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ORIGINAL ARTICLE

Advanced Liver Fibrosis Is Associated with Necroinflammatory Grade but Not Hepatic Steatosis in Chronic Hepatitis B Patients

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Abstract

Background and Aims Patients with chronic hepatitis B (CHB) are at an increased risk of disease progression. The influence of hepatic steatosis (HS) to liver fibrosis was controversial. We aim to investigate the association between HS and liver fibrosis and explore the predicting factors for advanced fibrosis.

Methods CHB patients undergoing liver biopsy with complete assessments of HS, necroinflammation grade [histological activity index (HAI) score], and fibrosis stage were retrospectively recruited. Logistic regression analysis was performed to determine the factors associated with advanced liver fibrosis.

Results In this cohort of 672 patients, 342 (50.9%) had HS and 267 (39.4%) were of advanced liver fibrosis. Age [odds ratio (OR) 1.026, 95% confidence interval (CI) 1.007–1.046, $p=0.008$], body mass index (BMI, OR 1.091, 95% CI 1.026–1.159, $p=0.005$), genotype (C vs. B) (OR 2.790, 95% CI 1.847–4.214, $p<0.001$), platelet (OR 0.986, 95% CI 0.982–0.991, $p<0.001$), and HAI score (OR 1.197, 95% CI 1.114–1.285, $p<0.001$) were independent factors for advanced liver fibrosis in multivariate logistic regression analysis. HAI score was also a significantly associated factor for significant liver fibrosis in non-cirrhotic subpopulation (OR 1.578, 95% CI 1.375–1.810, $p<0.001$). HS was not related to advanced/significant liver fibrosis in overall/non-cirrhotic population ($p>0.05$).

Conclusions Significant or advanced liver fibrosis is associated with grade of necroinflammation but not with HS in CHB patients.

Keywords Chronic hepatitis B · Hepatic steatosis · Advanced liver fibrosis · Histological activity index

Abbreviations

CHB	Chronic hepatitis B
HCC	Hepatocellular carcinoma
ALT	Alanine aminotransferase
NAFLD	Nonalcoholic fatty liver disease
MetS	Metabolic syndrome
LSM	Liver stiffness measurement
CAP	Controlled attenuation parameter
BMI	Body mass index
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus
AST	Aspartate aminotransferase
IQR	Interquartile ranges
OR	Odds ratio
CI	Confidence interval
HS	Hepatic steatosis
DM	Diabetes mellitus
NTCP	Na/taurocholate cotransporter

Introduction

Chronic hepatitis B (CHB) is an important global health issue which affects approximately 250 million people in the world [1]. People with CHB are at an increased risk of disease progression, and the subsequent untoward outcomes, such as hepatic decompensation, liver cirrhosis, and hepatocellular carcinoma (HCC), can occur in the natural course [2]. Older age, male gender, HBeAg positivity, increasing

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levels of alanine aminotransferase (ALT), highly active replication of HBV DNA, genotype, concurrent infection of other hepatitis viruses, and quantitative HBsAg have been reported to be associated factors for liver disease progression [3, 4]. Nonalcoholic fatty liver disease (NAFLD) is a significant chronic liver disease with a global prevalence of around 25% [5]. The prevalence of hepatic steatosis (HS) in CHB patients has been reported to range from 14 to 76% in past studies [6, 7].

CHB patients who had metabolic syndrome (MetS), being strongly associated with NAFLD, had been reported to have more histological liver cirrhosis (38 vs. 11%, $p < 0.001$), and MetS was an independent factor associated with probable cirrhosis [odds ratio (OR) 1.7, 95% confidence interval (CI) 1.1–2.6, $p = 0.03$] [8]. In another report on 663 CHB patients with paired liver stiffness measurements (LSM) after an interval of 44 months, it was also found that coincidental MetS was significantly associated with liver fibrosis progression (adjusted OR 2.0, 95% CI 1.1–3.5, $p = 0.015$) [9]. These findings linked fatty liver and liver fibrosis progression in CHB patients. Two studies in Hong Kong using transient elastography for LSM and controlled attenuation parameter (CAP) to evaluate liver fibrosis and HS have shown that severe steatosis was associated with an increased percentage of severe fibrosis when compared with mild/moderate steatosis. Severe steatosis was an independent factor predicting severe fibrosis with the OR ranging from 1.95 to 3.60 [10, 11]. A recent study in Malaysia also found that presence of HS assessed by CAP was independently associated with advanced fibrosis (OR 1.956, 95% CI 1.250–3.060, $p = 0.003$) [12]. However, liver fibrosis was reported not to be associated with histological HS in a meta-analysis of five studies with pooled standardized mean difference 0.22 (95% CI −0.84 to 0.41, $p = 0.495$) [7]. Similar results of negative association were also observed in subsequent studies in Greece [13], Hong Kong [8], Korea [14], and Thailand [15].

With the controversial relationship between HS and liver fibrosis, we therefore conducted a large-scale retrospective study on biopsy-proven CHB patients to investigate this issue.

Patients and Methods

Study Subjects

We retrospectively recruited CHB patients undergoing liver biopsy in pathological report system from 2003 May to 2019 December at Chang Gung Memorial Hospital, Lin Kou branch in Taoyuan, Taiwan. Liver biopsies were performed under the indications of clinical trial screening, disease status evaluation, and the purpose of reimbursement for antiviral treatment. All patients were HBsAg-positive for at least 6 months at liver biopsy. Those with coinfection with hepatitis C virus (HCV), hepatitis D virus (HDV), or human immunodeficiency virus (HIV); concomitant alcoholic liver disease or autoimmune liver disease; history of HCC; and those under antiviral treatment or on medications associated with fatty liver (such as estrogen, tamoxifen, corticosteroids, methotrexate, etc.) were excluded. This study was conducted under the approval of institutional review board (IRB No. 201701168B0).

Clinical and Laboratory Assessments

Demographic information of age, gender, body mass index (BMI), and medical history of diabetes mellitus (DM) were recorded from electronic medical records. Laboratory data including aspartate aminotransferase (AST), ALT, platelet count, fasting sugar, lipid profiles, HBeAg, anti-HBe, anti-HCV, anti-HDV, HBsAg, HBV DNA, and HBV genotype were collected. Dyslipidemia was defined as at least one component of abnormal lipids (i.e., total cholesterol ≥ 240 mg/dL, low density lipoprotein cholesterol [LDL-C] ≥ 160 mg/dL, high density lipoprotein cholesterol [HDL-C] < 40 mg/dL for men or < 50 mg/dL for women, triglyceride ≥ 200 mg/dL) [16]. Fibrosis 4 index (FIB-4) [17] was used based on the better performance in diagnostic accuracy of liver fibrosis in CHB [18, 19]. Stored serums, if available, were retrieved for assays of HBV genotype, HBsAg, and HBV DNA for any incomplete data. HBV genotype was determined by polymerase chain reaction-restriction fragment length polymorphism of the surface gene of HBV. Serum HBsAg levels were quantified using the Roche Elecsys HBsAg II quant assay (detection limit, 0.05–52,000 IU/mL; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Serum HBV DNA was assayed by COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, version 2.0 (lower limit of detection: 20 IU/mL, Roche Diagnostics, Mannheim, Germany). HBeAg, anti-HBe, and anti-HCV were tested with electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany). Anti-HDV was assayed with enzyme immunoassay kit (Abbott Diagnostics, North Chicago, IL or General Biologicals Corp., Hsinchu, Taiwan after 2018 June).

Histological Evaluation

Percutaneous liver biopsy was performed using a 18G cord biopsy needle and biopsy gun (Bard® Magnum®, Bard Peripheral Vascular, Inc. AZ, USA). All the specimens were stained with hematoxylin–eosin and Masson trichrome stains, and histological characteristics of necroinflammation, liver fibrosis, and HS were evaluated. The necroinflammatory score was graded by modified histological activity index

(HAI), including (A) periportal or periseptal interface hepatitis (score 0–4); (B) confluent necrosis (score 0–6); (C) focal (spotty) lytic necrosis, apoptosis, and focal inflammation (score 0–4); and (D) portal inflammation (score 0–4). The grade of necroinflammation was arbitrarily categorized by the sum of HAI scores into mild (0–6), moderate (7–13), and marked (14–18). Fibrosis score was staged by architectural changes, fibrosis, and cirrhosis (score 0–6) [20]. Significant fibrosis was defined as Ishak fibrosis score ≥ 3, advanced fibrosis as score ≥ 4 , and cirrhosis as score ≥ 5 [13, 21]. HS was defined as the presence of steatosis in over 5% of hepatocytes according to the Brunt criteria [22] and categorized into three groups [5–33% (mild), > 33–66% (moderate), and > 66% (severe)] [23]. Nonalcoholic steatohepatitis (NASH), which was additionally assessed since 2014 in pathological report system, was defined as the presence of HS and inflammation (lobular) with hepatocyte injury (ballooning, score 0–2), with or without any fibrosis (score 0–4) [5, 24].

Statistical Analysis

Continuous variables were expressed as means and standard deviations (SD) or medians and interquartile ranges (IQR) as appropriate after testing for normal distribution using the Kolmogorov–Smirnov test and were compared by independent Student's *t* test or Mann–Whitney *U* test between two different groups. One-way ANOVA or Kruskal–Wallis one-way ANOVA was performed to compare the clinical characteristics among patients with different degrees of HS or different stages of liver fibrosis. Categorical variables were presented as the number of cases (proportions) and compared by Chi-squared or Fisher's exact tests when appropriate. The serum HBsAg and HBV DNA levels were logarithmically transformed for analysis. Logistic regression analysis was performed to find the associated predictors for the severity of liver fibrosis. Variables with $p < 0.1$ in univariate analysis will be entered in multivariate analysis. Statistical analysis was performed by IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). A two-tailed $p < 0.05$ was considered statistically significant.

Results

A total of 672 consecutive CHB patients were enrolled in this study. The mean age was 46.7 ± 10.9 years and 533 (79.3%) were males. There were 342 (50.9%) HS, 267 (39.4%) advanced liver fibrosis, 87 (12.9%) DM, 221 (32.9%) positive HBeAg, and 451 (71.1%) genotype B. Lipid profiles were available in 259 (38.5%) patients, and dyslipidemia was identified in 93 (35.9%). HBV genotype was available in 636 (94.6%) patients, and only genotypes B and C were included for statistical analysis ($n = 634$, one genotype D and one genotype I excluded). HBsAg levels were available in 636 (94.6%) patients. The clinical characteristics are shown in Table 1.

Comparison of the Clinical Characteristics Between Patients with Non-advanced and Advanced Liver Fibrosis

Patients with advanced liver fibrosis were older (49.5 vs. 44.9 years, $p < 0.001$); had higher median levels of BMI (24.2 vs. 23.8 kg/m^2, $p = 0.009$), HAI score (6 vs. 5, $p < 0.001$), and FIB-4 (2.06 vs. 1.38, $p < 0.001$); higher proportions of DM (16.9 vs. 10.4%, $p = 0.020$) and genotype C (40.2 vs. 21.4%, $p < 0.001$); lower median levels of ALT (90 vs. 104 U/L, $p = 0.013$), platelet (160 vs. 193 10^9/L, $p < 0.001$), and HBsAg levels (3.19 vs. 3.29 log IU/mL, $p = 0.018$), when compared to those with non-advanced liver fibrosis (Table 1). The differences in gender, HBeAg positivity, AST, dyslipidemia, fasting sugar, HBV DNA levels, and HS between patients with and without advanced liver fibrosis were not statistically significant.

Mild, moderate, and marked necroinflammation existed in 496 (73.8%), 159 (23.7%), and 17 (2.5%) patients, respectively, in the overall population. The patients with advanced liver fibrosis had a significantly higher proportion of moderate and marked necroinflammation (38.2%, $N = 102$) than those with non-advanced fibrosis (18.3%, $N = 74$, $p < 0.001$) (Fig. 1A).

Factors Associated with Advanced Liver Fibrosis

The clinical variables (age, gender, BMI, DM, dyslipidemia, genotype, HBeAg, AST, ALT, platelet, sugar, HBsAg, HBV DNA, HS, HAI score, FIB-4) were analyzed using logistic regression analysis for the factors associated with advanced liver fibrosis. Age, BMI, DM, genotype, platelet, HBsAg, HAI score, and FIB-4 ($p < 0.1$ in univariate analysis) and HS (for comparison) were entered in multivariate analysis by two models. Model 1 included the components of FIB-4 (age and platelet). Model 2 selected FIB-4 without its components. Age (OR 1.026, 95% CI 1.007–1.046, $p = 0.008$, model 1), BMI (OR 1.091, 95% CI 1.026–1.159, $p = 0.005$, model 1; OR 1.074, 95% CI 1.014–1.138, $p = 0.016$, model 2), genotype (C vs. B) (OR 2.790, 95% CI 1.847–4.214, $p < 0.001$, model 1; OR 2.785, 95% CI 1.871–4.144, $p < 0.001$, model 2), platelet (OR 0.986, 95% CI 0.982–0.991, $p < 0.001$, model 1), HAI score (OR 1.197, 95% CI 1.114–1.285, $p < 0.001$, model 1; OR 1.133, 95% CI 1.053–1.219, $p = 0.001$, model 2), and FIB-4 (OR 1.589, 95% CI 1.323–1.908, $p < 0.001$, model 2) were independent factors for advanced liver fibrosis

Table 1 Comparison of clinical characteristics between patients with non-advanced and advanced liver fibrosis

	Overall	Non-advanced fibrosis	Advanced fibrosis	*p*
No	672	405	267	
Age, years	46.7 ± 10.9	44.9 ± 10.8	49.5 ± 10.5	< 0.001
Males	533 (79.3)	329 (81.2)	204 (76.4)	0.157
BMI, kg/m^2	24.1 (22.1–26.7)	23.8 (21.9–26.3)	24.2 (22.3–27.2)	0.009
DM	87 (12.9)	42 (10.4)	45 (16.9)	0.020
Dyslipidemia[†]	93 (35.9)	50 (33.3)	43 (39.4)	0.378
Genotype[‡]				< 0.001
B	451 (71.1)	301 (78.6)	150 (59.8)	
C	183 (28.9)	82 (21.4)	101 (40.2)	
HBeAg (+)	221 (32.9)	141 (34.8)	80 (30.0)	0.220
AST, U/L	57 (40–102)	56 (40–96)	60 (42–108)	0.233
ALT, U/L	98 (61–168)	104 (69–171)	90 (53–166)	0.013
Platelet, 10^9/L	180 (151–215)	193 (167–225)	160 (131–193)	< 0.001
Sugar, mg/dL	92 (86–103)	91 (85–102)	93 (87–105)	0.186
HBsAg, log IU/mL	3.25 (2.79–3.72)	3.29 (2.78–3.98)	3.19 (2.85–3.57)	0.018
HBV DNA, log IU/mL	6.14 (4.95–7.35)	6.18 (4.97–7.48)	6.12 (4.93–7.26)	0.499
HS	342 (50.9)	197 (48.6)	145 (54.3)	0.174
HAI score	5 (4–7)	5 (3–6)	6 (4–7)	< 0.001
FIB-4	1.63 (1.07–2.42)	1.38 (0.92–1.99)	2.06 (1.52–3.02)	< 0.001

Presented as mean ± SD, median (interquartile range) or number (%)

BMI, body mass index; HS, hepatic steatosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HS, hepatic steatosis; HAI, histological activity index; FIB-4, fibrosis 4 index

[†]Lipid profiles available in 259 patients (150 non-advanced fibrosis, 109 advanced fibrosis)

[‡]Genotype available in 636 patients (one genotype D and one genotype I excluded for analysis)

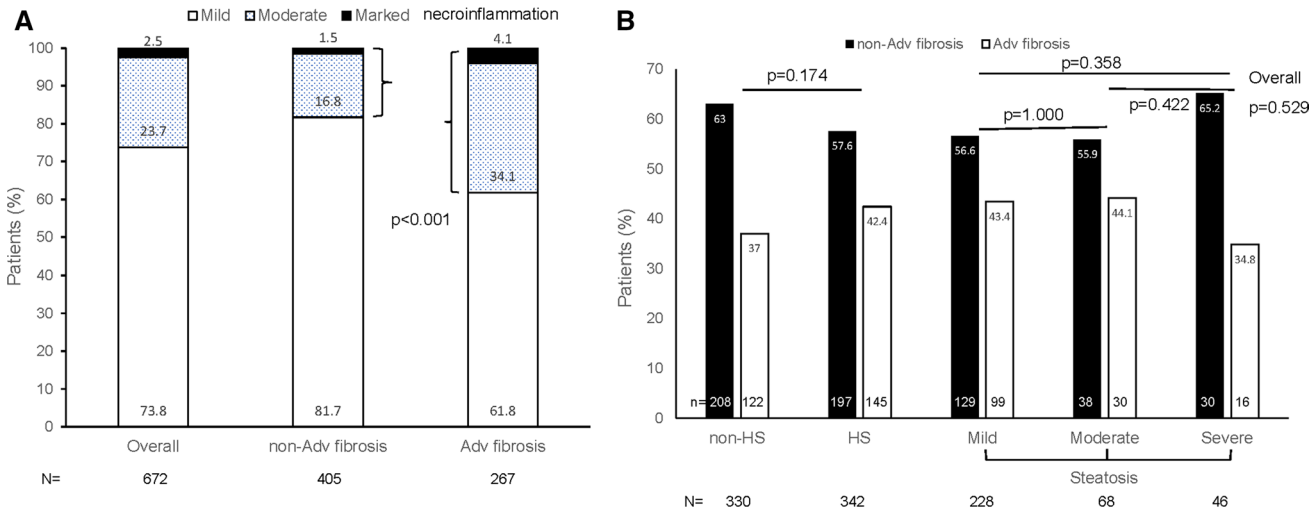

Fig. 1 The grade of necroinflammation and hepatic steatosis (HS) in patients with non-advanced (non-adv, Ishak fibrosis score 0–3) and advanced (adv, Ishak fibrosis score 4–6) fibrosis. **a** The distribution of mild [histological activity index (HAI) score 0–6], moderate (score 7–13) and marked (score 14–18) necroinflammation in patients with non-adv and adv fibrosis. Patients with adv fibrosis had significant higher proportion of moderate and marked necroinflammation than those with non-adv fibrosis (38.2% vs. 18.3%, $p < 0.001$); **b** Comparison between patients with and without HS and among different degrees of steatosis in non-adv and adv fibrosis. Patients with HS had a higher proportion of adv fibrosis (42.4%) than those with non-HS (37%) without statistically significant ($p = 0.174$). Patients with mild, moderate, and severe steatosis had adv fibrosis in 43.4%, 44.1%, and 34.8%, respectively, and the difference was not significant in overall and intergroup comparison

(Table 2 and supplementary Table 1). HS was not associated with advanced liver fibrosis.

Clinical Characteristics and Factors Associated with Significant Liver Fibrosis in Non-cirrhotic Patients

There were 155 patients with Ishak fibrosis score 0–2, and 322 patients with fibrosis score 3–4 (significant liver fibrosis). The comparison of clinical characteristics between patients with nonsignificant and significant liver fibrosis are shown in supplementary Table 2. The patients with significant liver fibrosis were older (46.4 vs. 43.4 years, $p=0.004$) and had higher median levels of AST (60 vs. 53 U/L, $p=0.005$), HAI score (5 vs. 4, $p<0.001$), FIB-4 (1.54 vs. 1.24, $p<0.001$), and lower median platelet level (186 vs. 200 10^9/L, $p=0.006$) than those with nonsignificant fibrosis. Age (OR 1.025, 95% CI 1.002–1.047, $p=0.029$, model 1), AST (OR 0.995, 95% CI 0.992–0.999, $p=0.010$, model 1), and HAI score (OR 1.578, 95% CI 1.375–1.810, $p<0.001$, model 1; OR 1.421, 95% CI 1.259–1.604, $p<0.001$, model 2) were the independent factors for significant liver fibrosis in multivariate logistic regression analysis (Model 1, components of FIB-4: age, AST, and platelet; model 2, FIB-4 without its components) (Supplementary Table 3).

Table 2 The independent factors associated with advanced liver fibrosis in multivariate logistic regression analysis

	Odds ratio	95% CI	p
Model 1			
Age	1.026	1.007–1.046	0.008
BMI, kg/m^2	1.091	1.026–1.159	0.005
Genotype (C vs. B)	2.790	1.847–4.214	< 0.001
Platelet, 10^9/L	0.986	0.982–0.991	< 0.001
HAI score	1.197	1.114–1.285	< 0.001
HS[†]	1.030	0.686–1.545	0.886
Model 2			
BMI, kg/m^2	1.074	1.014–1.138	0.016
Genotype (C vs. B)	2.785	1.871–4.144	< 0.001
HAI score	1.133	1.053–1.219	0.001
FIB-4	1.589	1.323–1.908	< 0.001
HS[†]	1.173	0.788–1.745	0.432

BMI body mass index, *HS* hepatic steatosis, *HAI* histological activity index, *FIB-4* fibrosis 4 index, *HS* hepatic steatosis

[†]HS was listed for comparison

Association Between Clinical Characteristics and HS

Patients with HS ($n=342$) were older (47.5 vs. 45.9 years, $p=0.048$), more males (82.5 vs. 76.1%, $p=0.041$), had higher median levels of BMI (25.4 vs. 22.9 kg/m^2, $p<0.001$), and sugar (93 vs. 89 mg/dL, $p=0.006$), higher proportions of DM (19 vs. 6.7%, $p<0.001$) and dyslipidemia (45.2 vs. 19.4%, $p<0.001$), lower median levels of AST (52 vs. 67 U/L, $p<0.001$), ALT (91 vs. 119 U/L, $p<0.001$), HBsAg (3.17 vs. 3.33 log IU/mL, $p=0.001$), HBV DNA (5.91 vs. 6.43 log IU/mL, $p<0.001$), and FIB-4 (1.58 vs. 1.64, $p=0.027$) than patients without HS. The clinical characteristics in patients with and without HS are shown in Table 3. The patients with mild, moderate, and severe HS had advanced liver fibrosis in 99 (43.4%), 30 (44.1%), and 16 (34.8%), respectively, and the difference was not significant in overall and intergroup comparison (all $p>0.05$). (Fig. 1B). The proportion of HS was 55.6%, 58.2%, 44.3%, 47.2%, 47.2%, 52.9%, and 66.1% in fibrosis score 0 ($n=9$), 1 ($n=67$), 2 ($n=79$), 3 ($n=250$), 4 ($n=72$), 5 ($n=136$), and 6 ($n=59$), respectively ($p=0.112$) (Supplementary Fig. 1).

Forty-four patients with HS had additional pathological assessment of NASH (31 NASH and 13 no NASH). Advanced liver fibrosis existed in 14 (45.2%) and 5 (38.5%) of patients with and without NASH, respectively, and the difference was not significant ($p=0.940$). Patients with ballooning score 0 ($n=13$) had no or mild NASH fibrosis (11 score 0 and 2 score 1), and 5 (38.5%) of them had advanced Ishak fibrosis. Among 20 patients with ballooning score 2, 4 (20%), 3 (15%), 6 (30%), and 7 (35%) were NASH fibrosis score 1, 2, 3, and 4, respectively, and 12 (60%) were advanced Ishak fibrosis. Patients with greater ballooning scores had higher chance of advanced NASH fibrosis ($p<0.001$) and advanced Ishak fibrosis ($p=0.064$). The ballooning scores in different stages of NASH fibrosis and Ishak fibrosis are shown in Supplementary table 4.

Discussion

This is a large-scale retrospective study investigating the association of histological HS with advanced liver fibrosis in CHB patients. Our results showed that neither advanced liver fibrosis in overall population nor significant liver fibrosis in non-cirrhotic subpopulation was related to histological HS in CHB patients. On the other hand, HAI score was an independent factor for advanced liver fibrosis in overall population and significant liver fibrosis in non-cirrhotic subpopulation.

The development of HS in CHB patients has been researched in viral and host factors. Hepatitis B protein X is reported to increase lipid accumulation in liver by the activation of fatty acid binding protein 1 (FABP1) promoter [25],

Table 3 The clinical characteristics in different degrees of hepatic steatosis (HS)

HS	< 5%	≥ 5%	*p*	Mild	Moderate	Severe
No	330	342		228	68	46
Age, years	45.9 ± 11.7	47.5 ± 10.0	0.048	48.6 ± 10.0	45.5 ± 10.3	45.2 ± 9.0
Males	251 (76.1)	282 (82.5)	0.041	190 (83.3)	58 (85.3)	34 (73.9)
BMI, kg/m^2	22.9 (21.4–25.0)	25.4 (23.2–28.1)	< 0.001	24.7 (22.9–27.2)	25.8 (23.8–29.4)	28.1 (25.2–31.0)
DM	22 (6.7)	65 (19.0)	< 0.001	35 (15.4)	15 (22.1)	15 (32.6)
Dyslipidemia[†]	18 (19.4)	75 (45.2)	< 0.001	40 (40.8)	16 (45.7)	19 (57.6)
Genotype[‡]						
B	237 (73.1)	214 (69.0)	0.291	148 (69.2)	46 (75.4)	20 (57.1)
C	87 (26.9)	96 (31.0)		66 (30.8)	15 (24.6)	15 (42.9)
HBeAg (+)	112 (33.9)	109 (31.9)	0.625	66 (28.9)	22 (32.4)	21 (45.7)
AST, U/L	67 (43–125)	52 (39–80)	< 0.001	53 (39–83)	47 (37–68)	58 (40–90)
ALT, U/L	119 (67–213)	91 (57–148)	< 0.001	90 (54–152)	86 (57–141)	111 (71–158)
Platelet, 10^9/L	179 (151–214)	181 (151–216)	0.630	175 (147–209)	189 (158–217)	206 (176–244)
Sugar, mg/dL	89 (85–99)	93 (87–106)	0.006	93 (86–104)	91 (87–105)	101 (89–142)
HBsAg, log IU/mL[§]	3.33 (2.89–3.88)	3.17 (2.67–3.65)	0.001	3.18 (2.78–3.60)	3.10 (2.59–3.64)	3.24 (1.50–3.98)
HBV DNA, log IU/mL	6.43 (5.22–7.64)	5.91 (4.61–7.18)	< 0.001	5.89 (4.87–7.16)	6.10 (4.42–7.22)	5.43 (2.55–7.44)
HAI score	5 (4–7)	5 (4–6)	0.160	5 (4–6)	5 (3–6)	5 (3–6)
FIB-4	1.66 (1.14–2.62)	1.58 (1.04–2.27)	0.027	1.70 (1.18–2.40)	1.26 (0.83–2.00)	1.23 (0.91–2.07)
Advanced fibrosis	122 (37.0)	145 (42.4)	0.174	99 (43.4)	30 (44.1)	16 (34.8)

Presented as mean ± SD, median (interquartile range) or number (%)

BMI body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *HS* hepatic steatosis, *HAI* histological activity index, *FIB-4* fibrosis 4 index

[†]Lipid profiles available in 259 patients (93 non-HS, 166 HS)

[‡]Genotype available in 634 patients

[§]HBsAg levels available in 636 patients

up-regulation of sterol regulatory element binding protein 1 (SREBP-1), and peroxisome proliferator activated receptor gamma (PPAR-λ) [26]. By binding to Na/taurocholate cotransporter (NTCP), HBV could alter bile acid metabolism and therefore induce increased uptake and synthesis of cholesterol and lipid accumulation in hepatocytes [27, 28]. However, some studies have shown that HS in CHB is likely to be a result of host metabolic factors rather than the effect of HBV itself [29–32]. Through the altered mediators such as decreased adiponectin, increased leptin, angiotensin II, connective tissue growth factor, advanced glycation end products and reactive oxygen species, MetS can activate hepatic stellate cells and thereafter modulate hepatic fibrogenesis [33]. Accordingly, MetS may be the primary culprit for liver fibrosis progression instead of HS, which may be regarded as a hepatic manifestation of MetS [34]. In contrast to recent studies showing that severe steatosis is an independent factor for severe liver fibrosis [10–12], our results found no statistical difference in advanced liver fibrosis between severe HS and mild or moderate HS ($p = 0.358$ and $p = 0.422$, respectively, Fig. 2B). Of note is that HS was also not associated with significant liver fibrosis in non-cirrhosis patients (supplementary Table 3) when cirrhotic patients were excluded to avoid the possible contribution bias of HS to liver fibrosis stage due to the disappearance of histological steatosis in liver cirrhosis [35]. The differences in study population and assessment methods for steatosis and fibrosis evaluation may be the reasons for these conflicting observations. In addition, moderate-severe steatosis may increase the LSM value by transient elastography in CHB patients without significant fibrosis and lead to overestimation of liver fibrosis [36]. Therefore, interpretation of LSM in patients with high CAP should be cautious.

HAI score for hepatic necroinflammatory grade includes portal, periportal, and intra-acinar inflammatory cell infiltration and various forms of liver cell necrosis which can reflect liver damages by chronic hepatitis of viral cause, autoimmune, and drugs [20]. In our results, median HAI score was an independent factor for advanced or significant liver fibrosis in overall population and in non-cirrhotic subpopulation, respectively. This finding was compatible with observations in previous studies [14, 31, 37, 38], and the results in a most recent report on a north American cohort of 466 CHB patients that high HAI (≥ 5) was associated with a threefold higher chance of advanced fibrosis [39]. Activation of hepatic stellate cells around necroinflammatory regions

may explain the fibrogenesis in necroinflammatory process [40]. Furthermore, necroinflammatory grade by HAI scores requires pathological assessment, and liver biopsy may still play an important role in disease status determination in chronic viral hepatitis despite the emergence of noninvasive methods.

The finding in our study that BMI was one of the independent factors for advanced liver fibrosis was in line with the studies on CHB patients receiving long-term nucleoside analogue [41] and HBeAg-negative patients [42]. An increase in leptin and a corresponding decrease in adiponectin in obese patients may drive the fibrogenesis in liver [43]. In our study, lipid profiles were available in 259 patients and dyslipidemia was significantly higher in patients with HS than those without (Table 3) but was not associated with advanced/significant liver fibrosis (supplementary Tables 1 and 3). Abnormal lipid profiles (cholesterol, triglyceride, HDL-C, LDL-C) or dyslipidemia have been analyzed for the association with advanced liver fibrosis, and their relationship was not significant [9, 12, 13, 15, 41].

NASH has been reported as an independent predictor of significant fibrosis (OR 10.0; 95% CI, 2.08–48.5) and advanced fibrosis (OR 3.45; 95% CI, 1.11–10.7) after adjusting for viremia levels and features of the MetS [15]. Coexisting NASH in CHB may be associated with advanced liver fibrosis when compared to simple steatosis (RR 1.89, 95% CI, 0.94–3.80, $p=0.07$) [39]. A recent cohort study of 1089 CHB patients has shown a higher rate of advanced fibrosis in those with NASH (39.5% vs. 24.5% in no NASH, $p<0.001$) [44]. Among the 44 patients with NASH assessment in our study, the advanced liver fibrosis was not different between NASH and non-HASH ($p=0.940$). Of interest was that those with greater ballooning score had a higher chance for advanced NASH fibrosis (score 3 and 4) ($p<0.001$) and a trend for advanced Ishak fibrosis ($p=0.064$) (supplementary table 4). The association between NASH or its histological components and viral fibrosis stage could not be explored by this small patient number, but is warranted to be investigated in large-scaled studies in the future.

The main strengths of our study are the inclusion of a large cohort of CHB patients with liver histological assessment, 95% with HBsAg levels, and HBV genotype available. With complete histological evaluation of fat composition and necroinflammatory grades, we could therefore explore the relationship among steatosis, grade of necroinflammation, and fibrosis stage. Beyond the reach of current noninvasive methods, HAI score was found to be an independent factor for significant/advanced liver fibrosis in our study (Table 2, supplementary Table 1 and 3). The results that genotype was an independent predictor for liver fibrosis progression and not associated with HS [3, 7] were further confirmed in our study. In addition to the inverse relationship between HS and HBV viremia in past studies [7, 10] and ours, we also found that quantitative HBsAg levels were negatively associated with HS (Table 3), which was rarely discussed in past literature. This finding was similar to the result in our HBeAg-positive cohort [45].

Some limitations in our study included a retrospective study design, selection bias for liver biopsy in CHB patients, small patient number with NASH assessment, limited data on lipid profiles, and shortage of insulin resistance (IR) test. Nowadays, large-scaled studies in CHB patients with liver biopsy will be difficult because liver histology for fibrosis staging is limited by potential complications and gradually replaced by noninvasive assessments. There was no difference in advanced liver fibrosis between patients with and without NASH in our study (only 44 patients analyzed), whereas emerging data have shown a positivity association between concomitant NASH and liver fibrosis in CHB [15, 39, 44]. Even though the patients with lipid profiles was limited in our study (38.5%), the finding of negative association with liver fibrosis was compatible with previous studies [9, 13, 15, 41]. As a retrospective study, insulin level was lacking for IR estimation, and demographic data were insufficient for MetS assessment. IR has been early reported to be associated with liver fibrosis progression in chronic HCV patients [46], but its relationship to liver fibrosis in chronic HBV patients was not clearly elucidated with both positive [47] and negative [15, 48] relationship in past reports.

In conclusion, our study investigated the influence of clinical and pathological characteristics to liver fibrosis in CHB patients. HS may just be a hepatic manifestation of underlying MetS and was not associated with the severity of liver fibrosis. HAI scores representing necroinflammation was the independent predictor for advanced or significant liver fibrosis.

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Author's contribution Y-C C was involved in concept and design; Y-C C, C-W H, W-J J, C-Y L helped in data acquisition, interpretation, and analysis; Y-C C was involved in drafting of the manuscript; Y-C C, C-W H, W-J J, C-Y L contributed to critical revision of the manuscript for important intellectual content; Y-C C helped in statistical analysis. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of interests The authors have nothing to declare.

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