approach to identify increased T1 signal intensity in the brain using FreeSurfer.

Method: 3T brain MRI and single -voxel proton MRS on 26 participants with cirrhosis secondary to non-alcoholic steatohepatitis (NASH), 7 with hepatic encephalopathy (HE) and 19 without, and 30 healthy age matched controls. Cerebral cortex, white matter, putamen, globus pallidus and brainstem are the five relevant regions of interest selected. Concentration of Ins, T1 signal intensity of these five regions, and orthotic normalized signal ratio of basal ganglia region over non basal ganglia are obtained.

Results:

Conclusion:

Increased T1 signal intensity was noted in all brain regions studied in those with cirrhosis, with or without HE, compared to controls (p<<0.001). A decrease in T1 signal was noted in all brain regions in NASH+HE compared to NASH only, which may indicate presence of brain edema.

Introduction:

Liver disease impairs brain function and may lead to development of hepatic encephalopathy. A commonly used brain imaging marker for the effects of liver failure is an increase in T1 signal intensity within the basal ganglia. Multiple radiological – pathologic correlation studies have demonstrated that manganese deposition is responsible for T1 shortening among those with advanced liver disease 1-4. In cirrhotic patients, increased manganese deposition in the brain is related to increased concentration in the bloodstream due to portal shunting and failure of hepatobiliary clearance of metabolites from the intestines3.   Daily dietary intake of Manganese is typically 2.5–3 mg of which the hepatic-biliary system normally drains up to 96% from the systemic circulation5. The etiology of manganese deposition due to portosystemic shunting has been confirmed in congenital portal-systemic bypass and portal hypertension from non-cirrhotic portal vein thrombosis3,6,7. Iatrogenic manganese deposition is also demonstrated by increase of basal ganglia T1 SI after trans-jugular intrahepatic portosystemic shunt placement2. Outside of the presence of liver disease, a high manganese concentration in the blood via parenteral nutrition also induces basal ganglia T1 shortening8.

Hepatic encephalopathy is a complex disease with contributions from multiple pathophysiologic processes27. Magnesium deposition in the brain may promote neurocognitive abnormalities among people with portal hypertension regardless of its relationship to hepatic encephalopathy. The neurocognitive effects of manganese toxicity are not confined to parkinsonian motor functions typically associated with the basal ganglia, and may also contribute to cognitive and psychiatric impairments28. Manganese toxicity disrupts glutamine/glutamate-γ-aminobutyric acid (GABA) neurotransmitter cycling between astrocytes and neurons which are critical to brain function29.

There is considerable interest in development of automated tools to assist with disease screening and diagnostics, particularly in resource limited situations13,14. There have been continued advances in computational power and machine learning to assist with radiological evaluation of diseases impacting different parts of the body including in the chest, the brain and the liver14-17. Automated approaches are already routinely employed for brain imaging research which have potential for clinical translation. In this project, we sought to assess an automated evaluation for manganese deposition which is one of the complications of advanced liver disease in the brain. We sought to determine whether T1 shortening on FSPGR images could be identified automatically using the Freesurfer image analysis program in patients with NASH cirrhosis, with and without HE, compared to a control group without history of liver disease.

Method:

In this IRB-approved study with written consent, 26 NASH (7 HE, 19 Non-HE) patients and 30 age-matched controls were examined on a General Electric (GE) 3 Tesla scanner, demographics are shown in Table 1. Standard 3D T1 FSPGR (TE:2.41ms; TR:6.75ms; TI:600ms; slice thickness=1.2mm; matrix 256 x 256; flip angle: 8) and single voxel, short echo time point-resolved spectroscopy (PRESS) in the posterior gray matter (PGM) and parietal white matter (PWM) were obtained. Hepatic encephalopathy is brain dysfunction caused by liver insufficiency and/or PSS; it manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma.

Averaged T1 intensity values of the left and right side of cerebral cortex (CC), cerebral white matter (CWM), globus pallidus (GP), putamen and brainstem were obtained from automated Freesurfer image analysis suite 6.010. The image used to obtain SI come from the brain extracted image (brain.mgz) using the segmentation labels (aparg+aseg.mgz). Average intensity ratios of GP and putamen with respect to CC, CWM, and brainstem were also calculated. Spectroscopy data was analyzed using Linear Combination Model (LC Model) to quantify brain metabolites, including myo-inositol (Ins).

ANOVA was applied to analyze SI ratio of GP and putamen with respect to CC, CWM, and brainstem and SI of CC, CWM, GP, putamen and brainstem regions between controls and NASH, and NASH+HE. ANOVA p-values were adjusted for false discovery rate and level of significance was set at p<.05. There was no additional independent effect related to sex, and it was not included in the models. Where ANOVA was significant, between-group differences were assessed using tukey’s honest significant difference test. Two-tailed tests with multiple comparison correction using the Benjamin-Hochberg False Discovery Rate (FDR)11, where p-values presented are already FDR corrected. General linear regression is used to evaluate T1 SI of these 5 regions and Ins concentration with class difference adjusted. All statistical analysis was performed with JMP Pro version13 (SAS, Cary NC).

Results:

Patients with advanced liver disease have signal shortening at the globus pallidus shown in T1 sequence12,13. Figure 1 shows unadjusted window or level of a sample brain axial views, control on the lest and a cirrhotic participant on the right. Table 2 reported both the normalized T1 SI ration and the T1 SI of all five region under the ANOVA test showed between group differences among control, NASH and NASH+HE. Figure 2 reports normalized T1 SI ratios for between group difference among controls, NASH and NASH+HE. Although the SI ratio of putamen over CC (Figure 2A) or GP over CC (Figure 2D) did not show a significant in between group difference for the control versus NASH and that of putamen over CWM (Figure 2E) only showed a trend, SI ratio indicates significant difference among controls, NASH and NASH+HE (Figure 2B, 2C and 2F). Further, the SI ratio increase from NASH to NASH+HE among the cirrhosis patients and some actually reached between group differences, such as that of GP with respect to CC (Figure 2A), GP with CWM (Figure 2B), and putamen with CWM (Figure 2E).

Figure 3 looked at the SI of putamen (Figure 3A), GP (Figure 3B), CC (Figure 3C), CWM (Figure 3D), and brainstem (Figure 3E) independently. It shows the consistent result of significant SI abnormality between control and cirrhosis. However, it presented two additional information. One, there is significant SI difference between NASH and NASH+HE in the CC (Figure 3D) and CWM (Figure 3E) regions. And there is a trend between group difference between NASH and NASH+HE in putamen (Figure 3A) brainstem area. On the side, although the difference is not significant, the GP area show a observable SI decrease from NASH to NASH+HE (Figure 3B).

SI increase on FSPGRE 3D sequence seems to increase with Ins concentration as measured by MRS (Table 3). This table also present that SI of CC CWM, putamen, GP and brainstem is significantly correlated with Ins concentration. Further, after adjusting the influence of Ins on SI, the SI difference between NASH and NASH+HE diminished. Ever more noticeable is the F-distribution of putamen, CC, and brainstem is nearly 1 (Table 3).

**Discussion**:

In this study, we used automated approaches to identify the signal intensity changes on T1 brain MRI with cirrhosis and HE. The automated approach showed expected SI ratio increases in GP and putamen with respect to the CC, CWM, or brainstem for cirrhosis with respect to control and cirrhosis with HE compared to without as shown in Figure 2. GP / ??? was the best at identifying between-group differences and showed a significant increase in cirrhosis compared with control as well as in cirrhosis with HE compared to cirrhosis without HE.

Looking at the signal intensities without normalization to another brain region demonstrated a T1 SI increase among cirrhosis with and without HE compared with controls for all the brain regions evaluated. This indicates widespread manganese deposition beyond areas typically associated with cirrhosis in imaging studies. Further, the T1 signal intensities tended to be lower in cirrhosis with HE compared to without.

We did not anticipate an increase in T1 signal intensity in all brain regions we evaluated but this finding is concordant with multiple radiological-pathological correlation studies which noted higher manganese deposition throughout the brain in patients with cirrhosis or portal-systemic shunts1-4. Our findings are also in agreement with a 1996 AJNR study that quantified T1 relaxation time and showed T1 shortening in the cortex and white matter as well as the basal ganglia of patients with chronic liver disease4. In addition, *Iwasa et al*. performed a study in 1998 evaluating magnetization transfer contrast of different brain regions between control and cirrhotic patients and found abnormal magnetization transfer ratio in otherwise normal appearing cerebral regions 18.

This raises the question of why other studies have not reported T1 signal abnormalities outside the basal ganglia. The manganese concentrations are highest in the basal ganglia and T1 shortening is most apparent in this location, likely explaining why imaging studies have focused on this region as a marker of cirrhosis. Studies were then limited in their ability to identify diffuse signal changes since they utilized background tissue signal as a normalization factor to create intensity ratios for the basal ganglia. This normalization is necessary in clinical studies that include images obtained on different scanners that make direct comparison of intensity values unreliable. Since all areas exhibited some degree of T1 signal intensity change, only areas with even more exaggerated differences can therefore be identified. Unfortunately, even for the basal ganglia, this still can alter the nature of associations or cause a decrease in statistical power as the normalization factor is also impacted by the disease process being studied. Our findings therefor point to limitations of the current clinical approach to evaluation of SI using ratios for evaluation of diffuse disease processes.

Another phenomenon we observe from this study is the decreased SI intensity of cirrhosis to cirrhosis+HE in all five brain regions (Figure 3). Surprised by this observation, we hypothesized that SI decrease in T1 might be influenced by edema in cirrhosis+HE. In proton magnetic resonance spectroscopy, Ins is an abundant neuro-biomarker that has previously been associated with brain edema in patients with HE, such as that of astrocyte swelling as a by-product of hyper-ammonic states 14. Therefore, we selected Ins as proxy to track brain swelling in a continuous fashion proportional to severity of HE. After adjusting for Ins concentration on T1 SI for both NASH and NASH+HE, there were almost no differences in manganese concentration between NASH vs. NASH+HE (Table 3). Rather, we found that Ins concentration appears to increase proportionally during acute hepatic encephalopathy, which may represent astrocyte swelling. This radiographic association suggests that cerebral edema provides a second hit for clinically significant hepatic encephalopathy that has not been previously reported.

The imaging software, FreeSurfer, provides some degree of standardization by performing automatic correction and standardization of image intensity values [REF]; analyzing signal intensities across scanners will likely require more standardization such as with the use of phantoms and may still require scanner specific reference ranges. New imaging sequences such as synthetic MRI19 which provide T1 and T2 quantification may excel at identifying manganese related T1 shortening.

We propose distinct effects are occurring related to T1 shortening due to manganese deposition in chronic liver disease and T1 prolongation with superimposed brain edema that occurs with HE. In cirrhosis +HE patients, T1 intensity was lower than cirrhosis without HE which is consistent with development of subclinical brain edema among those with hepatic encephalopathy2. Variable impact of edema versus manganese in different brain regions may account for inconsistencies in prior studies evaluating correlations between T1 signal and HE [REF]. Automatic identification of T1 SI increase allows for a simple, yet rapid diagnosis and characterization of manganese deposition in advanced liver disease and other pathologies using a relatively ubiquitous imaging modality. Several issues will need to be addressed for widespread clinical adoption. The automated approach used in this study to identify T1 shortening will also reflect gadolinium deposition that may occur after MRI scans with contrast 24. This was not an issue in our current study as our participants were followed clinically with ultrasound and CT for surveillance of hepatocellular cancer and had not received gadolinium. Our practice follows the current American Association for the Study of Liver Diseases guidelines that favor ultrasound rather than gadolinium-contrast for surveillance for hepatocellular carcinoma in patients with cirrhosis25. Confounding due to effects of gadolinium may be less of an issue in the future as linear contrast agents implicated in tissue deposition 26 are replaced with macrocyclic agents.

In this study, we are able to assess T1 intensity for individual regions as we used the same scanner and the same sequence. FSPGR technique provides high-resolution images needed for automatic segmentation. This sequence has different signal characteristics compared to standard spin echo sequences typically obtained in the clinic and may be more sensitive to the effects of T1 shortening related to manganese, similar to what has been reported for detection of gadolinium30. Further work is needed to determine the sensitivity of different T1 weighted sequences in identifying manganese. Additionally, we used T1 signal intensity values generated as part of the Freesurfer image analysis pipeline, which normalizes images intensity to further minimize remaining technical differences and corrects for fluctuations in signal intensity that may interfere with intensity-based segmentation31. A standardized approach to generating signal intensities of MRI images is need to help facilitate clinical use across different scanners which may involve standardizing T1 values using a phantom scanned at each site. Alternatively, newer synthetic MRI sequences calculate T1 and T2 relaxation which are objective properties that should not vary between scanners given appropriate quality controls.

Conclusion:

we demonstrate that there is significant increase in T1 shortening that precedes encephalopathy. with automatic process, we provide

1. We can automate detection of basal ganglia T1 increased SI indicating cirrhosis and HE.
2. Looking directly at SI for different brain regions shows diffuse T1 shortening consistent with diffuse manganese deposition.
3. T1 SI was lower in HE than cirrhosis without HE. The decrease was related to presence of brain osmotic stress as indicated by decreased Ins, an osmotic marker. After adjusting for ins no significant difference was seen for HE compared to cirrhosis without HE except for the white matter.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study | Control | NASH | NASH+HE | *ANOVA Prob > F* |
| (N=30) | (N=19) | (N=7) |
| Age | 62.8±2.4 | 63.1±5.1 | 62.4±5.1 |  |
| Female: Male | 15:15 | 16:3 | 4:3 |  |
|  |  |  |  |  |
| Diabetes Mellitus Type II | 0 | 17 | 6 |  |
|  |  |  |  |  |
| Laboratory and clinical parameters |  |  |  |  |
| Albumin | N/A | 3.98±0.1 | 3.31±0.18 | 0.004 |
| INRa | N/A | 1.09±0.03 | 1.267±0.04 | 0.001 |
| Alanine transaminase (ALT) | N/A | 34.5±3.6 | 29.6±6.2 | 0.5 |
| Aspartate transaminase (AST) | N/A | 40.8±4.7 | 39.4±7.9 | 0.9 |
| Creatinine | N/A | 0.74±0.08 | 1.24±0.13 | 0.002 |
| Sodium | N/A | 140.2±0.7 | 137.5±1.3 | 0.08 |
| a INR: international normalized ratio | | | | |

Table 1: Study population demographics, clinical laboratory parameters.

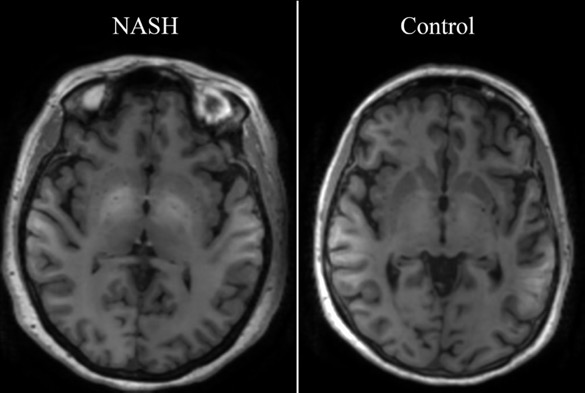


Figure1: Sample 3D T1 FSGPR axial view of Control and a NASH subjects without adjusting image signal intensity.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Control | NASH | NASH+HE | ANOVA | Control vs  NASH | Control vs  NASH+HE | NASH vs  NASH+HE |
|  | n=30 | n=19 | n=7 | prob > F | p-value | p-value | p-value |
| Normalized T1-Singal Ratio |  |  |  |  |  |  |  |
| Globus Pallidus / Cerebral Cortex | 0.96±0.0086 | 1.04±0.011 | 1.11±0.018 | <0.0001 | 0.29 | <0.0001 | <0.0001 |
| Globus Pallidus / Cerebral WM | 1.36±0.023 | 1.42±0.029 | 1.66±0.048 | <0.0001 | <0.001 | <0.0001 | 0.0073 |
| Globus Pallidus / Brainstem | 1.10±0.016 | 1.17±0.020 | 1.21±0.033 | <0.001 | 0.027 | 0.006 | 0.41 |
| Putamen / Cerebral Cortex | 0.84±0.0067 | 0.86±0.0085 | 0.90±0.014 | <0.001 | 0.101 | <0.001 | 0.1 |
| Putamen / Cerebral WM | 1.15±0.020 | 1.17±0.026 | 1.35±0.042 | <0.001 | 0.053 | <0.001 | 0.002 |
| Putamen / Brainstem | 0.93±0.0086 | 0.97±0.011 | 0.98±0.018 | <0.001 | 0.069 | 0.039 | 0.82 |
|  |  |  |  |  |  |  |  |
| Non-normalized T1-Signal Intensity |  |  |  |  |  |  |  |
| Putamen | 72.98±2.92 | 121.35±3.67 | 106.45±6.05 | <0.0001 | <0.0001 | <0.0001 | 0.098 |
| Globus Pallidus | 86.19±3.78 | 146.10±4.75 | 132.83±7.83 | <0.0001 | <0.0001 | <0.0001 | 0.32 |
| Cerebral Cortex | 63.35±2.54 | 103.45±3.19 | 81.22±5.26 | <0.0001 | <0.0001 | 0.0019 | 0.0095 |
| Cerebral WM | 87.25±3.35 | 140.71±4.22 | 119.68±6.95 | <0.0001 | <0.0001 | 0.0003 | 0.033 |
| Brainstem | 78.92±3.23 | 126.08±4.06 | 108.95±6.69 | <0.0001 | <0.0001 | 0.0005 | 0.082 |

Table 1: Normalized T1 SI ratio of globus pallidus and putamen over the cerebral cortex, cerebral white matter, and brain stem and non-normalized T1 SI values of those brain regions (putamen, globus pallidus, cerebral cortex, cerebral WM, and brainstem) show differences among control, NASH and NASH+HE.

A close up of a map

Description automatically generated

Figure 2: Normalized T1 SI of globus pallidus and putamen with respect to cerebral cortex, cerebral white matter and brainstem after adjusted for false discover rate.



Figure 3: Signal intensity without normalization from five regions of interest: putamen (A), globus pallidus (B), cerebral cortex (C), cerebral white matter (D), and brainstem (E) . p values adjusted for false discover rate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | [Ins]a | | | NASH vs NASH+HEb |
|  | r2 | pe±se | p-value | ANOVA |
| Non-normalized T1 SI |  |  |  |  |
| Putamen | 0.4 | 51.6±12.7 | 0.0005 | 0.99 |
| Globus Pallidus | 0.4 | 61.8±16 | 0.001 | 0.50 |
| Cerebral Cortex | 0.6 | 48.7±10.1 | <0.0001 | 0.99 |
| Cerebral WM | 0.5 | 44.3±12.3 | 0.002 | 0.07 |
| Brainstem | 0.3 | 35.3±11.8 | 0.005 | 0.99 |

a: p-value adjusted for between group difference

b: ANOVA between NASH and NASH+HE

pe: parameter estimate

se: standard error

Table 3: General linear regression of myo-inositol (Ins) concentration as dependent variable and non-normalized T1 SI as independent variable. ANOVA analysis of NASH and NASH+HE between group difference are shown in the last column adjusted for Ins concentration.

References:

1. Inoue E, Hori S, Narumi Y, et al. Portal-systemic encephalopathy: presence of basal ganglia lesions with high signal intensity on MR images. *Radiology.* 1991;179(2):551-555.

2. Rovira A, Alonso J, Cordoba J. MR imaging findings in hepatic encephalopathy. *AJNR Am J Neuroradiol.* 2008;29(9):1612-1621.

3. Klos KJ, Ahlskog JE, Kumar N, et al. Brain metal concentrations in chronic liver failure patients with pallidal T1 MRI hyperintensity. *Neurology.* 2006;67(11):1984-1989.

4. Rose C, Butterworth RF, Zayed J, et al. Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction. *Gastroenterology.* 1999;117(3):640-644.

5. Choy G, Khalilzadeh O, Michalski M, et al. Current Applications and Future Impact of Machine Learning in Radiology. *Radiology.* 2018;288(2):318-328.

6. Ashburner J, Neelin P, Collins DL, Evans A, Friston K. Incorporating prior knowledge into image registration. *Neuroimage.* 1997;6(4):344-352.

7. Wang S, Summers RM. Machine learning and radiology. *Med Image Anal.* 2012;16(5):933-951.

8. Shen D, Wu G, Suk HI. Deep Learning in Medical Image Analysis. *Annu Rev Biomed Eng.* 2017;19:221-248.

9. Jiang F, Jiang Y, Zhi H, et al. Artificial intelligence in healthcare: past, present and future. *Stroke Vasc Neurol.* 2017;2(4):230-243.

10. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage.* 1999;9(2):179-194.

11. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological).* 1995;57(1):289-300.

12. Pujol A, Pujol J, Graus F, et al. Hyperintense globus pallidus on T1-weighted MRI in cirrhotic patients is associated with severity of liver failure. *Neurology.* 1993;43(1):65-69.

13. Vymazal J, Babis M, Brooks RA, et al. T1 and T2 alterations in the brains of patients with hepatic cirrhosis. *AJNR Am J Neuroradiol.* 1996;17(2):333-336.

14. Huda A, Gupta RK, Rajakumar N, Thomas MA. Role of Magnetic Resonance in Understanding the Pathogenesis of Hepatic Encephalopathy. *Magn Reson Insights.* 2008;2:109-122.

15. Maeda H, Sato M, Yoshikawa A, et al. Brain MR imaging in patients with hepatic cirrhosis: relationship between high intensity signal in basal ganglia on T1-weighted images and elemental concentrations in brain. *Neuroradiology.* 1997;39(8):546-550.

16. Krieger D, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H. Manganese and chronic hepatic encephalopathy. *Lancet.* 1995;346(8970):270-274.

17. Pinto RB, Froehlich PE, Pitrez EH, et al. MR findings of the brain in children and adolescents with portal hypertension and the relationship with blood manganese levels. *Neuropediatrics.* 2010;41(1):12-17.

18. Iwasa M, Kinosada Y, Nakatsuka A, Watanabe S, Adachi Y. Magnetization transfer contrast of various regions of the brain in liver cirrhosis. *AJNR Am J Neuroradiol.* 1999;20(4):652-654.

19. Tanenbaum LN, Tsiouris AJ, Johnson AN, et al. Synthetic MRI for Clinical Neuroimaging: Results of the Magnetic Resonance Image Compilation (MAGiC) Prospective, Multicenter, Multireader Trial. *AJNR Am J Neuroradiol.* 2017;38(6):1103-1110.

20. Cordoba J, Blei AT. Brain edema and hepatic encephalopathy. *Semin Liver Dis.* 1996;16(3):271-280.

21. Crippin JS, Gross JB, Jr., Lindor KD. Increased intracranial pressure and hepatic encephalopathy in chronic liver disease. *Am J Gastroenterol.* 1992;87(7):879-882.

22. Thuluvath PJ, Edwin D, Yue NC, deVilliers C, Hochman S, Klein A. Increased signals seen in globus pallidus in T1-weighted magnetic resonance imaging in cirrhotics are not suggestive of chronic hepatic encephalopathy. *Hepatology.* 1995;21(2):440-442.

23. Geissler A, Lock G, Frund R, et al. Cerebral abnormalities in patients with cirrhosis detected by proton magnetic resonance spectroscopy and magnetic resonance imaging. *Hepatology.* 1997;25(1):48-54.

24. Kang KM, Choi SH, Hwang M, Yun TJ, Kim JH, Sohn CH. T1 Shortening in the Globus Pallidus after Multiple Administrations of Gadobutrol: Assessment with a Multidynamic Multiecho Sequence. *Radiology.* 2018;287(1):258-266.

25. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2018;67(1):328-357.

26. Choi JW, Moon W-J. Gadolinium Deposition in the Brain: Current Updates. *Korean J Radiol.* 2019;20(1):134-147.

27. Al-Busafi SA, McNabb-Baltar J, Farag A, Hilzenrat N. Clinical manifestations of portal hypertension. *International journal of hepatology.* 2012;2012:203794-203794.

28. Klos KJ, Ahlskog J, Josephs KA, Fealey RD, Cowl CT, Kumar N. Neurologic spectrum of chronic liver failure and basal ganglia t1 hyperintensity on magnetic resonance imaging: Probable manganese neurotoxicity. *Archives of Neurology.* 2005;62(9):1385-1390.

29. Sidoryk-Wegrzynowicz M, Aschner M. Manganese toxicity in the central nervous system: the glutamine/glutamate-gamma-aminobutyric acid cycle. *J Intern Med.* 2013;273(5):466-477.

30. Crombé A, Saranathan M, Ruet A, et al. MS Lesions Are Better Detected with 3D T1 Gradient-Echo Than with 2D T1 Spin-Echo Gadolinium-Enhanced Imaging at 3T. *Am J Neuroradiol.* 2015;36(3):501-507.

31. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage.* 2012;61(4):1402-1418.