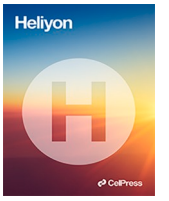
Heliyon 10 (2024) e28653

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

# The efficacy of antiviral treatment in chronic hepatitis B patients with hepatic steatosis

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ARTICLE INFO

*Keywords:*
Antiviral therapy
Chronic hepatitis B
Hepatic steatosis
Fibrosis progression

ABSTRACT

*Background & aims:* With a drastic increase in the number of chronic hepatitis B (CHB) patients with coexisting nonalcoholic fatty liver disease (NAFLD), there is an urgent need to evaluate antiviral treatment effects in this special population.
*Methods:* CHB patients with hepatic steatosis (CHB + HS) were prospectively recruited with followed-up of 3 years. HS and liver fibrosis were assessed by transient elastography. HS was defined as controlled attenuation parameter (CAP) ≥248 dB/m, and fibrosis progression was defined with ≥1-stage fibrosis increment. Multivariate and propensity score matching (PSM) analysis were used to evaluate antiviral therapy effects on fibrosis progression.
*Results:* In total 212 recruited CHB + HS patients (median age 36 years, median ALT 59 U/L), 49.1% (104/212) received antiviral therapy and 50.9% (108/212) did not. Among patients with antiviral therapy, rates of serum HBV DNA undetectable, HBeAg and HBsAg loss, and ALT normalization at year 3 were 88.5%, 31.0%, 8.7% and 70.2%, respectively. Patients with mild-moderate HS didn't differ patients with severe HS regarding biochemical and virological responses. Antiviral therapy was independently associated with a lower risk of fibrosis progression among the entire cohort (odds ratio 0.473, 95% CI 0.245–0.911, $P = 0.025$). This finding was further verified by PSM analysis. When stratified by the severity of HS, the antiviral therapy benefits in reducing fibrosis progression were mainly seen in patients with mild-moderate HS.
*Conclusions:* Among CHB + HS patients, long-term antiviral treatment effectively inhibits HBV replication and reduces fibrosis progression. Our findings have implications for the optimal management of this population.

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https://doi.org/10.1016/j.heliyon.2024.e28653
Received 30 September 2023; Received in revised form 20 March 2024; Accepted 21 March 2024
Available online 23 March 2024

## 1. Introduction

Chronic hepatitis B (CHB) virus infection remains a major cause leading to liver fibrosis, cirrhosis, hepatocellular carcinoma and death, with an estimated 316 million HBV-infected patients globally, including more than 70 million patients in China [1,2]. Antiviral therapy can effectively reduce liver disease progression and overall mortality in CHB patients [3–5]. However, it is estimated only around 5% of treatment eligible CHB patients have received antiviral therapy worldwide [1].

Nonalcoholic fatty liver disease (NAFLD) is becoming the fastest rising cause of chronic liver disease, with an overall global prevalence of 32.4% [6]. It also seems to be a growing prevalence of NAFLD for Asia regions, with the 29.0% reported rate in CHB patients (rang from 13.5% to 56.0%) [7]. Ilkay et al. also reported a high rate of positive HBV core antibody among patients with fatty liver [8]. In addition, although debate exists, several studies have showed the diverse impacts of NAFLD on hepatic outcomes in CHB: for one thing coexisting NAFLD can reduce viral replication activity and promote HBsAg clearance, but for another, their coexistence may present faster progression of liver disease than CHB alone [9–11], urging for a specific attention for these patients.

Elevated serum ALT levels were related to HBV immune activation and have been adopted as a major criteria to guide treatment decisions for non-cirrhotic CHB patients by current international guidelines [12–14]. Nevertheless, in real-world clinical practice, serum ALT levels can be influenced by several factors, eg, NAFLD [15,16]. Therefore, ALT elevation in patients with concurrent NAFLD and CHB cannot be solely interpreted as a reflection of hepatic inflammation caused by HBV infection. Liver biopsy may be required to identify the possible causes leading to elevated ALT levels to guide the initiation of antiviral therapy. However, according to a recent East Asia expert opinion, liver biopsies for all such patients are unrealistic and may also not distinctly pinpoint the exact cause leading to ALT elevation [16]. Additionally, there are no specific recommendations for antiviral therapy among exclusively patients with coexisting CHB and NAFLD by current practice guidelines [16,17]. This conundrum poses a dilemma for clinicians regarding the management or treatment initiation of this special population in clinical practice. Given that low therapeutic coverage aggravated by restrictive treatment guidelines may promote disease progression in such patients, it is urgent to determine whether antiviral therapy may benefit CHB patients with hepatic steatosis (HS).

In this study, we investigated antiviral effects in a real-world cohort of non-cirrhotic CHB patients with varying degrees of HS, assessed by transient elastography (TE) with controlled attenuation parameter (CAP).

## 2. Methods

### 2.1. Study participants

Adult patients (HBsAg positive >6 months) with CHB with HS (CHB + HS) were prospectively enrolled in the Department of Infectious Diseases of Tongji Hospital from October 2018 to July 2019. HS was defined by CAP ≥248 dB/m [18]. From initial 1005 patients with CHB + HS, we identified treatment-naive non-cirrhotic patients with elevated ALT and HBV DNA who started antiviral therapy or not, to enter into the observational study. Patients were further followed up and received laboratory, clinical and TE reevaluation at year 3. Due to no shared guidelines determining the antiviral treatment initiation in these patients, the decision of treatment initiation was made based on the consideration of general consensus CHB guidelines, physicians' discretion, and patients' preference. Consenting patients declining antiviral therapy were enrolled in a natural history arm (untreated group).

Patients with chronic hepatitis C virus (HCV) infection, chronic schistosoma liver disease, autoimmune liver disease, malignancies and having excessive alcohol intake (for women, ≥20 g/day; for men, ≥30 g/day) were excluded. Patients having ALT >5 times ULN (upper limit of normal) were also excluded due to inadequate accuracy of TE at such high ALT levels [19].

### 2.2. Clinical and laboratory assessment

Clinical and laboratory assessments were made for all enrolled patients at both baseline and follow-up visits. Patients' medical history was recorded with a short questionnaire. Anthropometric measurements such as body weight, body height, body mass index (BMI), waist circumference, systolic and diastolic blood pressure were recorded. Hypertension and diabetes mellitus was recorded based on self-reported medical history. Dyslipidemia and central obesity (base on waist circumference, for females, ≥80 cm; for males, ≥90 cm) were defined as previously reported [20]. The ULN of ALT was 40 U/L [14]. Serum HBsAg and HBV DNA levels were detected by Elecsys HBsAg II assays (detection limit: 0.05 IU/mL; Roche Diagnostics Gmbh, Mannheim, Germany), and Cobas TaqMan assay kit (detection limit: 20 IU/mL; Roche Diagnostics, Branchburg, NJ), respectively.

### 2.3. Transient elastography

All operators received adequate training in the beginning of this study. Fasting patients were given TE examinations (Fibroscan, Echosens®, Paris, France) with the M probe. When the M probe failed or BMI was >30 kg/m$^2$, the XL probe was used [21] Liver stiffness (LS) was considered reliable when at least 10 validated LS measures had less than 30% of the median quartile range. Fibrosis staging was assessed as previously reported [19]: no significant fibrosis (F0–F1), LS < 6.0 kPa; the 'grey area', 6.0≤ LS ≤ 9.0 kPa for patients with normal ALT or 9.0≤ LS ≤ 12.0 kPa for patients with ALT 1-5 × ULN; advanced liver fibrosis (F3), LS > 9.0 kPa for patients with normal ALT or LS > 12.0 kPa for patients with ALT 1-5 × ULN; liver cirrhosis (F4), LS > 12.0 kPa for patients with normal ALT or >13.4 kPa for patients with ALT 1-5 × ULN [22]. HS was categorized based on CAP value: mild (248≤ CAP ≤267 dB/m), moderate (268≤ CAP ≤279 dB/m) or severe (CAP ≥280 dB/m) [18]. Fibrosis progression was defined as an increment of ≥1 fibrosis stage

compared with baseline [10,23].

### 2.4. Statistical analysis

Data were showed as median (interquartile rang) or percentages (%). For statistical significance, differences in continuous or categorical variables were performed by Student *t*-test (or Mann-Whitney *U* test) or chi-square test (or Fisher's exact test). Factors with a $P < 0.1$ in univariate analysis entering into multivariate analysis were performed using binary logistic regression. To minimize the potential confounder, propensity score matching (PSM) analysis in a 1:1 ratio was used to generate well matched groups of treated and untreated patients (variables matched: baseline age, gender, diabetes, serum HBeAg, HBV DNA, ALT, AST and LS). The PSM was assessed by nearest-neighbour matching with a caliper size of 0.1. Statistical significance was defined as $P$ value $< 0.05$. Data were analyzed by SPSS version 26.0.

## 3. Results

### 3.1. Baseline characteristics

After excluding 31 patients receiving antiviral treatment during follow-up or lost follow-up, 212 non-cirrhotic patients with CHB + HS having elevated HBV DNA and ALT were finally included for analysis: 104 patients who started antiviral therapy at baseline (treated group), 108 patients who declined antiviral therapy at baseline and remained treatment-naive at 3 years (untreated group). The patient disposition was depicted in Fig. 1. In the treated group, 96.2% (100/104) patients received the treatment of nucleos(t)ide analogues (NAs), including tenofovir alafenamide (TAF), entecavir (ETV) and tenofovir disoproxil fumarate (TDF), 3.8% (4/104) patients were treated with interferon-based or combination NAs.

Patients' baseline characteristics are presented in Table 1. The median age was 36 years and 91.0% were males without significant differences between the untreated and treated groups. Compared to untreated group, the treated group showed higher serum HBV DNA levels (4.4 vs. 2.1 log10 IU/ml, $P < 0.001$) and higher HBeAg positivity rate (40.4% vs. 15.7%, $P < 0.001$). Additionally, the treated group displayed higher ALT, AST, LS, APRI (AST to platelet ratio index), while lower albumin (all $P < 0.05$). We didn't find any differences in terms of total bilirubin, platelet, CAP, and most metabolic variables except triglyceride (TG) and waist circumference. The distribution of HS grade consisted of 17.6% (19/108) mild, 12.0% (13/108) moderate, and 70.4% (76/108) severe in untreated group vs. 18.3% (19/104) mild, 17.3% (18/104) moderate, and 64.4% (67/104) severe in treated group ($P = 0.523$). The rate of mild-moderate HS and severe HS was comparable between the two groups ($P = 0.356$). Accordingly, fibrosis stages were distributed as follows: F0/F1: 52.8% (57/108), grey area: 47.2% (51/108) and F3: 0% (0/108) in untreated group, while F0/F1: 29.8% (31/104), grey area: 63.5% (66/104), and F3: 6.7% (7/104) in treated group ($P < 0.001$).

### 3.2. Virological and biochemical responses to antiviral treatment

Among the treated group, the cumulative incidences of HBV DNA undetectable (52.9%, 69.2%, and 88.5%) (Fig. 2, A), HBeAg loss (19.0%, 23.8%, and 31.0%) (Fig. 2, B), and HBsAg loss (1.0%, 1.9%, and 8.7%) (Fig. 2, C) at 1, 2 and 3 years were shown. ALT

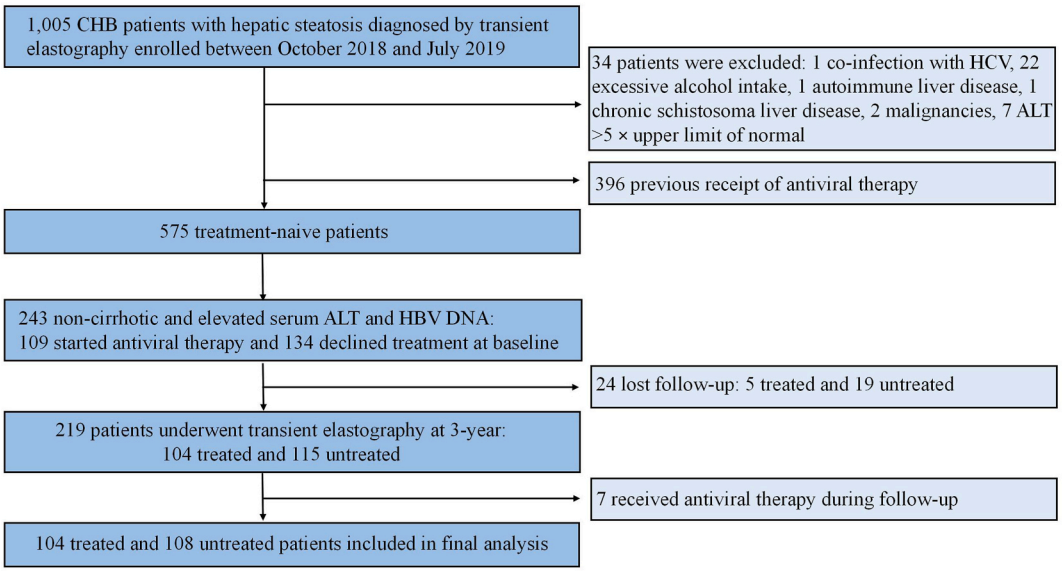


**Fig. 1.** Patients' disposition.

**Table 1**
Baseline characteristics of the total cohort of non-cirrhotic chronic hepatitis B (CHB) patients with hepatic steatosis (HS).

| Variable | Total cohort (n = 212) | CHB + HS untreated (n = 108) | CHB + HS treated (n = 104) | *P* value |
|---|---|---|---|---|
| Age, years | 36 (30–44) | 37 (31–44) | 36 (30–45) | 0.975 |
| Sex, male (%) | 193 (91.0%) | 100 (92.6%) | 93 (89.4%) | 0.419 |
| Body mass index (kg/m$^2$) | 26.4 (25.0–28.5) | 26.6 (25.5–28.8) | 26.1 (24.7–28.1) | 0.059 |
| BMI category (%) | | | | 0.206 |
| Normal | 11 (5.2%) | 4 (3.7%) | 7 (6.7%) | |
| Overweight | 41 (19.3%) | 17 (15.7%) | 24 (23.1%) | |
| Obese | 160 (75.5%) | 87 (80.6%) | 73 (70.2%) | |
| Waist circumference (cm) | 94 (90–99) | 95 (91–100) | 93 (90–98) | 0.022 |
| Central obesity (%) | 180 (84.9%) | 94 (87.0%) | 86 (82.7%) | 0.377 |
| Systolic blood pressure (mmHg) | 129 (121–137) | 130 (121–137) | 129 (120–137) | 0.856 |
| Diastolic blood pressure (mmHg) | 82 (76–89) | 84 (78–91) | 82 (76–88) | 0.197 |
| Total cholesterol (mmol/L) | 4.5 (4.1–5.1) | 4.6 (4.1–5.2) | 4.5 (4.1–4.9) | 0.248 |
| Triglyceride (mmol/L) | 1.7 (1.2–2.5) | 1.7 (1.3–2.9) | 1.5 (1.1–2.3) | 0.031 |
| HDL-cholesterol (mmol/L) | 1.1 (1.0–1.2) | 1.1 (1.0–1.3) | 1.1 (1.0–1.2) | 0.729 |
| LDL-cholesterol (mmol/L) | 2.8 (2.4–3.3) | 2.8 (2.3–3.5) | 2.9 (2.4–3.2) | 0.606 |
| Fasting glucose (mmol/L) | 5.2 (4.8–5.5) | 5.2 (4.9–5.6) | 5.1 (4.8–5.4) | 0.335 |
| Dyslipidemia (%) | 119 (56.1%) | 57 (52.8%) | 62 (59.6%) | 0.316 |
| Hypertension (%) | 62 (29.2%) | 33 (30.6%) | 29 (27.9%) | 0.669 |
| Diabetes (%) | 13 (6.1%) | 5 (4.6%) | 8 (7.7%) | 0.353 |
| HBeAg positive (%) | 59 (27.8%) | 17 (15.7%) | 42 (40.4%) | <0.001 |
| HBV DNA ($\log_{10}$ IU/ml) | 3.1 (2.1–5.4) | 2.1 (2.0–3.3) | 4.4 (3.1–6.4) | <0.001 |
| HBsAg ($\log_{10}$ IU/ml) | 3.5 (2.5–4.2) | 3.0 (2.0–3.8) | 3.9 (3.2–4.2) | <0.001 |
| ALT (U/L) | 59 (47–78) | 56 (45–73) | 65 (50–103) | 0.006 |
| AST (U/L) | 35 (29–48) | 33 (28–40) | 38 (29–58) | 0.001 |
| Albumin (g/L) | 48 (46–50) | 48 (46–50) | 47 (46–49) | <0.001 |
| Total bilirubin (μmol/L) | 11.5 (8.3–15.2) | 11.2 (8.3–14.1) | 12.1 (8.2–16.3) | 0.353 |
| Platelet ( $\times 10^9$/L) | 207 (182–245) | 207 (186–248) | 203 (178–234) | 0.263 |
| AST to platelet ratio index | 0.42 (0.31–0.65) | 0.38 (0.30–0.51) | 0.45 (0.34–0.77) | 0.004 |
| CAP (dB/m) | 302 (271–338) | 308 (271–345) | 293 (272–330) | 0.272 |
| Hepatic steatosis (%) | | | | 0.356 |
| Mild-moderate steatosis (%) | 69 (32.5%) | 32 (29.6%) | 37 (35.6%) | |
| Severe steatosis (%) | 143 (67.5%) | 76 (70.4%) | 67 (64.4%) | |
| Liver stiffness (kPa) | 6.4 (5.3–8.1) | 5.8 (5.1–7.8) | 6.6 (5.7–8.2) | 0.005 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HDL, high density lipoprotein; LDL, low density lipoprotein.

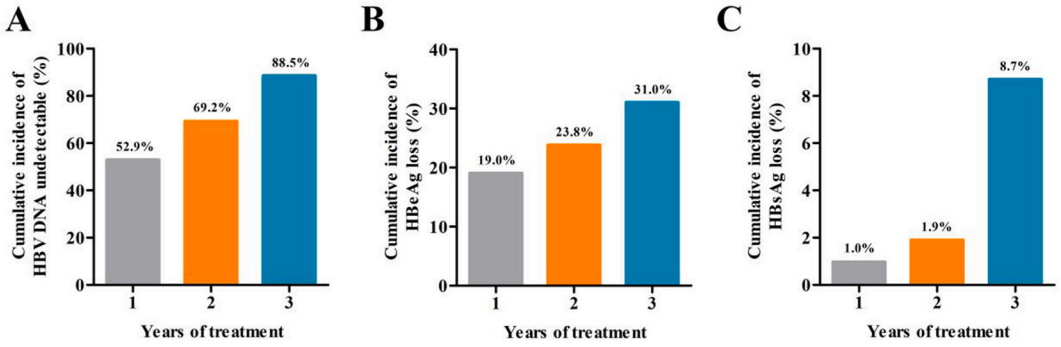


**Fig. 2.** Virological outcomes in treated patients with CHB + HS during a 3-year follow-up. The cumulative incidences of HBV DNA undetectable (**A**), HBeAg (**B**) and HBsAg loss (**C**) were shown.

normalization rate was 70.2% in the treated group during a follow-up of 3 years.

Subgroup analysis was further performed to examine for any differences in the severity of HS on treatment outcomes. No significant differences regarding baseline age, sex, HBV DNA, HBsAg, and ALT were observed between mild-moderate HS and severe HS groups (all $P > 0.05$). We found similar cumulative incidences of HBV DNA undetectable (56.8% vs. 50.7% at year 1, 73.0% vs. 67.2% at year 2, 91.9% vs. 86.6% at year 3) (Fig. 3, A), HBeAg loss (17.6% vs. 20.0% at year 1, 23.5% vs. 24.0% at year 2, 23.5% vs. 36.0% at year 3) (Fig. 3, B), and HBsAg loss (2.7% vs. 0% at year 1, 5.4% vs. 0% at year 2, 13.5% vs. 6.0% at year 3) (Fig. 3, C) in mild-moderate HS group vs. severe HS group (all $P > 0.05$). No difference regarding ALT normalization rate at year 3 was observed between mild-moderate HS and severe HS groups (73.0% vs. 68.7%, $P = 0.645$).

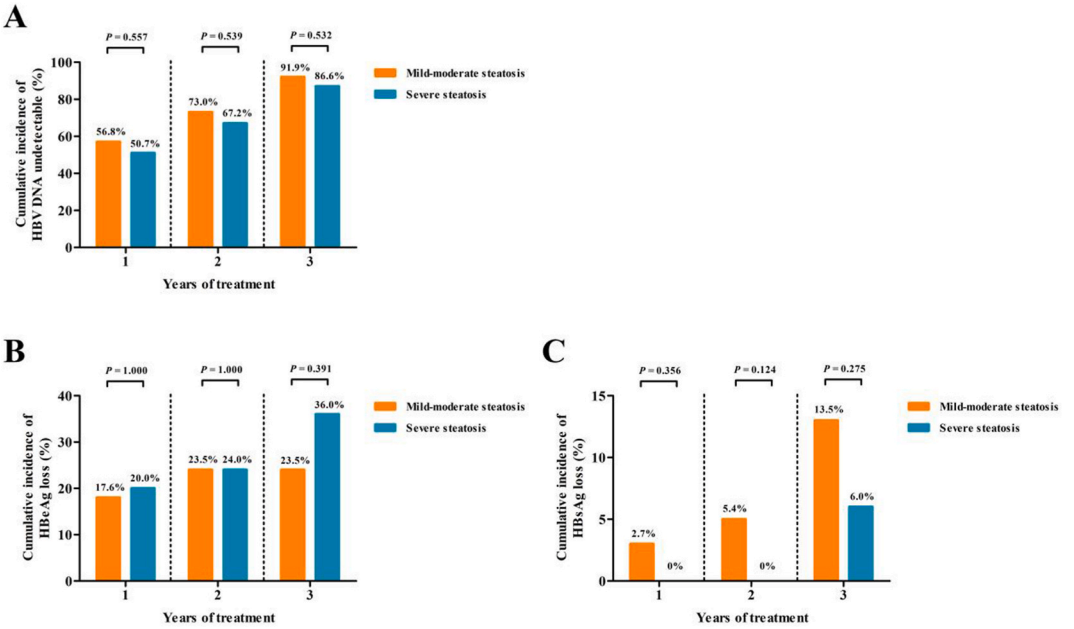


**Fig. 3.** Virological outcome in treated patients with CHB + HS stratified by baseline HS during a 3-year follow-up. The cumulative incidences of HBV DNA undetectable (**A**), HBeAg (**B**) and HBsAg loss (**C**) at 1, 2 and 3 years in mild-moderate and severe HS patients were shown.

### *3.3. Antiviral therapy effects on fibrosis progression compared to no treatment*

At year 3, HS grades were distributed as follows: 2.8% (3/108) no, 16.7% (18/108) mild, 15.7% (17/108) moderate and 64.8% (70/108) severe in untreated group, and 10.6% (11/104) no, 21.2% (22/104) mild, 14.4% (15/104) moderate and 53.8% (56/104) severe in treated group, respectively ($P = 0.087$). Fibrosis stages were distributed as follows: 38.9% (42/108) F0/F1, 50.9% (55/108) grey area, 8.3% (9/108) F3, and 1.9% (2/108) F4 in untreated group; 65.4% (68/104) F0/F1, 26.9% (28/104) grey area, 6.7% (7/104) F3, and 1.0% (1/104) F4 in treated group ($P = 0.001$). Treated group had lower fibrosis progression in comparison with untreated group (18.3% vs. 35.2%, $P = 0.005$) (Fig. 4, A). Consistently, the treated group had lower median LS value than untreated group at 3 years (5.6 vs. 6.2 kPa, $P < 0.001$). When stratified by baseline HS degree, significant differences regarding risk of fibrosis progression between treated and untreated groups were only observed in patients with mild-moderate HS (5.4% vs. 28.1%, $P = 0.010$), but not in severe HS patients (25.4% vs. 38.2%, $P = 0.102$) (Fig. 4, B).

### *3.4. Antiviral therapy effects on fibrosis progression after multivariate-adjusted analysis*

Binary logistic regression analysis was applied to identify factors independently associated with fibrosis progression (Table 2). In univariate analysis, antiviral therapy [odds ratio (OR) 0.412, 95% CI 0.218–0.777, $P = 0.006$] and higher ALT (OR 0.988, 95% CI

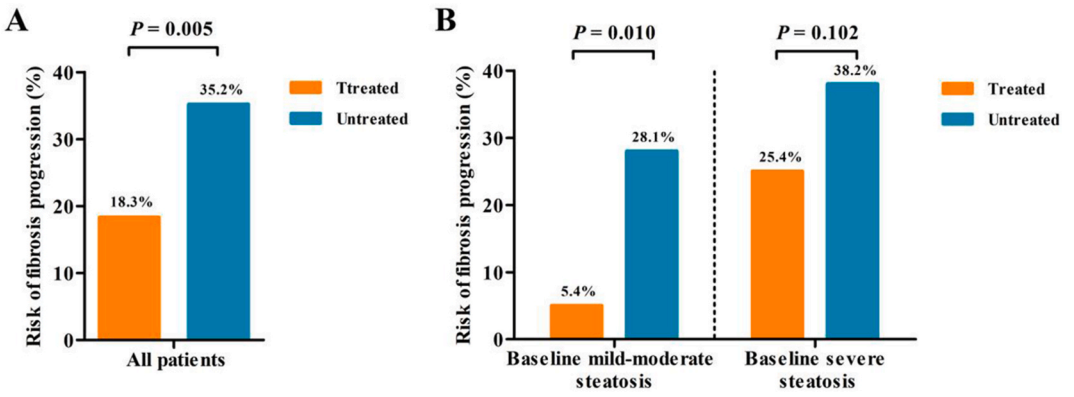


**Fig. 4.** Risk of fibrosis progression in patients with CHB + HS. (A) Fibrosis progression risk in treated and untreated patients. (B) Fibrosis progression risk in treated and untreated patients stratified by baseline HS.

**Table 2**
Predictors of fibrosis progression in the total cohort of non-cirrhotic chronic hepatitis B patients with hepatic steatosis at 3-years.

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| Age | 0.983 | 0.951–1.017 | 0.332 | | | |
| Sex, male | 1.420 | 0.451–4.472 | 0.549 | | | |
| BMI ≥23 kg/m$^2$ | 1.695 | 0.355–8.093 | 0.508 | | | |
| Central obesity | 2.921 | 0.977–8.737 | 0.055 | | | |
| Antiviral therapy | 0.412 | 0.218–0.777 | 0.006 | 0.473 | 0.245–0.911 | 0.025 |
| HBeAg positive | 1.143 | 0.586–2.232 | 0.694 | | | |
| HBV DNA | 0.983 | 0.836–1.155 | 0.831 | | | |
| HBsAg | 1.043 | 0.774–1.406 | 0.783 | | | |
| ALT | 0.988 | 0.977–0.999 | 0.030 | 0.989 | 0.977–1.000 | 0.053 |
| AST | 0.986 | 0.971–1.002 | 0.085 | | | |
| Albumin | 0.937 | 0.849–1.034 | 0.195 | | | |
| Total bilirubin | 1.009 | 0.983–1.036 | 0.504 | | | |
| Dyslipidemia | 1.337 | 0.727–2.459 | 0.350 | | | |
| Diabetes | 1.241 | 0.329–4.682 | 0.750 | | | |
| Severe steatosis | 2.500 | 1.200–5.209 | 0.014 | 2.581 | 1.213–5.493 | 0.014 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen.

0.977–0.999, $P = 0.030$) were associated with lower risk of fibrosis progression, while severe HS (OR 2.500, 95% CI 1.200–5.209, $P = 0.014$) was associated with a higher risk of fibrosis progression. After adjusting other covariates by multivariate analysis, antiviral therapy (OR 0.473, 95% CI 0.245–0.911, $P = 0.025$) was independently associated with a lower fibrosis progression risk, while severe HS (OR 2.581, 95% CI 1.213–5.493, $P = 0.014$) was independently associated with a higher fibrosis progression risk.

To further understand whether antiviral therapy benefits differed by the severity of baseline HS, we further stratified the multivariate binary logistic regression models into mild-moderate HS group (n = 69) and severe HS group (n = 143) (Table 3). The benefit was observed mainly in those with mild-moderate HS, but not in those with severe HS. As shown in Table 3, antiviral therapy led to 85.4% reduction in risk of fibrosis progression (OR 0.146, 95% CI 0.029–0.738, $P = 0.020$) in mild-moderate HS patients.

### 3.5. Antiviral therapy effects on fibrosis progression by PSM analysis

PSM analysis was applied to further confirm our findings, yielding 55 pairs of untreated and treated patients (Table S1). Regarding baseline characteristics, there were no significant differences between the two groups, except HBsAg levels (3.1 vs. 3.6 log10 IU/ml, $P = 0.034$) in the PSM cohort. The treated group had lower fibrosis progression compared to untreated group (18.2% vs. 36.4%, $P = 0.032$) (Figure S1). To strengthen our findings, APRI score was analyzed. In treated group, the median year-3 APRI score decreased compared to baseline (from 0.42 to 0.30, $P = 0.004$), but not in untreated group ($P = 0.489$). We also found that year-3 FIB-4 score trended to be declined in treated group (from 0.93 to 0.71, $P = 0.065$), but not in untreated group ($P = 0.908$).

### 3.6. Antiviral therapy effects on hepatic steatosis and glycolipid metabolism

Among the PSM cohort, we further compared the distribution of HS and glycolipid metabolism between patients with and without antiviral therapy at year 3 (Figure S2). HS grades were distributed as follows: 5.5% (3/55) no, 18.2% (10/55) mild, 16.3% (9/55) moderate and 60.0% (33/55) severe in untreated group vs. 7.3% (4/55) no, 23.6% (13/55) mild, 12.7% (7/55) moderate and 56.4% (31/55) severe in treated group ($P = 0.838$). Levels of plasma TG, total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) were comparable between treated and untreated groups (all $P > 0.05$). To explore whether antiviral medication may have effects on metabolic parameters and HS in these patients, subgroup analysis for patients receiving TAF, TDF or ETV was performed (Figure S3). We found TC and TG levels were lower in TDF treated patients compared to those in untreated patients (4.5 vs. 3.8 mmol/L, $P = 0.006$; 1.7 vs. 1.2 mmol/L, $P = 0.009$, respectively), but not for HDL-C, LDL-C and glucose levels and HS severity (all $P > 0.05$).

**Table 3**
Evaluating the impact of antiviral therapy on fibrosis progression in the total cohort stratified by baseline hepatic steatosis at 3-years.

| | Fibrosis progression | | | | | |
|---|---|---|---|---|---|---|
| | Mild-moderate steatosis | | | Severe steatosis | | |
| | Multivariate analysis (Model 1) | | | Multivariate analysis (Model 2) | | |
| | **OR** | **95% CI** | ***P* value** | **OR** | **95% CI** | ***P* value** |
| Antiviral therapy | 0.146 | 0.029–0.738 | 0.020 | 0.551 | 0.268–1.131 | 0.104 |

Note: Multivariate model 1 and model 2 were adjusted for age, sex, BMI ≥23 kg/m$^2$, central obesity, HBeAg positive, HBV DNA, HBsAg, albumin, total bilirubin, ALT, AST, dyslipidemia, diabetes.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen.

Additionally, these metabolic parameters and HS severity were comparable between treated (TAF or ETV) and untreated groups (all $P > 0.05$).

## 4. Discussion

In this study, we showed antiviral therapy effectively inhibited HBV replication in CHB + HS patients, regardless of HS severity. In addition, antiviral therapy was independently associated with a lower risk of fibrosis progression, especially in those with mild-moderate HS. Our results were consistently validated by multivariate and PSM analysis.

Overall, our study complements and extends existing literature by identifying antiviral benefit in patients with CHB + HS. We specifically focused on non-cirrhotic CHB + HS patients with ALT elevation, and observed the achievement of high rates of virological response. In addition, our real-world cohort observed a significant reduction in fibrosis progression due to antiviral treatment. Our results suggested that antiviral therapy was effective in this special population. These data may be helpful to guide clinical practice for patients with CHB + HS, an unmet need that has been highlighted by a recent East Asia expert opinion [16]. At the moment, given the increasing prevalence of CHB + HS patients and its related worrisome consequences, aggressive control of steatosis, in addition to antiviral therapy, should also be mandatory to prevent liver disease progression.

The finding of antiviral benefit in reducing fibrosis progression in mild-moderate HS but not in those with severe HS is intriguing. Severe HS has been reported to be associate with increased severe fibrosis or fibrosis progression in CHB patients [10,24,25]. A recent review has concluded that severe HS might exacerbate hepatic fibrosis in Asian CHB patients [7]. Consistently, we found severe HS was associated with increased risk of fibrosis progression in CHB + HS patients. Li et al. identified that fatty liver was not related with complete response rates to antiviral treatment in CHB patients [26]. Interestingly, we found cumulative incidences of undetectable HBV DNA, HBeAg and HBsAg loss within 3 years were comparable between mild-moderate HS and severe HS groups, suggesting that HS severity was not associate with the efficacy of antiviral therapy. Thus, it is possible that fibrosis progression may be largely determined by severe HS, other than viral factors in this particular subgroup. However, this observation needs to be assessed in cohorts with longer follow-up.

Notably, a high HBsAg loss rate within 3 years was found in treated patients (8.7%). To our knowledge, data on the rate of HBsAg loss of newer CHB treatment in the presence of HS are scarce, since most studies only focused on examining patients with CHB [16]. In addition, we found patients without antiviral treatment also had a high HBsAg loss rate at year 3 (5.6%) (data not shown), which exceeds the 1.02% annual reported rate of CHB natural history [27]. Indeed, higher HBsAg loss rates have been reported in CHB + HS patients than in CHB alone by several studies [10,28,29]. In *Journal of Hepatolgy*, Lung-Yi Mak et al. have recently reported that the 3-year HBsAg seroclearance rate reached 9.9% in treatment-naive CHB patients having HS and elevated HBV DNA from Hong Kong [10]. It was presumed that the presence of fat in hepatocytes and its associated metabolic stress may reinforce HBV-suppressed innate immunity, therefore restoring antiviral molecules production such as interferon and tumor necrosis factor-α, and activating lymphocytes, ultimately promoting the clearance of HBsAg [30].

Although HCV infection was reported associated with HS [31,32], whether HBV infection may interfere with lipid metabolism and the steatogenic process remains inconclusive [33,34]. Moreover, whether antiviral therapy would have possible impact on lipid metabolism and HS remains unknown in patients with CHB + HS. We didn't find any difference on HS and glycolipid metabolism between treated and untreated groups in our PSM cohort. We have previously demonstrated that HBV infection didn't alter metabolic factors and HS in mice model [35]. Therefore, HS and glycolipid metabolism were more likely to be determined by fatty liver disease, rather than viral factors. Antiviral drugs such as TDF and TAF may have effects on metabolic factors and HS in CHB patients [36–38], however, their effects in patients with CHB + HS remains unclear. Here, we found TC and TG levels, but not LDL-C, HDL-C, glucose levels and HS severity, were lower in TDF treated patients compared to untreated patients. Regarding TAF and ETV treatment, HS and glycolipid metabolism were comparable between untreated and treated patients. However, these findings should be interpreted with caution, as the relatively small sample size when further classified treated patients into TDF, TAF, and ETV treated groups. The effects of different antiviral drugs on metabolic factors in these patients need to be addressed in large-scale cohort studies.

There are several ways to identify HS including liver histology, TE, ultrasound, computed tomography (CT) as well as magnetic resonance imaging (MRI). Although liver histological examination is traditionally considered to be a gold standard, it is invasive and costly, limiting the widespread utilization in real-world clinical practice [39]. Ultrasound is a common tool to detect HS. However, ultrasound has low sensitivity and high observer variability, and cannot accurately evaluate the degree of HS [40]. CT and MRI can also be used to diagnose HS, however, they are unsuitable for routine clinical practice due to relatively high cost and requirement for specific facility [40]. In our study, TE with associated CAP was used to define HS. It has been shown that CAP is well related with liver fat quantity both in Western and Asian populations [41,42]. In particular, TE can simultaneously measure liver stiffness and is now identified as a non-invasive criteria for assessing fibrosis to guide treatment and disease surveillance [43]. Because TE with CAP is non-invasive, accurate and reproducible, it allows the opportunity to examine hepatic fibrosis and HS longitudinally in a considerable number of patients.

To our knowledge, it is the first time to comprehensively assess antiviral therapy effects on virological response and liver fibrosis, as well as lipid metabolism in CHB patients with different degrees of HS. Our current study has the strength of respectable length of follow-up, improving the statistical power and reliability of our results. However, this study also has several limitations. First, liver biopsy was not performed. Serial liver biopsies may not be acceptable to patients given the costs and risks of repeated liver biopsy, particularly in developing countries, in which the prevalence of HBV infection is high. Instead, we adopted one of the most reliable, reproducible, and non-invasive tools for hepatic fibrosis assessment recommended by a variety of international guidelines [19,44]. Overweight and HS are considered to be confounding factors for the unreliable liver stiffness values [45]. However, this confounding

effect should be minimal in our study, as there were no differences regarding these confounding factors between treated and untreated patients both at baseline and follow-up visit. Furthermore, serum indices of hepatic fibrosis, eg, APRI score provides extra evidence to support our findings. Second, our fi ndings may have potential selection and confounding biases as an observational study. However, multiple strategies were tried to adjust the differences at baseline, including multivariate-adjusted and PSM analysis. Third, although a male predominance in this special population has been reported by several studies [11,46], the higher percent of male patients included in our cohort may lead to a potential bias. Finally, unmeasured confounding factors, such as homeostatic model assessment of insulin resistance, couldn't be stated completely in an observational study.

In conclusion, we demonstrated antiviral therapy was effective in CHB + HS patients. Also, our results showed that HS severity didn't affect HBV treatment outcomes of biochemical response or virological suppression. Finally, the impact of antiviral therapy on fibrosis progression is limited in those with severe HS, suggesting that not only antivirals but strict lifestyle interventions using exercise and diet change are necessary for this special population.

## Funding

This work was funded by the National Key Research and Development Program of China (No. 2021YFC2600200), and the National Natural Science Foundation of China (No. 82170596).

## Ethics statement

The present study was conducted in line with the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology (2017S277). Written informed consent was obtained from every patient.

## Data availability

Data supporting the findings in this study are available from the corresponding author upon reasonable request.

## CRediT authorship contribution statement

**Danqing Hu:** Writing – original draft, Data curation. **Peng Wang:** Data curation. **Xiaojing Wang:** Formal analysis. **Xue Hu:** Data curation. **Da Huang:** Data curation. **Weiming Yan:** Formal analysis. **Dong Xi:** Formal analysis. **Meifang Han:** Writing – review & editing, Supervision, Conceptualization. **Qin Ning:** Writing – review & editing, Supervision, Conceptualization. **Hongwu Wang:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors would like to thank the medical staffs in the Department and Institute of Infectious Disease of Tongji Hospital for their essential support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28653.

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