Liver iron overload and fat content analyzed by magnetic resonance contribute to evaluating the progression of chronic hepatitis B

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Abstract. Chronic hepatitis B (CHB) and its complications still have a major role in liver-related mortality. It has been indicated that hepatic iron and steatosis may influence liver fibrosis and carcinogenesis. The present study aimed to assess the liver iron and fat in patients with CHB by MRI in order to estimate the associations among liver iron, fat and the severity and progression of liver fibrosis. In the present retrospective study, consecutive patients with CHB examined from August 2018 to August 2020 were analyzed. Liver iron and fat content were assessed by MRI, which was measured as liver iron content (LIC) and proton density fat fraction (PDFF). A total of 340 patients were included in the current study. For LIC, the median value was 1.68 mg/g and elevated LIC was seen in 122 patients (35.9%). For liver fat content, the median value of PDFF was 3.1%, while only 15.0% of patients had liver steatosis (PDFF ≥5%). Age, total bilirubin and sex were independent predictive factors of liver iron overload [odds ratio (OR)=1.036, 1.005 and 8.834, respectively]. A higher platelet count (OR=1.005) and no portal hypertension (OR=0.381) independently predicted liver steatosis. The areas under the receiver operating characteristic curves of PDFF for the identification of liver cirrhosis estimated by different non-invasive tools ranged from 0.629 to 0.704. It was concluded that iron overload was common in patients with CHB, particularly in those with older age, male sex and high total bilirubin level, and liver steatosis was less common in CHB. Liver iron and fat content analyzed by MRI may contribute to the evaluation of the severity and progression of CHB.

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Abbreviations: CHB, chronic hepatitis B; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; PVD, portal vein diameter; SVD, splenic vein diameter; LIC, liver iron content; MRI, magnetic resonance imaging; PDFF, proton density fat fraction; PH, portal hypertension; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; PLT, platelet count; TB, total bilirubin; ALB, albumin; INR, international normalized ratio; FER, serum ferritin; Cr, creatinine; FIB-4, fibrosis index based on four factors; APRI, AST-to-PLT ratio index; ALBI, ALB-bilirubin index; AAR, AST-to-ALT ratio; GPR, GGT-to-PLT ratio; OR, odds ratio

Key words: chronic hepatitis B, liver iron overload, liver steatosis, magnetic resonance

Introduction

Chronic hepatis B (CHB) remains the main cause of liver cirrhosis and liver cellular carcinoma (1). The progress of liver fibrosis and hepatic cellular carcinoma (HCC) is often unpredicted due to viral and host factors (2). It has been indicated that hepatic iron and steatosis may have a role in liver fibrosis and carcinogenesis (3-5). Iron overload is common in hemochromatosis, which is one of the etiologies of cirrhosis, but it may also worsen liver injury in other chronic liver diseases (3,4). Certain studies suggested that elevated serum ferritin (FER) or liver iron were associated with a diminished likelihood of response to antiviral therapy (6-8). A further study examined the association between hepatic iron grade and HCC in patients with end-stage liver disease of diverse etiologies, indicating that any iron overload was significantly associated with HCC (3). However, the prevalence and clinical significance of iron overload in CHB have remained elusive. For liver steatosis, the prevalence of non-alcoholic fatty liver disease (NAFLD) is currently increasing, and so is the coexistence of NAFLD and hepatitis B virus (HBV) infection (5). However, the interplay between these two diseases remains unclear (9). Both may lead to liver injury and augment the risk of liver cirrhosis and HCC. Conversely, NAFLD may have a positive role in HBV antiviral therapy (10-13). More information on the prevalence of NAFLD in CHB and its relationship with the progress of the underlying disease is needed.

Histopathological visualization of hepatocellular fat droplets remains the gold standard for the assessment of liver steatosis, as well as the liver iron concentration. However, it is an invasive method that may potentially be associated with significant complications, such as infection and

bleeding (14). On the other hand, it also has disadvantages including being semi-quantitative, prone to sampling variability and observer-dependent (15). MRI is a non-invasive tool that can measure liver iron and fat by R2* relaxometry and proton density fat fraction (PDFF) (16). In the present study, it was attempted to measure the content of liver iron and fat noninvasively by MRI and then compare them with the clinical characteristics, to predict the prevalence of iron overload and NAFLD in CHB and the relationships between iron overload, NAFLD, the severity of liver fibrosis and progression of CHB.

Patients and methods

Study design and participants. the study protocol was approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University [approval no. (2022)02-328-01]. The requirement of written informed consent was waived. Patients with CHB were retrospectively enrolled consecutively from August 2018 to August 2020. CHB was defined as positive hepatitis B surface antigen (HBsAg) or HBV DNA for at least 6 months (17). The exclusion criteria are provided in Appendix S1.

Data collection. Baseline demographic, clinical and laboratory characteristics, along with MRI features, were collected. The following data were included: Age, sex, clinical presentation and blood biochemical indices. The fibrosis index based on four factors (FIB-4), aspartate aminotransferase (AST) to platelet (PLT) ratio index (APRI), albumin (ALB)-bilirubin score (ALBI), AST-alanine aminotransferase (ALT) ratio (AAR) and gamma glutamyl transpeptidase (GGT)-PLT ratio (GPR) were calculated (Table SI) (18-22).

MRI examination. MRI examination was performed at the same hospitalization within 30 days. The details of the MRI scanning and parameters are presented in Appendix S2. The percentage of liver fat content was measured under the fat fraction sequence estimated by MRI-PDFF, which does not exceed 5% in normal individuals (23). The iron content was measured by R2* relaxation rate image sequence and the liver iron content (LIC) was then measured according to Wood formula [(Fe) mg/g=R2* x 0.0254 + 0.202] (24). The severity of iron overload was rated as follows: No iron overload (<2 mg/g), insignificant (2-4 mg/g), mild (4-6 mg/g), moderate (6~8 mg/g), moderate-severe (8-16 mg/g) or severe (≥16 mg/g) (25). Portal hypertension (PH) was defined as portal vein or splenic vein dilation [portal vein diameter (PVD) >12 mm or splenic vein diameter (SVD) >8 mm] (26).

Statistical analysis. Quantitative variables were presented as the mean ± standard deviation or median (interquartile range) based on whether the data followed a normal distribution. Categorical variables were compared using the Chi-square or Fisher's exact test when appropriate, and quantitative variables were compared using Student's t-test or the Mann-Whitney U-test, as applicable. Correlation analysis was performed with Pearson's correlation test. Predictive factors of NAFLD and iron overload were evaluated using the 'enter' multivariate binary logistic regression model. Receiver operating characteristic (ROC) curve analysis was performed to identify the discriminative capacity of PDFF and LIC levels in predicting the degree of liver fibrosis, as well as FER levels in predicting the degree of liver iron overload. P-values for ROC curves were identified based on Wilcoxon's test and the Delong test was used to compare the area under the receiver operating characteristic curves (AUCs). A two-tailed $P<0.05$ was considered to indicate statistical significance. All data were analyzed by SPSS version 22.0 software (IBM Corp.) and R version 4.1.2 (R Core Team).

Results

Patient characteristics. Within the enrolment period for the study, 378 patients met the criteria of inclusion. Of these, 38 patients were excluded based on the exclusion criteria. As a result, 340 patients were available for analysis. Table I shows the characteristics of these patients. The mean age was 50.6±10.4 years (range, 18-77 years) with a male-to-female ratio of 6:1. The LIC had a median value of 1.68 mg/g, ranging from 0.79 to 9.90 mg/g and elevated LIC (LIC ≥2 mg/g) was seen in 122 patients (35.9%), while the prevalence of insignificant, mild, moderate and moderate-severe degree of iron overload was 28.2, 5.3, 1.8, 0.6%, respectively. Regarding the liver fat content, the median value of PDFF was 3.1%, ranging from 1.2 to 30%, while only 15.0% of patients had liver steatosis (MRI-PDFF ≥5%). Representative MRI images for liver iron and fat measurement are provided in Fig. 1.

Correlation between MRI features. After the intra-group consistency analysis and inter-group consistency analysis, the intra-class correlation values were 0.977 (95%CI 0.964-0.990) and 0.962 (95%CI 0.942-0.982), respectively. The correlation between PVD, SVD, LIC and PDFF were explored. PVD and SVD had a moderate correlation ($r=0.686$, $P<0.001$), while a slight negative correlation was observed between LIC and SVD ($r=-0.161$, $P=0.003$) (Fig. S1). There were no linear correlations between the LIC and PDFF, LIC and PVD, PVD and PDFF or SVD and PDFF ($r=0.016$, $P=0.773$; $r=-0.104$, $P=0.056$; $r=-0.082$, $P=0.129$; $r=-0.084$, $P=0.124$, respectively).

Correlation between MRI features and serum parameters and indices. The correlation between MRI features and serum indices was investigated using Pearson's correlation analysis. The PDFF showed a positive association with PLT and ALB ($r=0.240$, $P<0.001$; $r=0.214$, $P<0.001$. respectively), and a negative association with FIB-4, APRI, ALBI, AAR and GPR score ($r=-0.224$, $P<0.001$; $r=-0.164$, $P=0.002$; $r=-0.245$, $P=0.002$; $r=-0.146$ $P=0.007$; $r=-0.111$, $P=0.042$, respectively), The LIC was positively associated with AST, alkaline phosphatase, total bilirubin (TB), international normalized ratio (INR), FIB-4, APRI and ALBI score ($r=0.143$, $P=0.008$; $r=0.143$, $P=0.019$; $r=0.248$, $P<0.001$; $r=0.315$, $P<0.001$; $r=0.114$, $P=0.035$; $r=0.119$, $P=0.029$; $r=0.260$, $P<0.001$, respectively), and negatively associated with ALB ($r=-0.146$, $P=0.007$). For the PVD, positive correlations were indicated with FIB-4, APRI and GPR ($r=0.184$, $P=0.001$; $r=0.132$, $P=0.015$; $r=0.114$, $P=0.037$, respectively) while it was negatively associated with PLT ($r=-0.290$, $P<0.001$). For the SVD, a positive correlation was only obtained with FIB-4 ($r=0.178$, $P=0.001$) while a

Table I. Baseline clinical and MRI characteristics of the patients.

Variable[a]	Value
Age, years	50.6±10.4
Males/females	292/48
LIC, mg/g	1.68 (1.35, 2.41)
PDFF, %	3.1(2.5, 3.9)
PVD, cm	14.5±2.70
SVD, cm	9.57±2.59
ALT, U/l	34.5 (24.0, 59.0)
AST, U/l	41.0 (27.0, 63.0)
GGT, U/l	61.0 (32.5, 124.0)
ALP, U/l	92.0 (72.0, 123.0)
PLT, 10^9/l	92.0 (63.0, 143.0)
TB, μmol/l	17.8 (10.9, 42.7)
ALB, g/l	39.4±6.20
INR	1.44±0.46
FER, ng/ml	440.7 (160.5, 1446.0)
Cr, μmol/l	75.2±21.9
FIB-4	4.17 (2.24, 6.94)
APRI	1.28 (0.67, 2.20)
ALBI	-2.44±0.70
AAR	1.27±0.63
GPR	0.76 (0.33, 1.39)

Values are expressed as the median (interquartile range) or the mean ± standard deviation. [a]Normal ranges of the laboratory variables: ALT, 3-35 U/l; AST, 15-40 U/l; GGT, 10-60 U/l; ALP, 45-125 U/l; PLT, 100-350 10^9/l; TB, 4.0-23.9 μmol/l; FER, 29-322 μmol/l; Cr, 31.8-116.0 μmol/l. LIC, liver iron content; PDFF, proton density fat fraction; PVD, portal vein diameter; SVD, splenic vein diameter; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; ALP, alkaline phosphatase; PLT, platelet count; TB, total bilirubin; ALB, albumin; INR, international normalized ratio; FER, serum ferritin; Cr, creatinine; FIB-4, fibrosis index based on four factors; APRI, AST-to-PLT ratio index; ALBI, ALB-bilirubin index; AAR, AST-to-ALT ratio; GPR, GGT-to-PLT ratio.

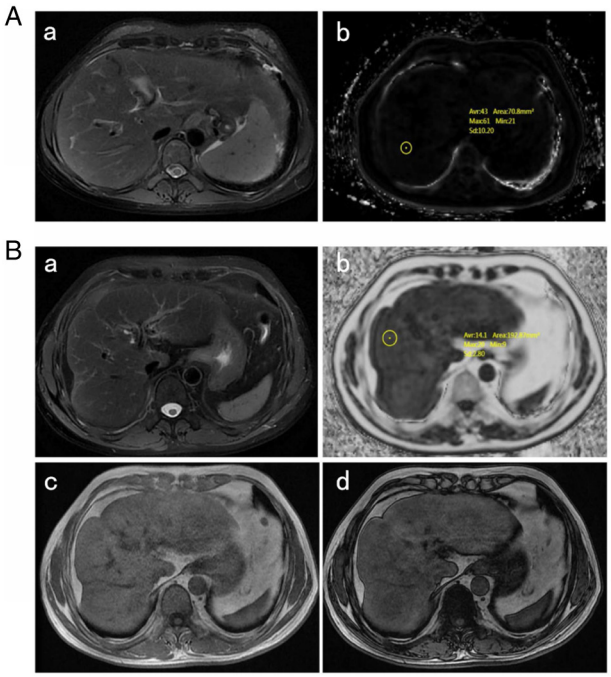

Figure 1. Examples of liver iron and fat measured by magnetic resonance imaging. (A) A 52-year-old male, hepatitis B cirrhosis for 22 years. (a) Axial T2WI and (b) R2* map of the IDEAL-IQ sequence shows the liver iron overload (R2* value, 301.2). (B) A 43-year-old male, hepatitis B cirrhosis for 17 years. (a) Axial T2WI and (b) FF map of the IDEAL-IQ sequence show that liver steatosis was observed as increased signal intensity (FF value, 14.1%). (d) The liver signal in the out-phase sequence is unevenly decreased in comparison to (c) that in the in-phase sequence, indicating that there is uneven liver steatosis in liver parenchyma. T2WI, T2-weighted imaging; FF, fat fraction; IDEAL-IQ, iterative decomposition of water and fat with echo asymmetry and least squares estimation quantification.

negative correlation with PLT, creatinine and FER (r=-0.325, P<0.001; r=-0.148, P=0.009; r=-0.227, P=0.006, respectively) was determined (Fig. S2).

Univariate and multivariate analysis between MR features and serum indices. In the liver steatosis subgroup (PDFF ≥5%), sex, PLT, ALB, proportion of PH, FIB-4, APRI, ALBI and GPR were significantly different from those without steatosis in the univariate analysis. Sex, age and factors such as ALT, AST, TB, ALB, PLT and PH were included in the multivariate logistic regression analysis, revealing that a higher PLT count [OR=1.005 (95%CI: 1.000 to 1.009), P=0.041] and PH [OR=0.381 (95%CI: 0.177 to 0.820, P=0.014)] independently predicted liver steatosis (Table II).

In the univariate analysis of the subgroup of liver iron overload (LIC ≥2 mg/g), sex, age, ALT, AST, TB, ALB and ALBI showed significant differences from those without iron overload. Sex, age and factors such as ALT, AST, TB, ALB, PLT and PH were included in the multivariate logistic regression analysis. Age, TB and sex were significant independent predictive factors of liver iron overload [OR=1.036 (95%CI: 1.011 to 1.062), P=0.005; 1.005 (95%CI: 1.002 to 1.009), P=0.004; 8.8344 (95%CI: 2.931 to 26.62), P<0.001, respectively] (Table III).

PDFF and LIC for predicting liver cirrhosis. Liver cirrhosis was previously identified by FIB-4 ≥3.25, APRI ≥2, ALBI ≥-2.190, AAR ≥1 or GPR >0.56 (18-22). As seen in Fig. 2, at different levels of FIB-4, APRI, ALBI, AAR and GPR, the PDFF showed significant differences in each of the two groups, while for LIC, significant differences were obtained in the APRI and ALBI groups. The ROC curves of LIC and PDFF for the identification liver cirrhosis estimated by different serum indices are shown in Fig. 3. The areas under the ROC curves for PDFF were 0.677 (95% CI: 0.620 to 0.734, P<0.001), 0.708 (95% CI: 0.647 to 0.768, P<0.001), 0.704 (95% CI: 0.647 to 0.768, P<0.001), 0.629 (95% CI: 0.568 to 0.689, P<0.001), 0.635 (95% CI: 0.575 to 0.695, P<0.001), respectively; while for LIC, the areas under the ROC curves were 0.574 (95% CI: 0.502 to 0.645, P=0.0328) (APRI ≥2 as cut-off value) and 0.637 (95% CI: 0.570 to 0.703, P<0.001) (ALBI ≥-2.190 as cut-off value). After logistic regression, predictive models for liver cirrhosis using a joint indicator of LIC and PDFF were established (liver cirrhosis was identified by APRI ≥2 and ALBI ≥-2.190). The areas under

Table II. Predictors of prevalence of fatty liver in patients with chronic hepatitis B.

	Univariate analysis			Multivariate analysis	
Variable	Fatty liver subgroup (n=51)	Non-fatty liver subgroup (n=289)	P-value	OR (95%CI)	P-value
Sex (male/female)	39/12	253/36	0.036	1.56 (0.688, 3.536)	0.287
Age, years	49.8±9.87	50.7±10.5	0.564	0.999 (0.968, 1.030)	0.941
ALT, U/l	39.5±27.6	61.6±154.6	0.310	1.007 (0.992, 1.021)	0.368
AST, U/l	39.0±22.5	66.5±108.9	0.074	0.984 (0.964, 1.004)	0.117
PLT, 10^9/l	148.0±78.8	103.0±64.5	<0.001	1.005 (1.000, 1.009)	0.041
TB, μmol/l	29.4±100.3	56.5±97.0	0.068	1.000 (0.995, 1.005)	0.935
ALB, g/l	42.7±6.42	38.8±5.99	<0.001	1.056 (0.992, 1.124)	0.090
PH (yes/no)	36/15	252/37	0.002	0.381 (0.177, 0.820)	0.014
FIB-4	3.22±2.72	5.76±5.07	0.001	-	-
APRI	0.97±0.95	2.18±3.42	0.013	-	-
ALBI	-2.88±0.64	-2.36±0.68	<0.001	-	-
AAR	1.13±0.55	1.30±0.64	0.101	-	-
GPR	0.73±0.91	1.11±0.05	0.016	-	-

Values are expressed as n or the mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet count; TB, total bilirubin; ALB, albumin; PH, portal hypertension; FIB-4, fibrosis index based on four factors; APRI, AST-to-PLT ratio index; ALBI, albumin-bilirubin index; AAR, AST-to-ALT ratio; GPR, gamma glutamyltransferase-to-platelet ratio; OR, odds ratio.

Table III. Predictors of prevalence of liver iron overload in patients with chronic hepatitis B.

	Univariate analysis			Multivariate analysis	
Variable	Iron overload subgroup (n=122)	No iron overload subgroup (n=218)	P-value	OR (95%CI)	P-value
Sex (male/female)	118/4	174/44	<0.001	8.834 (2.931, 26.62)	<0.001
Age, years	52.4±9.48	49.6±10.8	0.019	1.036 (1.011, 1.062)	0.005
ALT, U/l	86.2±231.0	42.7±39.5	0.007	1.005 (0.995, 1.014)	0.322
AST, U/l	88.93±153.6	47.4±47.1	<0.001	1.002 (0.993, 1.012)	0.623
PLT, 10^9/l	111.9±66.7	108.6±69.8	0.676	1.003 (0.999, 1.007)	0.180
TB, μmol/l	87.1±130.0	33.1±66.9	<0.001	1.005 (1.002, 1.009)	0.004
ALB, g/l	38.5±6.22	39.9±6.15	0.039	0.997 (0.952, 1.043)	0.891
PH (yes/no)	102/20	186/32	0.674	0.778 (0.386, 1.571)	0.484
FIB-4	5.97±5.23	5.05±4.64	0.093	-	-
APRI	2.44±2.98	1.74±3.31	0.054	-	-
ALBI	-2.21±0.74	-2.57±0.64	0.000	-	-
AAR	1.31±0.62	1.25±0.64	0.404	-	-
GPR	1.11±0.97	1.03±1.08	0.463	-	-

Values are expressed as n or the mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet count; TB, total bilirubin; ALB, albumin; FIB-4, fibrosis index based on four factors; APRI, AST-to-PLT ratio index; ALBI, albumin-bilirubin index; AAR, AST-to-ALT ratio; GPR, gamma glutamyltransferase-to-PLT ratio; OR, odds ratio.

the ROC curves for each predictive model were 0.717 (95% CI: 0.657-0.777) and 0.696 (95% CI: 0.636-0.757), P<0.001, respectively (Fig. S3). Both AUCs were higher than those of LIC only (P<0.004, <0.001), while there was no significant difference when compared with that of PDFF only (P=0.562, 0.812).

Association of FER with LIC. A total of 145 patients had FER assessment at the same time. FER elevation (upper limit of normal is 322 ng/ml) was seen in 88 patients (60.7%). A significant linear correlation was observed between FER and LIC (r=0.623, P<0.001) (Fig. S4A). When the ROC curve was

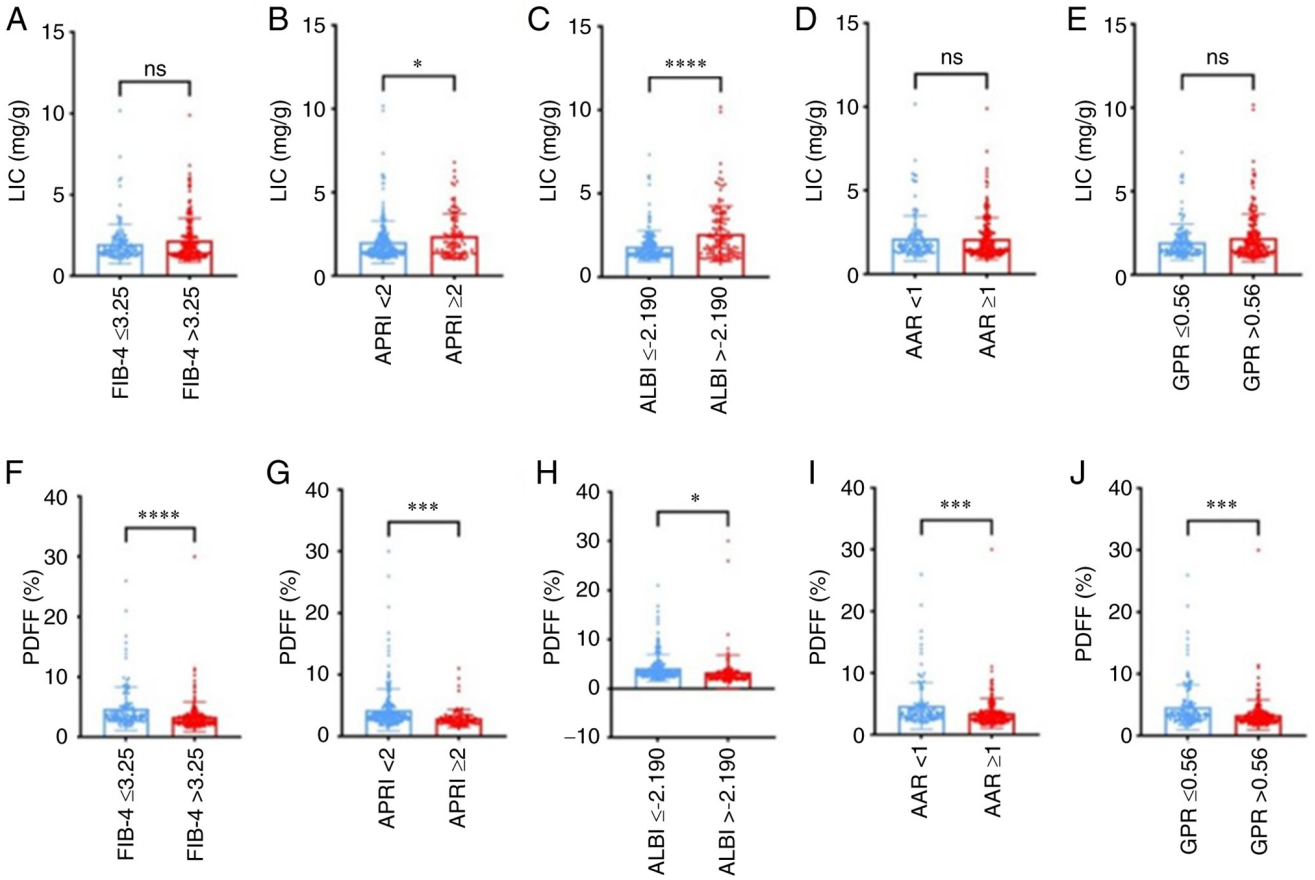

Figure 2. LIC and PDFF level in each liver fibrosis score estimated by FIB-4, APRI, ALBI, AAR and GPR. (A-E) LIC level estimated by (A) FIB-4, (B) APRI, (C) ALBI, (D) AAR and (E) GPR. There was no significant difference in LIC levels between two groups except for the APRI and ALBI (P=0.0218 and 0.001, respectively). (F-J) PDFF level in each liver fibrosis score estimated by (F) FIB-4, (G) APRI, (H) ALBI, (I) AAR and (J) GPR. Significant differences in PDFF levels were found between different groups according to FIB-4, APRI, ALBI, AAR and GPR (P<0.001, <0.001, 0.013, <0.001 and <0.001, respectively). ns, P>0.05; *P≤0.05; ***P≤0.001; ****P≤0.0001. LIC, liver iron content; PDFF, proton density fat fraction; FIB-4, fibrosis index based on four factors; APRI, aspartate aminotransferase-to-platelet ratio index; ALBI, albumin-bilirubin index; AAR, AST-to-ALT ratio; GPR, gamma glutamyltransferase-to-platelet ratio.

plotted to study the performance of FER for predicting liver iron overload, the AUC was 0.858 (95%CI: 0.790 to 0.910, P<0.001) (Fig. S4B), while the specificity and sensitivity was 90.6 and 70.0%, respectively, with a cut-off value of 885.3 ng/ml. According to the ROC curve, the patients were divided into two groups based on their FER status [FER ≥885.3 ng/ml (n=50) and FER <885.3 ng/ml (n=95)]. The data showed that the patients in the group with FER ≥885.3 ng/ml had higher levels of ALT, AST and TB and INR. The PVD and SVD were slightly but significantly lower in the same group (Table SII).

Discussion

The present study indicated that iron overload was common in CHB with a prevalence of 35.9%, particularly in those with older age, male sex and higher TB. The prevalence of NAFLD in patients with CHB was 15.0%, particularly in those with a high platelet count and without PH. There appeared to be a weak correlation between LIC and liver fibrosis, with a slight diagnostic ability for cirrhosis with the AUCs ranging from 0.5 to 0.7. The diagnostic ability of PDFF to distinguish cirrhosis from non-cirrhosis stages was slight to moderate and the AUCs were between 0.6 and 0.8.

Martinelli *et al* (27) found hepatic iron deposits in 48.7% of cases among 39 patients with HBV measured by liver biopsy, while Ko *et al* (3) examined the prevalence of hepatic iron overload in 5,224 patients undergoing liver transplantation; only 13.3% of patients with HBV infection had liver iron overload. Several studies have evaluated iron overload in hepatitis C (HCV), with an increased liver iron concentration in 10-36% of patients (28,29). Ito *et al* (30) evaluated MR images for diffuse hepatic iron deposition, indicating that 40% were positive in cirrhotic patients with HCC. The differences among those studies may be due to the various criteria used to define iron stores.

FER is regarded as the primary tissue iron-storage protein in the liver, which is induced in iron overload disorders of various etiologies, resulting in increased hepatic and circulating FER levels (31). Hyperferritinemia has been observed in chronic liver disease due to HCV and alcohol consumption (32-34), but its relationship with hepatic iron deposition in such situations has remained elusive. In the present study, FER elevation was seen in more than half of the patients with CHB, which had a strong correlation with the liver iron concentration measured by MRI. Ripoll *et al* (35) reported that 59% of cirrhotic patients had increased FER, which was similar to the present study. Furthermore, the markers of liver

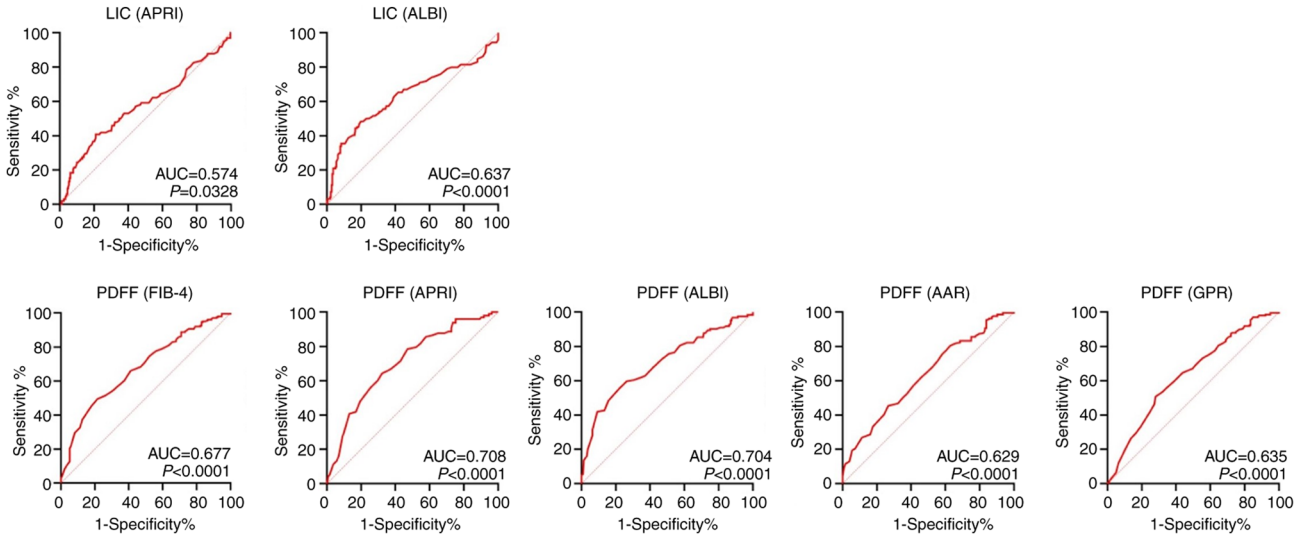

Figure 3. ROC curves of LIC and PDFF for the identification of liver cirrhosis estimated by different non-invasive fibrosis assessment tools (FIB-4 ≥3.25, APRI ≥2, ALBI ≥-2.190, AAR ≥1, GPR0.56) among patients with chronic hepatitis B. The diagonal line represents detection achieved by chance alone (AUC=0.50); the ideal AUC is 1.00. The AUCs for LIC were 0.574 (LIC-APRI, P=0.0328), 0.637 (LIC-ALBI, P<0.001), the areas under the ROC curves of PDFF were 0.677 (P<0.001), 0.708 (P<0.001), 0.704 (P<0.001), 0.629 (P<0.001) and 0.635 (P<0.001), respectively. LIC, liver iron content; PDFF, proton density fat fraction; FIB-4, fibrosis index based on four factors; APRI, aspartate aminotransferase-to-platelet ratio index; ALBI, albumin-bilirubin index; AAR, AST-to-ALT ratio; GPR, gamma glutamyltransferase-to-platelet ratio; ROC, receiver operating characteristic; AUC, area under the ROC curve.

failure, such as bilirubin and INR score, were observed to be significantly different in those patients with elevated FER levels. The markers of liver inflammation, such as AST and ALT, were also elevated in the high-level FER group, but were not independent factors that predicted liver iron overload. It may be explained by FER being induced by systemic inflammation, so that the situation of HBV replication and other inflammation may lead to increased FER. This suggests that when the FER level is used to evaluate the liver iron overload, the inflammation status should be considered.

Several physiological mechanisms, particularly reactive oxygen species accumulation and damage, may explain the iron overload in liver fibrosis; low to moderate levels of excess iron are sufficient to support the pathological progression (36). A previous study indicated that iron could increase HBV mRNA expression in HepG2 cells (37), which may contribute to sustenance of infection and inflammation, thereby potentiating fibrosis. The iron-related parameters aid in the prediction, diagnosis, staging and prognosis of liver fibrosis, when used in combination with the routine markers of liver dysfunctionality. Metwally *et al* (38) found that increased hepatic iron deposition may be associated with more advanced hepatic fibrosis in patients with CHC infection. Martinelli *et al* (27) also demonstrated that patients of CHB with liver iron deposits exhibited significantly higher scores for necroinflammatory activity and fibrosis than those without iron deposits. In addition, patients with moderate liver iron deposits had a significantly higher histologic activity index (12.8±3.2 and 7.3±3.7, respectively, P=0.001) and liver fibrosis (2.3±0.8 and 1.5±0.6, respectively, P=0.02) scores compared with those with absent or mild liver iron deposits. In the present study, the LIC was not strongly positively associated with the severity of liver fibrosis, which was opposite to the above studies. The reason may be that the LIC does not reflect the whole iron overload status and extrahepatic iron load can also lead to the progression of liver fibrosis. Further studies are needed to evaluate the value of combining iron deposition parameters and other noninvasive indices in the prediction of liver fibrosis.

In the present study, only 15.0% of patients had liver steatosis as measured by MRI-PDFF, while the steatosis was mild in most cases. The global prevalence of NAFLD is currently estimated to be 24%, while it is 27% in Asia, and it is increasing year by year due to the change in lifestyle (39). The prevalence of NAFLD is estimated to be 14-67% in Asian individuals with CHB, similar to the data in Western countries (40,41). In former studies, mounting evidence tends to support a potentially negative association between CHB and NAFLD in terms of hepatitis B serum markers, as well as onset of NAFLD (9). Some research demonstrated a significantly higher incidence of HBsAg clearance in HBeAg-seronegative patients with CHB with hepatic steatosis than in those without, and hepatic steatosis was further identified as an independent predictor (hazard ratio=1.222) of spontaneous HBsAg seroclearance in patients with CHB (11). On the other hand, there has been evidence that indicates the association of chronic HBV infection with a reduction in either hyperlipidemia or NAFLD incidence, confirmed by the present data. A cross-sectional study in 7,695 Taiwanese adults showed that HBV-infected individuals exhibited a lower risk of hypercholesterolemia (OR=0.8), hypertriglyceridemia (OR=0.7) and high low-density lipoprotein cholesterol level (OR=0.8) (42). A large cross-sectional study in Hong Kong found a significantly lower risk of NAFLD in HBsAg-positive subjects (adjusted OR=0.42) (43). Another cross-sectional study in Taiwan also found a negative association of HBV infection with NAFLD, particularly in individuals with BMI >22.4 kg/m^2 or age >50 years (44).

Furthermore, a higher PLT and no PH were predictive factors of liver steatosis in patients with CHB. The PDFF was lower in cirrhotic patients than in those without cirrhosis. In

the ROC curve analysis, PDFF showed a slight to moderate diagnostic ability to distinguish cirrhosis from non-cirrhosis patients. This allows for the conclusion that, as liver fibrosis and PH progress, the prevalence of liver steatosis decreases. However, the above findings appear to be opposite to those of certain other studies. A cohort study evaluated 459 HBeAg-negative patients across a 10-year interval and found that hepatic steatosis was associated with fibrosis progression in patients with CHB (OR=7.799) (45). A clinical study from Thailand identified steatohepatitis as an independent predictor of significant fibrosis (adjusted OR=10) and advanced fibrosis (adjusted OR=3.45) (46). Due to these contradictory results, more studies are needed to offer high-level evidence in terms of the correlation with NAFLD and CHB in the whole course of liver pathologic and immune progression.

Of note, the present study has certain limitations. The pathologic data of live iron concentration, fat deposit and fibrosis stage were not used as the gold standard for diagnosis of iron overload, steatosis and liver fibrosis, which may lead to bias distortion. Furthermore, the absence of a follow-up process in the present study limits the ability to determine more precise causal relationships through before-and-after comparisons. Since MRI is a non-invasive and convenient tool, liver biopsy and MRI measurement will be further combined and the role of MRI in identifying the severity, complications, therapy response and progress in cirrhosis of various causes will be comprehensively evaluated.

In conclusion, in patients with CHB, iron overload was common and should be evaluated particularly in those with older age, male sex and high TB levels. Liver steatosis is less common and the steatosis was usually mild. Liver iron and fat measured by MRI may reflect the severity of liver fibrosis in patients with CHB.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

ST planned and designed the study. JL, ZL and QW collected and analyzed the data. JL and ST drafted the manuscript. All authors have read and approved the submitted manuscript. JL and ST checked and confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

The study protocol was approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University [Guangzhou, China; approval no. (2022)02-328-01]. The current study complied with the Declaration of Helsinki. The requirement of written informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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