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^{1}H Magnetic Resonance Spectroscopy Predicts Hepatocellular Carcinoma in a Subset of Patients With Liver Cirrhosis

A Randomized Trial

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Abstract: The goal of this study was to investigate the utility of ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) to quantify the differences in liver metabolites. Magnetic resonance spectroscopy was used as a means of predicting the probability of developing hepatocellular carcinoma (HCC) in patients with liver cirrhosis secondary to chronic hepatitis B.

This study included 20 healthy volunteers, 20 patients with liver cirrhosis secondary to chronic hepatitis B (cirrhosis group), and 20 patients with small HCC secondary to cirrhosis liver parenchyma (HCC group). All patients underwent routine MRI and ^{1}H-MRS scanning. LCModel software was used to quantify Cho (Choline), Lip (lipid), and Cho/Lip in the 3 groups, and a one-way ANOVA was used to compare the differences in these metabolites between groups.

Choline levels were significantly different between the control and HCC group and between the cirrhosis group and the HCC group (all $P < 0.001$). There was also a significant difference in Lip levels between the control and cirrhosis group and the control and HCC groups (all $P < 0.001$). There were also differences in Cho/Lip between the control and cirrhosis groups, the control and HCC groups, and the cirrhosis and HCC groups (all $P < 0.001$).

^{1}H-MRS followed by the analysis with LCModel can be used to measure changes in hepatic metabolite levels in patients with liver cirrhosis secondary to chronic hepatitis B and HCC. Thus, ^{1}H-MRS may be helpful in monitoring HCC and liver cirrhosis development.

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Abbreviations: ^{1}H-MRS = ^{1}H magnetic resonance spectroscopy, Alb = albumin, ALT = alanine aminotransferase, ANOVA = analysis of variance, AST = aspartate aminotransferase, Cho = choline, DB = direct bilirubin, HCC = hepatocellular carcinoma, Lip = lipid, MRS = magnetic resonance spectroscopy, ROIs = regions of interest, TB = total bilirubin, TP = total protein.

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INTRODUCTION

Liver cirrhosis secondary to chronic hepatitis B can have serious complications. Approximately, 20% to 25% of patients with cirrhosis may develop into hepatocellular carcinoma (HCC), thus monitoring disease progression is clinically important.[1] Liver biopsy remains the gold standard of diagnosing chronic liver disease; however, it is invasive and difficult to get consent from some patients. Other tests, such as serological methods and diagnostic imaging are continuously being developed and improved.[1–3] Magnetic resonance spectroscopy (MRS) is currently the only noninvasive technique that can identify metabolite profiles in vivo, providing quantitative or semiquantitative measures of some metabolites. Magnetic resonance spectroscopy has already been used in central nervous system, breast, and prostate disease, showing significant value in diagnosis malignant tumors.[4] The use of MRS in the liver is relatively understudied, and there is some controversy as to its ability to quantify metabolite levels in liver lesions.[4–6] It is widely accepted that LCModel software could quantify the metabolite in vivo. Its theroy is based on the basis of in vitro test set acquisition, automatic fitting lines, and get metabolite concentrations approximate maximum likelihood estimation. LCModel software could complete baseline correction, the eddy current correction, phase correction, etc., to obtain an ideal fit lines and quantify the metabolite concentrations. In this study, we used MRS and LCModel software to quantify the metabolic changes associated with liver cirrhosis secondary to chronic hepatitis B and to predict the probability of patient progression to develop HCC.

MATERIALS AND METHODS

Study Population

Besides, 3 patients with liver cirrhosis and 2 patients with HCC were excluded because the patients could not hold their breath and the resulting spectrum baseline instability and excessive noise impacted further MRS evaluation. This study enrolled 20 healthy volunteers (control group), 20 patients with liver cirrhosis secondary to chronic hepatitis B (cirrhosis group), and 20 patients with small HCC secondary to cirrhosis (HCC group). The general patient characteristics are described in Table 1. All subjects underwent ultrasound examination to exclude fatty liver. The control group had no history of liver disease and underwent a physical examination, laboratories, and imaging tests to confirm the absence of liver disease. Liver functions tests included analysis of the following parameters:alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (Alb),total bilirubin (TB), direct bilirubin (DB), choline (Chol). Patients diagnosed with cirrhosis were dependent on medical history, clinical

TABLE 1. General Characteristics of the Study Population

	Control Group	Liver Cirrhosis after Chronic B Hepatitis Group	HCC Liver Parenchyma Group	*P*		
				*P*1	P2	*P*3
Number	N = 20	N = 20	N = 20			
Age (years)	45.1 ± 11.9	54.15 ± 7.67	53.35 ± 5.82			
Sex (male/female)	8:12	13:7	12:8			
Child-Pugh (number)						
A	0	8	6			
B	0	12	14			
Total cholesterol (mmol/L)	4.63 ± 1.12	5.56 ± 1.22	5.54 ± 1.27	0.01844	0.01994	0.97496
High-density lipoprotein (mmol/L)	1.05 ± 0.26	1.18 ± 0.25	1.15 ± 0.28	0.11386	0.23241	0.69161
Low-density lipoprotein (mmol/L)	3.01 ± 0.93	3.45 ± 0.73	3.47 ± 0.76	0.0966	0.07662	0.9097

P1, control group and the cirrhosis group; P2, control group and HCC group; P3, cirrhosis group and HCC group. HCC, hepatocellular carcinoma.

manifestations, laboratory tests, and imaging data. Twelve patients of HCC were confirmed by surgery or ultrasound biopsy and 8 patients of HCC were confirmed by imaging diagnosis. All of the patients in the HCC group had tumor less than 3 cm in diameter. Radiologic examination was performed to rule out the presence of significant ascites.

We obtained written informed consent before the MR imaging examinations from volunteers and the patients themselves for the use of their data. This study was reviewed and approved by the Ethics Review Board of the Shanghai Sixth People's Hospital Affiliated with Shanghai Jiao Tong University.

MRS Examinations

All subjects underwent MRS in the early morning before breakfast, fasting at least 8 h, and using the segmented breath-hold method. A 3.0T MR Scanner (Magnetom Verio, Siemens Healthcare, Erlangen, Germany) and 16-channel body coil were used for all examinations. Regions of interest (ROIs) were selected trying to avoid visible blood vessels and bile ducts (Figures 1–3). Magnetic resonance spectroscopy examination using a single-voxel point-resolved spectroscopy sequence included the following parameters: TR 1280 ms; TE 135 ms; averages, 16; voxel size, 20 × 20 × 20 mm. This sequence was done twice, one is unsuppressed water and another is water-suppressed. A conventional automatic prescan was completed with shimming and water suppression, with the method of subsection breathless complete scan, while the saturated zone was placed surrounding the collection range.

Statistics Analysis

A radiologist major in abdominal imaging (with 5 years of experience) manually outlined the ROIs (ROI size is 2 × 2 cm^2) in T2W images to target the raw MR spectrum images, and then using the LCModel software to quantify metabolites of the raw MR spectrum images were by fitting experimental data in the frequency domain.[7,8]

A One-way ANOVA was used to compare differences in the Cho, Lip, and Cho/Lip values between the 3 groups using Fisher test. A $P < 0.05$ was considered to reflect a statistically significant difference between groups.

RESULTS

Magnetic resonance spectroscopy examination was performed in all study participants and the data analyzed using LCModel software to generate data on the level of Cho and Lip in the liver as well as the Cho/Lip ratio (Table 2, Figure 4A-C). A significant difference in the level of Cho was detected between the control and HCC group, and between the cirrhosis and HCC group (all $P < 0.001$). There was no significant difference between the Cho level in the control and cirrhosis group ($P = 0.17$). A significant difference in the level of Lip was detected between the control and cirrhosis groups and the control and HCC groups (all $P < 0.001$). There was no significant difference between the cirrhosis and HCC groups ($P = 0.14$). With regard to the Cho/Lip ratio, there were significant differences between the control and cirrhosis groups ($P < 0.001$), the control and HCC groups ($P < 0.001$), and the cirrhosis and HCC groups ($P = 0.011$).

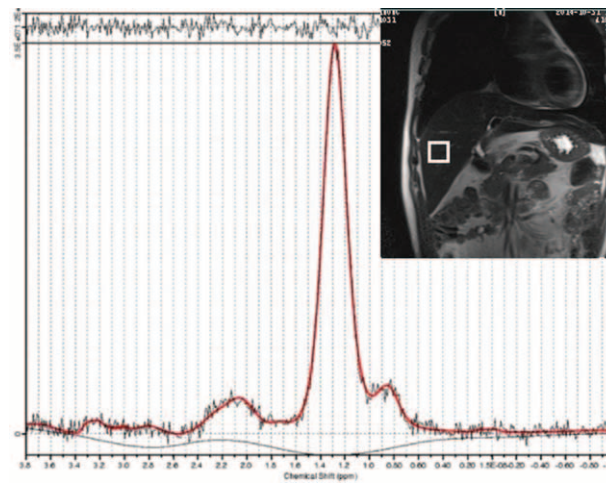

FIGURE 1. ^{1}H magnetic resonance spectrum and voxel localization images of a 48-year-old healthy volunteer. The ROI is targeted to the lesion and measured 2 × 2 cm^2. ^{1}H-MR, ^{1}H magnetic resonance spectroscopy; ROI, region of interest.

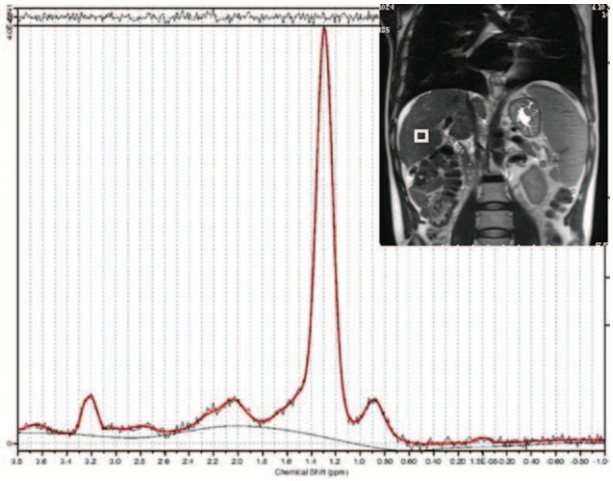

FIGURE 2. ^{1}H magnetic resonance spectrum and voxel localization images of a 58-year-old liver cirrhosis patient secondary to chronic hepatitis B. The ROI is targeted to the lesion and measured $2 \times 2\,cm^2$. ^{1}H-MR,^{1}H magnetic resonance spectroscopy; ROI, region of interest.

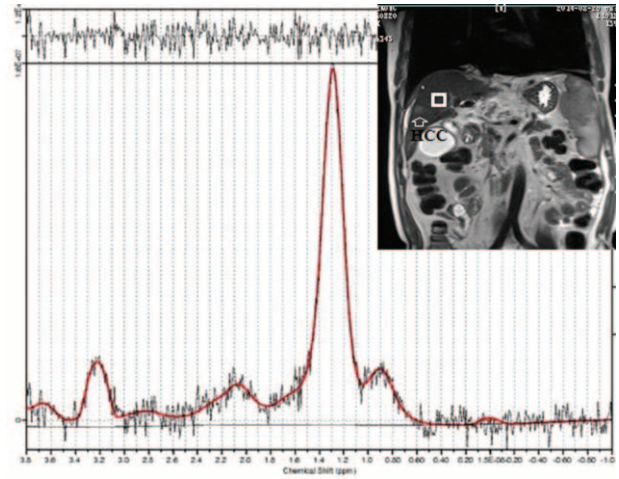

FIGURE 3. ^{1}H magnetic resonance spectrum and voxel localization images of a 57-year-old patient with small HCC. The ROI is targeted to the lesion and measured $2 \times 2\,cm^2$. HCC, hepatocellular carcinoma; ^{1}H-MR,^{1}H magnetic resonance spectroscopy; ROI, region of interest.

DISCUSSION

Chronic hepatitis B is one of the high risk factor for liver cirrhosis and HCC, although how to monitor liver disease progression in these patients is a focus of research in the field. It is believed that the main reason that chronic hepatitis B predisposes the patient to the development of HCC is the fact that the DNA of the hepatitis B virus directly integrates in the host DNA. The proteins encoded by this viral DNA then interfere with liver cell signaling pathways and can promote uncontrolled cell growth and carcinogenesis.[9,10] The goal of this study was to explore the possibility of using metabolic changes in the cirrhotic liver (ie, Cho and Lip levels) to predict the occurrence of HCC. We chose to study Cho because its levels are directly related to cell proliferation, which is itself linked with carcinogenesis.

Cho is one of the components of the cell membrane associated with phospholipid metabolism and is an effective indicator of cell proliferation. In normal liver tissue and benign tumors, Cho levels are low, but Cho are high in cancer.[11] Our MRS analysis of Cho revealed that in the HCC group, Cho levels were significantly higher than the control and cirrhosis groups. Indeed, the HCC group had the highest Cho content. We interpret the high level of Cho in the HCC group as indicating that these livers were undergoing continuous cell proliferation—characteristic of carcinogenesis—which coincided with the histopathology. The cirrhosis group had a higher Cho content than the normal group, but this difference was not statistically significant. The variable increase in Cho levels in the cirrhosis group may reflect the fact that some patients had entered the stage of liver cell proliferation associated with the future development of HCC, whereas other patients in the cirrhosis group had a relatively stable stage of liver cell regeneration while other patients may have been experiencing the continuous destruction of liver cells and entering liver failure.

The liver is the primary organ that mediates lipid metabolism and lipid transport in the body. Lipid metabolism largely depends on normal liver function. In patients with liver disease such as cirrhosis, the damage of the liver cells is bound to affect lipid synthesis, resulting in abnormal lipid metabolism.[3,4,12] With regard to our analysis of hepatic Lip levels, we found no significant difference between the cirrhosis group and the HCC group, although the level of Lip in these 2 groups were significantly lower than the control group.

With regard to the Cho/Lip ratio, in both the cirrhosis group and the HCC group, the Cho/Lip levels were significantly higher than the control group, although there was no significant difference between the cirrhosis and HCC groups. This finding is consistent with previous studies.[13,14] For example, Kuo et al[13] concluded that the Cho/Lip ratio in benign lesions is significantly lower than HCC tissues.[14] On the contrary, Dong et al[14]

TABLE 2. MRS Results and Comparison Between Study Groups

	Control Group	Liver Cirrhosis After Chronic B Hepatitis Group		HCC Liver Parenchyma Group			*P*	
						*P*1	*P*2	*P*3
Cho (a.u.)	8.8791	3.06151	11.20322	5.03483	21.93529	6.9783	0.1688	<0.001	<0.001
Lip (a.u.)	720.8368	283.8721	298.9156	90.01525	387.6701	128.5421	<0.001	<0.001	0.13946
Cho/Lip	0.01471	0.00815	0.04229	0.02827	0.06504	0.03745	0.00244	<0.001	0.01133

P1, control group and the cirrhosis group; P2, control group and HCC group; P3, cirrhosis group and HCC group. Cho, choline; HCC, hepatocellular carcinoma; Lip, lipid; MRS, magnetic resonance spectroscopy.
a.u. is the unit of measurement by LCmodel software.

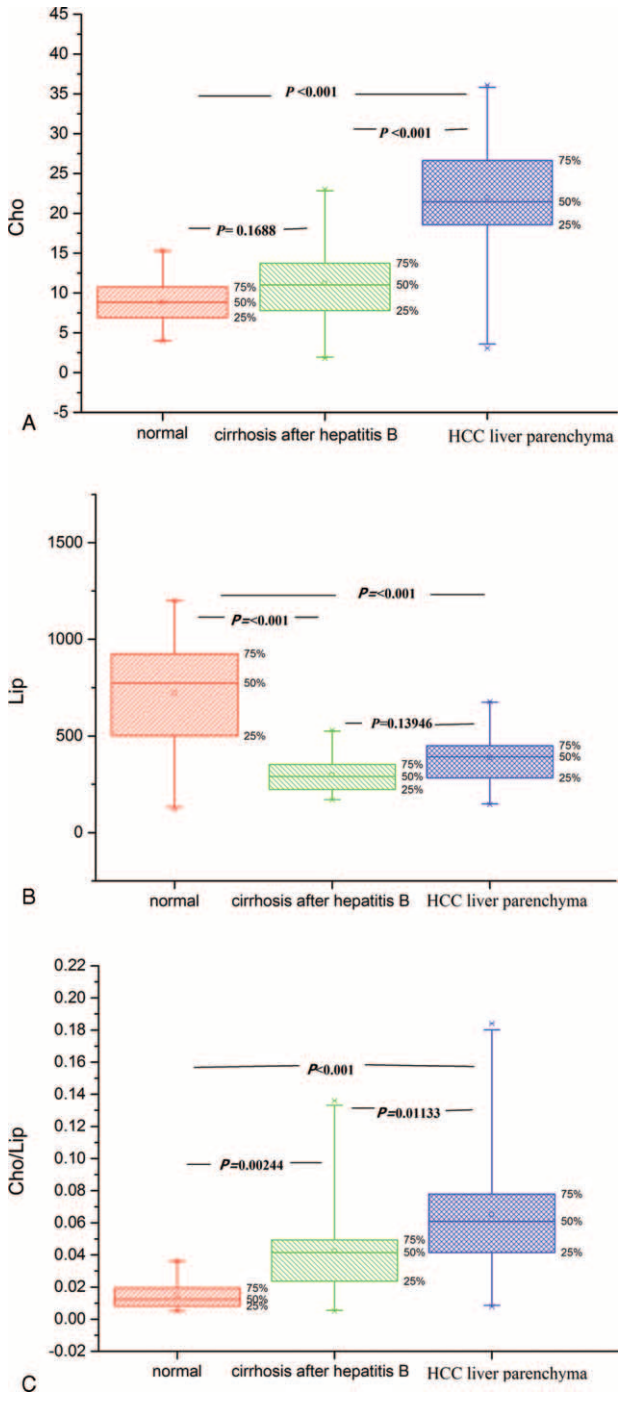

FIGURE 4. Data showing the Cho (A), Lip (B), and Cho/Lip ratio (C) in the control, cirrhosis, and HCC liver parenchyma groups are also presented. Cho, choline; HCC, hepatocellular carcinoma; Lip, lipid.

also showed that the Cho/Lip ratio was significantly higher in HCC tissue compared with those associated with cholangiocarcinoma or normal livers.[13] Although Fischbach et al[15] reported that Lip levels in malignant liver tumors showed no significant difference versus the liver parenchyma. Given that there may be big differences in lipid metabolism between patients, the variable results reported regarding the Cho/Lip ratio in patients with cirrhosis and HCC is understandable. We speculate that our results can be explained by the fact that we excluded patients with fatty liver in our study, thus lipid metabolism in our patient population was basically at the same level. This can be seen from the blood lipid indicators in Table 1. It has previously been reported that Lip-related indicators of liver cirrhosis and HCC showed no significant differences;[12] therefore, we believe that the Cho level is the determining factor with regard to disease progression.

At the time of this writing, the biggest controversy regarding MRS to assess liver metabolism is that the constant changes in metabolism are dependent on blood flow changes.[3,12,16] This variability can be caused by eating, exercise, and time of the day. We selected the early morning to carry out our MRS analyses in this study because early morning is a relatively stable period in liver metabolism and is less affected by other factors. In this study, patients with liver function rated grade A-B in the Child-Pugh scale were selected and patients with fatty liver were excluded. This was done to exclude the effect of the other original metabolites, whereas this study was selected for hepatitis B patients with relatively consistent pathologic outcomes.

In this study, we used ^{1}H-MRS to reflect pathologic metabolism associated with liver cirrhosis secondary to hepatitis B and HCC. This method has previously been applied to evaluate the early response after chemoembolization of HCC, whereas ATP/Pi is often chosen as a useful indictor in ^{31}P-MRS studies[17–19]; ^{13}C-MRS metabolites has been used in a rat HCC model to measure pyruvate.[20] In this study, ^{1}H-MRS examination technical success rate was high and possible reasons for this include using a large area of interest to ensure acquiring a strong MRS signal; use of the segmented breath-hold method to ensure constant data acquisition and reduce the impact of respiratory motion; saturated zone with the protection of the area of interest, and shielding the signal around the chemical substances. Former prescan spectral data were collected to ensure uniformity of the magnetic field and the degree of suppression water standards; single-voxel technology was selected and the scanning time was relatively short thus less affected by movement.

Our study had the following limitations: the uneven distribution of liver disease within the liver may lead to errors in our results, although multipoint scanning was used. We did not use multi-voxel scanning because it requires much longer scanning time and therefore longer breath-holding time, which was difficult in our patient population. This study excluded patients with a fatty liver. We also excluded patients with ascites because these individuals could not hold their breath for a long time, which is required for the MR scanning. The study also enrolled only a relatively small number of cases, thus further study in a larger sample size is needed.

In conclusion, using MRS to quantify hepatic Cho, Lip, and Cho/Lip levels in patients with cirrhosis secondary to chronic hepatitis B and HCC, we found that the patients with higher Cho value may be more likely to develop liver cancer and need follow-up review.

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