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Original Article

Adverse Effect of Nonalcoholic Fatty Liver Disease on the Therapeutic Response in Patients with Chronic Hepatitis B

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Abstract

Background and Aims: The impact of nonalcoholic fatty liver disease (NAFLD) on the treatment outcome of chronic hepatitis B (CHB) is undefined and deserves an in-depth investigation. ***Methods:*** Histologically-proven CHB receiving first-line antiviral regimens as initial therapy was enrolled and grouped by the concurrence of NAFLD, and followed up at six monthly intervals. Therapeutic response related data were recorded and compared at multiple time points. Kaplan-Meier and Cox regression analyses were utilized to estimate the impact of NAFLD on complete virological response (CVR). ***Results:*** We enrolled 267 patients (CHB: 164; CHB with NAFLD: 103) with comparable follow-up durations. They were also comparable in baseline HBV DNA levels and HBeAg positivity. Patients with concomitant NAFLD showed less significant decline in HBV DNA, qHBsAg, pgRNA, and liver enzyme levels over time; moreover, their cumulative incidences of CVR were significantly lower and that of low-level viremia (LLV) were significantly higher at 6, 12, 18, 24 months. First CVR of CHB was delayed with the presence NAFLD (11.0 vs. 7.0 months, $p<0.001$) and further prolonged with higher grade of liver steatosis (Grade 2–3 vs. 1: 13.0 vs. 9.0 months). On multivariate analysis, HBeAg positivity (HR: 0.650, $p=0.036$), grade of steatosis (G2 [HR: 0.447, $p=0.004$]; G3 [HR: 0.085, $p=0.002$]) and HBV DNA (log10 IU/mL) (HR: 0.687, $p<0.001$) were significantly associated with delayed CVR, whereas grade of necroinflammation (HR: 1. 758, $p<0.001$) accelerated the CVR. ***Conclusions:*** In CHB patients receiving initial antiviral therapy, NAFLD was associated with higher levels of HBV DNA, pgRNA, and liver enzymes, and higher incidence of LLV and delayed CVR.

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Introduction

Chronic hepatitis B (CHB) is a major global public health challenge, with an estimated 292 million cases worldwide.[1] Nonalcoholic fatty liver disease (NAFLD) is another major cause of chronic liver disease worldwide due to increasing rates of obesity and metabolic syndrome (MetS), with a global prevalence of 25%.[2] Both CHB and NAFLD are responsible for considerable morbidity and the associated economic burden. Patients with concomitant CHB and NAFLD are frequently encountered in clinical practice. There is an emerging interest in the potential interactions between CHB and NAFLD. Nonetheless, the role of NAFLD in CHB is not well characterized.

NAFLD may affect viral replication through modulation of host immunity. Rex *et al*.[3] found that increasing steatosis was independently associated with lower serum HBV DNA levels in a cohort of 1,202 controlled attenuation parameter (CAP)-diagnosed NAFLD patients. Other studies have also found an inverse association of HBV viral load (VL) with concomitant NAFLD.[4,5] However, a number of biopsy-based studies did not find any association between NAFLD and HBV VL.[6,7] In addition, there is no clear consensus on the effect of NAFLD on the levels of serum aminotransferases. In a cross-sectional study, biopsy-proven hepatic steatosis and metabolic disorders were associated with elevated aminotransferases in HBeAg-negative patients, particularly those with low HBV DNA levels.[8] In two longitudinal studies, CHB patients with hepatic steatosis had lower rates of complete virological response (CVR) and alanine transaminase (ALT) normalization and/or delayed biochemical response to nucleoside analog (NA) therapies.[9,10] However, other studies found no impact of NAFLD on long-term biochemical or virologic response.[11,12] There were several limitations of

Keywords: Chronic hepatitis B; Nonalcoholic fatty liver disease; Therapeutic response; HBV pregenomic RNA; Low-level viremia.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CVR, complete virological response; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; HRs, hazard ratios; LLOQ, lower limit of quantitation; LLV, low-level viremia; MetS, metabolic syndrome; NA, nucleoside analog; NAFLD, nonalcoholic fatty liver disease; pgRNA, pregenomic RNA; VL, viral load.

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previous studies that compared the therapeutic efficacy in CHB patients with and without NAFLD, such as inclusion of nonbiopsy diagnosed patients and/or small sample size.

Furthermore, emerging evidence suggests the contribution of liver steatosis and other metabolic parameters on the process of fibrosis and hepatocarcinogenesis in CHB, even after antiviral treatment.[13–16] There is a need for more robust indicators to evaluate the effect of NAFLD on virological response in patients with CHB, to further reduce the risk of hepatocellular carcinoma (HCC). Delayed CVR (48 weeks) and/or virologic breakthrough during antiviral therapy indicates poor therapeutic response and is associated with high risk of liver fibrosis and HCC. Low-level viremia (LLV), defined as detectable HBV DNA either persistent or intermittent episodes of <2,000 IU/mL, is a manifestation of delayed CVR and/or virologic breakthrough. It has been shown to be associated with a higher risk of HCC, especially in patients with cirrhosis.[17,18] Besides, serum HBV RNA detection, in the form of 3.5 kb encapsidated virion-containing pregenomic RNA (pgRNA), has been widely described recently. Serum pgRNA levels were shown to be higher than DNA levels in most CHB patients receiving nucleoside reverse transcriptase inhibitor (NRTI) treatment.[19] In addition, serum pgRNA level was shown to predict antiviral treatment efficacy[20,21] and post-NRTI cessation virological relapse.[22] Moreover, it is a surrogate marker of covalently closed circular DNA (cccDNA) activity.[23] In a study by Lung-Yi *et al*.[24] more than 50% CHB patients on entecavir with HBV DNA below the lower limit of quantitation (LLOQ) by standard assay had persistent viraemia as determined by a more sensitive assay; in addition, detectable HBV DNA or HBV pgRNA was associated with HCC development. Therefore, incidence of LLV and HBV pgRNA levels are novel markers to evaluate the effect of NAFLD on the virological response in CHB patients, particularly in those with delayed CVR and/or virologic breakthrough. Although some studies have reported the potential association between NAFLD and CHB, the clinical impact of hepatic steatosis on the therapeutic response to NAs in patients with histologically-proven CHB has not been sufficiently clarified, especially as assessed by the incidence of LLV and HBV pgRNA levels.

Methods

This study was approved by the Ethics Committee of the Third Hospital of Hebei Medical University (K2019-014-2) and Ethics Committee of the Henan Provincial People's Hospital (2020-184), and was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent for use of their data or blood samples (anonymously) for research purposes.

Patients

Patients with biopsy-proven CHB with or without concomitant NAFLD who were admitted to the Third Hospital of Hebei Medical University or Henan Provincial People's Hospital between July 2015 and May 2021 were reviewed. The inclusion criteria were: (a) ≥18 years of age; (b) treatment naïve; (c) initial first-line NA treatment; (d) detectable HBV DNA at baseline; (e) at least one follow-up after the initial treatment; (f) LLOQ 20 IU/mL. The exclusion criteria were: (a) history of decompensated cirrhosis, HCC, liver transplantation; (b) coinfection(s) with hepatitis D, hepatitis C, HEV, HIV, or superimposed autoimmune liver disease, extra-hepatic malignancy or severe hematologic disease at screening; (c) significant alcohol intake (>30 g/day for men, >20 g/day for women); (d) current pregnancy. Patients who qualified all the study selection criteria were eligible for inclusion. They were followed up at six monthly intervals after the initial NA therapy. Besides, serum samples were collected for monitoring pgRNA levels at baseline, 6, 12, 18, and 24 months. All samples were tested using a diagnostic kit for hepatitis B virus pgRNA (PCR-Fluorescence Probing; Hotgen, Beijing, China).

Assessment

Demographic characteristics, medical history, and liver biopsy reports at baseline were collected. Biochemical tests, HBV DNA levels, HBV serological markers, and liver-related outcomes were also recorded and evaluated during follow-up. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and patients were categorized in accordance with Asian-Pacific classification for adult Asians: obese (≥25.0 kg/m^2) and overweight (≥23 kg/m^2). MetS was defined according to the International Diabetes Federation definition.[25] Liver biopsy examinations were carried out using standard techniques. All liver samples were evaluated according to the Scheuer scoring system for grade of liver inflammation and METAVIR scoring system for stage of liver fibrosis. The histological severity of hepatic steatosis was classified as grade 0 (<5%), grade 1 (5–33%), grade 2 (>33%–66%), and grade 3 (>66%).

Complete virologic response was defined as detectable serum HBV DNA at baseline which is suppressed to<20 IU/mL during follow-up for the first time. HBeAg seroconversion was defined as the first detection of antibody to HBeAg (anti-HBe). Virological breakthrough was defined as an increase in HBV DNA by >1 $\log_{10}$ IU/mL from nadir or redetection of HBV DNA. Functional cure was defined as negative or undetectable HBsAg following a previously positive result.

Statistical analysis

Continuous variables were expressed as means ± standard deviation or medians and interquartile range (IQR), as appropriate. Between-group differences were assessed using Student *t*-test or Mann-Whitney *U* test for continuous variables and chi-squared test or Fisher's exact test for categorical variables. Kruskal-Wallis tests were used to assess the dynamic changes in pgRNA levels in each group. Kaplan-Meier survival analysis was used to estimate the rates of first CVR over time. Hazard ratios (HRs) were determined by Cox proportional-hazards regression models. Two-tailed *p*-values ≤0.05 were considered statistically significant. The statistical analysis was performed with IBM SPSS Statistics 23.0 and plotted by R version 4.1.2.

Results

Study cohort

A total of 267 patients were enrolled (164 had CHB alone and 103 had CHB with concomitant NAFLD). The baseline characteristics of the study population are summarized in Table 1. Both groups were comparable in terms of age and sex distribution. Patients with concomitant NAFLD had significantly higher BMIs (25.7 vs. 23.4 kg/m^2, p<0.001) and higher prevalence of MetS (41.7% vs. 17.7%, p<0.001). Leukocyte counts, hemoglobin, albumin, lactate dehydrogenase, total cholesterol and uric acid were also significantly higher in patients with concomitant NAFLD. Of note, there was no significant between-group differences with respect

Table 1. Baseline characteristics of the study population

Variable	CHB (*n*=164)	CHB with NAFLD (*n*=103)	*p*
Age, years	37.0 (30.0, 47.0)	36.0 (31.0, 45.3)	0.854
Male	111 (67.7)	75 (72.8)	0.375
BMI, kg/cm^2	23.4 (21.0, 26.6)	25.7 (23.4, 28.1)	<0.001
Metabolic syndrome	29 (17.7)	43 (41.7)	<0.001
Obesity	55 (33.7)	58 (56.3)	<0.001
Raised triglycerides	28 (17.1)	37 (35.9)	<0.001
Reduced HDL-cholesterol	53 (32.3)	50 (48.5)	0.008
Raised blood pressure	9 (5.5)	18 (17.5)	0.002
Raised fasting plasma glucose	80 (48.8)	72 (69.9)	0.001
Laboratory tests			
Leukocytes, $\times10^9$/L	5.04 (4.10, 5.86)	5.88 (4.69, 6.77)	<0.001
Hemoglobin, g/L	147.4 (133.0, 158.4)	153 (142.5, 161.7)	0.007
Platelets, $\times10^9$/L	172.0 (130.0, 212.0)	192.0 (163.5, 242.5)	0.007
Albumin, g/L	44.2 (39.9, 47.0)	45.2 (43.1, 47.6)	0.004
ALT, U/L	62.0 (33.0, 124.3)	54.5 (34.8, 105.3)	0.714
AST, U/L	40.5 (28.0, 71.8)	34.0 (25.8, 58.3)	0.146
GGT, U/L	30.0 (20.0, 65.8)	33.0 (22.0, 51.0)	0.722
Total bilirubin, μmol/L	15.4 (12.4, 22.1)	13.9 (10.7, 19.6)	0.025
Lactate dehydrogenase, U/L	174.5 (146.0, 203.0)	188.0 (161.0, 215.5)	0.011
Total cholesterol, mmol/L	4.2 (3.8, 4.6)	4.9 (4.1, 5.3)	<0.001
Uric acid, μmol/L	305.5 (246.5, 364.3)	341.0 (291.0, 389.0)	0.002
qHBsAg, $\log_{10}$ IU/mL	3.7 (3.2, 4.2)	4.0 (3.4, 4.4)	0.060
HBV DNA, $\log_{10}$ IU/mL	6.59 (4.65, 7.89)	6.86 (4.55, 8.20)	0.397
HBeAg positive	100 (61.0)	64 (62.1)	0.850
Histologic evaluation			
Grade of steatosis (1/2/3)	–	53/41/9	–
Grade of inflammation (0–1/2/3/4)	42/88/30/4	35/58/8/2	0.085
Stage of fibrosis (0–1/2/3/4)	57/43/38/26	50/34/14/5	0.004
Follow-up, months	25.0 (15.0, 39.0)	25.0 (14.0, 43.0)	0.968
CVR	146 (89.0)	77 (74.8)	0.002
CVR, months	6.0 (3.0, 10.0)	8.0 (4.0, 14.5)	0.039
LLV*	49/151 (32.5)	50/86 (58.1)	<0.001
HBeAg seroconversion	26/100 (26.0)	9/64 (14.1)	0.069
HBeAg seroconversion, months	18.0 (6.0, 30.3)	35.0 (14.5, 42.0)	0.121
Virologic relapse	0/146 (0.0)	1/77 (1.3)	0.345
Virologic breakthrough	21/164 (12.8)	19/103 (18.4)	0.196
Functional cure	5 (3.0)	2 (1.9)	0.710

Data are n, n (%), n/N (%), or median (interquartile range) as shown; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CVR, the accumulative incidence of first complete viral response; GGT, gamma-glutamyltransferases; LLV*, the accumulative incidence of low-level viraemia at or after 1 year of nucleoside analog therapy; NAFLD, nonalcoholic fatty liver disease.

to the levels of liver enzymes, HBV DNA, quantitative HBsAg and the proportion of HBeAg-positive patients at baseline (p>0.05 for all).

On histological evaluation, in the concomitant NAFLD group, 53 (51.5%) patients had steatosis grade 1, 41 patients (39.8%) had steatosis grade 2, and nine patients (8.7%) had steatosis grade 3. The distribution of the grade of inflammation in the two groups showed no significant difference (p=0.085); however, significant and advanced liver fibrosis accounted for a greater proportion of patients in the

Table 2. Comparison of virological and biochemical response in HBV-infected patients based on the concurrence of nonalcoholic fatty liver disease

	Variable	CVR	LLV	HBeAg* seroconversion	ALT† normalization	AST† normalization	GGT† normalization
Month 6	CHB (*n*=164)	76 (46.3)	92 (56.1)	7/100 (7.0)	82/100 (82.0)	74/90 (82.2)	43/47 (91.5)
	CHB+NAFLD (*n*=103)	32 (31.1)	72 (69.9)	2/64 (3.1)	41/61 (67.2)	35/41 (85.4)	13/18 (72.2)
	p	0.013	0.024	0.485	0.032	0.655	0.101
Month 12	CHB (*n*=151)	117 (77.5)	43 (28.5)	9/91 (9.9)	81/93 (87.1)	75/83 (90.4)	42/44 (95.5)
	CHB+NAFLD (*n*=86)	46 (54.8)	41 (48.8)	1/51 (2.0)	41/52 (78.8)	32/36 (88.9)	12/16 (75.0)
	p	<0.001	0.003	0.095	0.192	0.753	0.038
Month 18	CHB (*n*=127)	108 (85.0)	21(16.5)	11/76 (14.5)	67/76 (88.2)	64/71 (90.1)	31/35 (88.6)
	CHB+NAFLD (*n*=75)	49 (65.3)	29 (38.7)	1/44 (2.3)	38/48 (79.2)	32/35 (91.4)	10/14 (71.4)
	p	0.002	<0.001	0.032	0.176	1.000	0.202
Month 24	CHB (*n*=105)	96 (91.4)	14 (13.3)	10/64 (15.6)	60/65 (92.3)	58/60 (96.7)	30/31 (96.8)
	CHB+NAFLD (*n*=65)	49 (75.4)	20 (31.3)	3/35 (8.6)	33/43 (76.7)	29/33 (87.9)	10/14 (71.4)
	p	0.004	0.006	0.372	0.022	0.181	0.027

Data are n, n (%), n/N (%), or median (interquartile range) as shown. *Patients who were seropositive for HBeAg and negative for anti-HBe at baseline; †Patients with ALT, or GGT at baseline above the lab criteria (ALT ≤50 U/L for men and ≤40 U/L for women; AST ≤40 U/L for men and ≤35 U/L for women; GGT ≤60 U/L for men and ≤45 U/L for women). ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVR, the accumulative incidence of first complete viral response; CHB, chronic hepatitis B; GGT, gamma-glutamyltransferases; LLV*, the accumulative incidence of low-level viraemia at or after 1 year of nucleoside analog therapy; NAFLD, nonalcoholic fatty liver disease.

CHB alone group (65.2% vs. 51.5%, p=0.025 and 39.0% vs. 18.4%, p<0.001, respectively, Table 1).

Virological response

Assessment of CVR and associated factors: In patients with concomitant NAFLD, 31.1% of patients showed decrease in HBV DNA level to <20 IU/mL at 6 months, 54.8% at 12 months, 65.3% at 18 months, and 75.4% at 24 months. In contrast, 46.3% of patients with CHB alone had VL <20 IU/mL at 6 months, with 77.5%, 85.0%, and 91.4% at 12 months, 18 months, and 24 months, respectively (Table 2). In the concomitant NAFLD group, the HBV DNA levels at 6, 12, 18, and 24 months were significantly higher than those in the CHB alone group (Fig. 1A). The median duration of follow-up in both groups was 25.0 months (p=0.968). At the end of follow-up, the cumulative incidence of CVR in the concomitant NAFLD group was significantly lower than that in CHB alone group (74.8% vs. 89.0%, p=0.002). In addition, the median time to first CVR in the concomitant NAFLD group was significantly longer than that in the CHB alone group (8.0 vs. 6.0 months, p=0.039) (Table 1). On K-M analysis, the estimated median time to CVR in the concomitant NAFLD group was significantly longer (11.0 vs. 7.0 months, p<0.001, Fig. 2A). Furthermore, the same trend was observed between patients with steatosis grade 1 and steatosis grade 2–3 (9.0 and 13.0 months) (Fig. 2B).

To identify baseline factors associated with CVR, Cox proportional-hazards regression was performed. The results of univariate and multivariate analysis are shown in Table 3. On multivariate analysis, after adjusting for age, sex, BMI, MetS, hemoglobin, platelet, albumin, ALT, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total bilirubin, qHBsAg, and antiviral regimens, HBeAg positivity (HR: 0.650, 95% CI: 0.435–0.972, p=0.036), HBV DNA ($\log_{10}$ IU/mL) (HR: 0.687, 95% CI: 0. 0.616–0.766, p<0.001), grade of hepatic steatosis (G2 [HR: 0.447, 95% CI: 0.259–0.773, p=0.004]; G3 [HR: 0.085, 95% CI: 0.019–0.392, p=0.002]) and leukocyte count (HR: 0.836, 95% CI: 0.743–0.0.939, p=0.003) were independently associated with longer time to achieve first CVR, whereas grade of necroinflammation (HR: 1.758, 95% CI: 1.336–2.312, p<0.001) predicted a shorter duration to CVR.

Incidence of LLV, virologic breakthrough, and dynamic changes in pgRNA levels: With the delayed CVR in patients with concomitant NAFLD, the cumulative incidence of LLV at or after 1 year of NA therapy was significantly higher (58.1% vs. 32.5%, p<0.001, Table 1). The incidence of LLV at 12 months in this group was 48.8%, and decreased to 38.7% and 31.3% at 18 and 24 months, respectively. In contrast, 28.5% of patients with CHB alone had LLV at 12 months, with 16.5% and 13.3% at 18 and 24 months, respectively (Table 2). Virologic breakthrough was recorded in 21 (14.4%) patients in the CHB group vs. 19 (25.0%) patients in the concomitant NAFLD group, with no significant between-group difference (p=0.196, Table 1). To assess the impact of NAFLD on virological response using a more sensitive assay, pgRNA detection was performed, and the results are depicted in Figure 3. Serum samples from 90 patients (50 with CHB alone and 40 with concomitant NAFLD) were collected. The pgRNA levels declined along with the anti-HBV therapy in patients with CHB alone, and the levels at 6, 12, 18, and 24 months were significantly lower than that at baseline (Fig. 3A). Patients with $\log_{10}$ pgRNA of<2.0 increased from 8.8% (n=3) to 29.6% (n=8) in this group, and patients with $\log_{10}$ pgRNA of ≥5.0 decreased from 55.9% (n=19) to 22.2% (n=6, Fig. 3B). On the contrary, no such obvious tendencies were observed in patients with concomitant NAFLD (Fig. 3A). Patients with $\log_{10}$ pgRNA of<2.0 showed a slight increase, but patients with $\log_{10}$ pgRNA of ≥5.0 persisted, accounting for a high proportion after 24 months of therapy (39.4% at baseline and 38.5% at 24 months).

Serological response

During follow-up, functional cure was recorded in 2 (1.9%) patients in the CHB with NAFLD group vs. five (3.0%) patients in the CHB alone group, with no significant between-

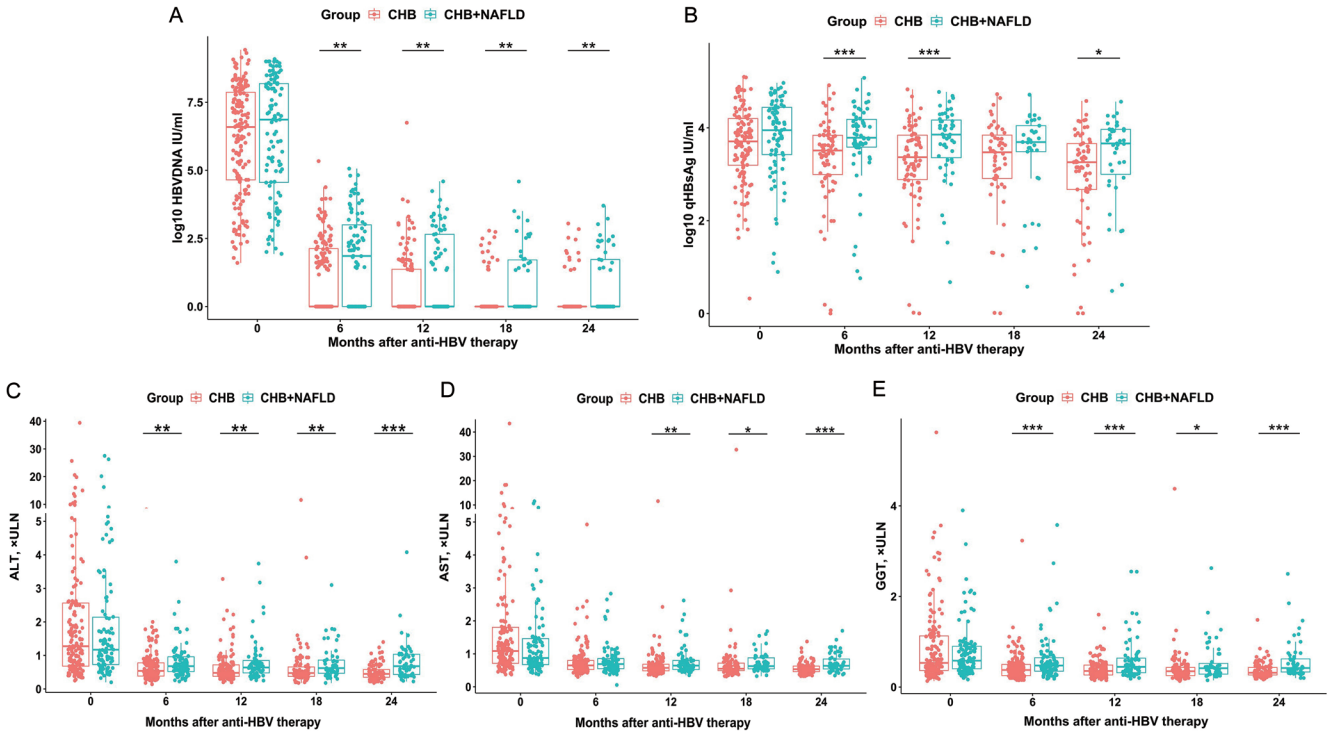

Fig. 1. Dynamic changes of virological (A, HBVDNA; B, HBsAg) and biochemical (C, ALT; D, AST; E, GGT) parameters in HBV-infected patients with and without concomitant hepatic steatosis. ALT, alanine transaminase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; GGT, gamma-glutamyltransferase; NAFLD, nonalcoholic fatty liver disease.

group difference (p=0.710, Table 1). The median serum qHBsAg in patients with concomitant NAFLD at 6, 12, and 24 months were numerically greater than those in patients with CHB alone, which declined from comparable levels at baseline (Fig. 1B). In the CHB alone group, the HBeAg seroconversion rate was slightly higher and the median time to achieve HBeAg seroconversion was shorter; however, these differences were not statistically significant (26.0% vs. 14.1%, p=0.069; 18.0 vs. 35.0 months, p=0.121, Table 1). Besides, the rates of HBeAg seroconversion among HBeAg-positive patients were higher at 6, 12, 18, 24 months and the end of follow-up, and the rate achieved statistical significance at 18 months (14.5% vs. 2.3%, p=0.032, Table 2).

Biochemical response

Although there were no significant between-group differences with respect to ALT, AST, and GGT levels at baseline, these indices were significantly higher in CHB with NAFLD group at multiple time points during follow-up. In the CHB with NAFLD group, the differences of ALT normalization rates achieved statistical significance at 6 and 24 months when compared with patients with CHB alone (67.2% vs. 82.0%, p=0.032 and 76.7% vs. 92.3%, p=0.022, respectively, Table 2). Among these patients, ALT levels were higher not only at 6 and 24 months, but also at 12 and 18 months (Fig. 1C). These tendencies were also in keeping with AST levels between the two groups at 12, 18, and 24 months (Fig. 1D). However, AST normalization rates at the four time points were comparable in the two groups (Table 2). In addition, GGT levels at 6, 12, 18, and 24 months were also significantly higher in the CHB with NAFLD group (Fig. 1E). The GGT normalization rates at 12 and 24 months were significantly lower in the CHB with NAFLD group (75.0% vs. 95.5%, p=0.038 and 71.4% vs. 96.8%, p=0.027, respectively, Table 2).

Discussion

Although the effects of obesity, MetS, and liver steatosis on the progress of CHB-related fibrosis and HCC have been previously described, our study identified hepatic steatosis as a risk factor for virological, serological, and biochemical response to NA therapy. In this study, we performed in-depth analysis of the impact of histologically-proven NAFLD on multiple parameters in CHB patients at different time points. The assessment not only included conventional parameters, such as incidence of CVR, virologic breakthrough, HBeAg seroconversion, serological changes of qHBsAg, HBV DNA, ALT, AST, and GGT, but also novel variables of LLV rates and pgRNA levels. To the best of our knowledge, this is the first study to compare LLV rates and pgRNA levels between CHB patients with and without NAFLD.

There are several other strengths of our study. Firstly, our study included patients with histologically-proven CHB and/or NAFLD. This is especially relevant as earlier studies mainly included CAP and/or ultrasonography diagnosed NAFLD patients rather than biopsy-proven. Owing to the limited precision of CAP and ultrasonography in detecting and grading liver steatosis,[26] our study provides more robust evidence of the influence of NAFLD on treatment outcomes. In addition, our study exclusively enrolled treatment-naïve patients who received initial first-line antiviral regimens at baseline, which helped minimize the influence of potential resistance and limited efficacy of non-first-line regimens, and provided more robust evidence of treatment outcomes in CHB patients with coexisting NAFLD.

Updated guidelines from the American Association for

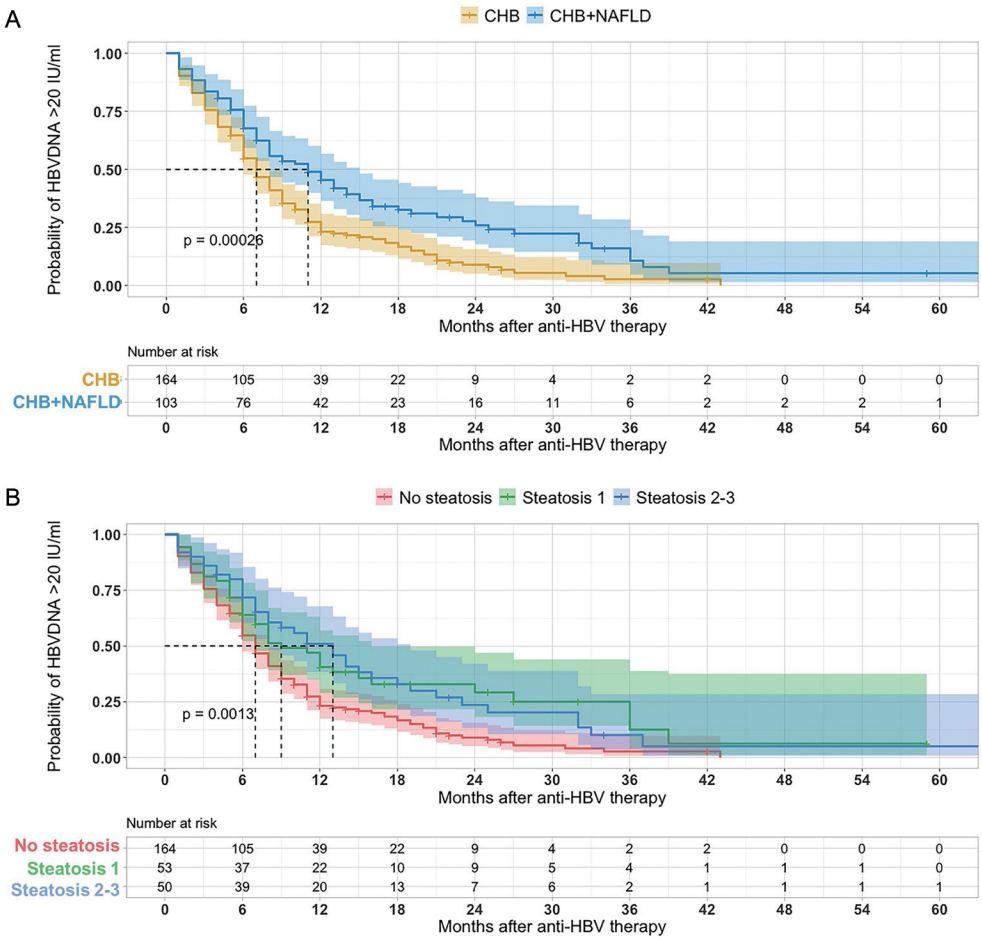

Fig. 2. Kaplan-Meier survival curves of percent complete virological response (CVR) in HBV-infected patients disaggregated by the concurrence and grade of hepatic steatosis. (A) Difference between patients with and without concomitant steatosis. (B) Difference between patients with grade of steatosis of 0, 1, and 2–3. CHB, chronic hepatitis B; NAFLD, nonalcoholic fatty liver disease; CVR, complete virological response.

Table 3. Univariate and multivariate analysis of baseline parameters associated with the complete viral response in chronic hepatitis B patients by Cox proportional-hazards regression

U Variates	Univariate			Multivariate		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
qHBsAg (log_{10} IU/mL)	0.581	0.494–0.683	<0.001			
HBeAg positive	0.338	0.247–0.444	<0.001	0.650	0.435–0.972	0.036
HBV DNA (log_{10} IU/mL)	0.736	0.690–0.785	<0.001	0.687	0.616–0.766	<0.001
Grade of necroinflammation	1.231	1.009–1.502	0.040	1.758	1.336–2.312	<0.001
Stage of fibrosis	1.155	1.013–1.318	0.032			
Grade of steatosis						
No steatosis			Reference			
1	0.599	0.417–0.859	0.005	0.822	0.512–1.319	0.416
2	0.678	0.459–1.000	0.050	0.447	0.259–0.773	0.004
3	0.397	0.184–0.856	0.018	0.085	0.019–0.392	0.002
Hepatic steatosis	0.611	0.461–0.811	0.001			
Steatosis 2–3	0.685	0.480–0.976	0.036			
Leukocyte counts, $\times 10^9$/L	0.901	0.823–0.987	0.024	0.836	0.743–0.939	0.003
Total bilirubin, μmol/L	1.004	1.000–1.008	0.030			
GGT, U/L	1.003	1.001–1.006	0.012			

GGT, gamma-glutamyltransferase.

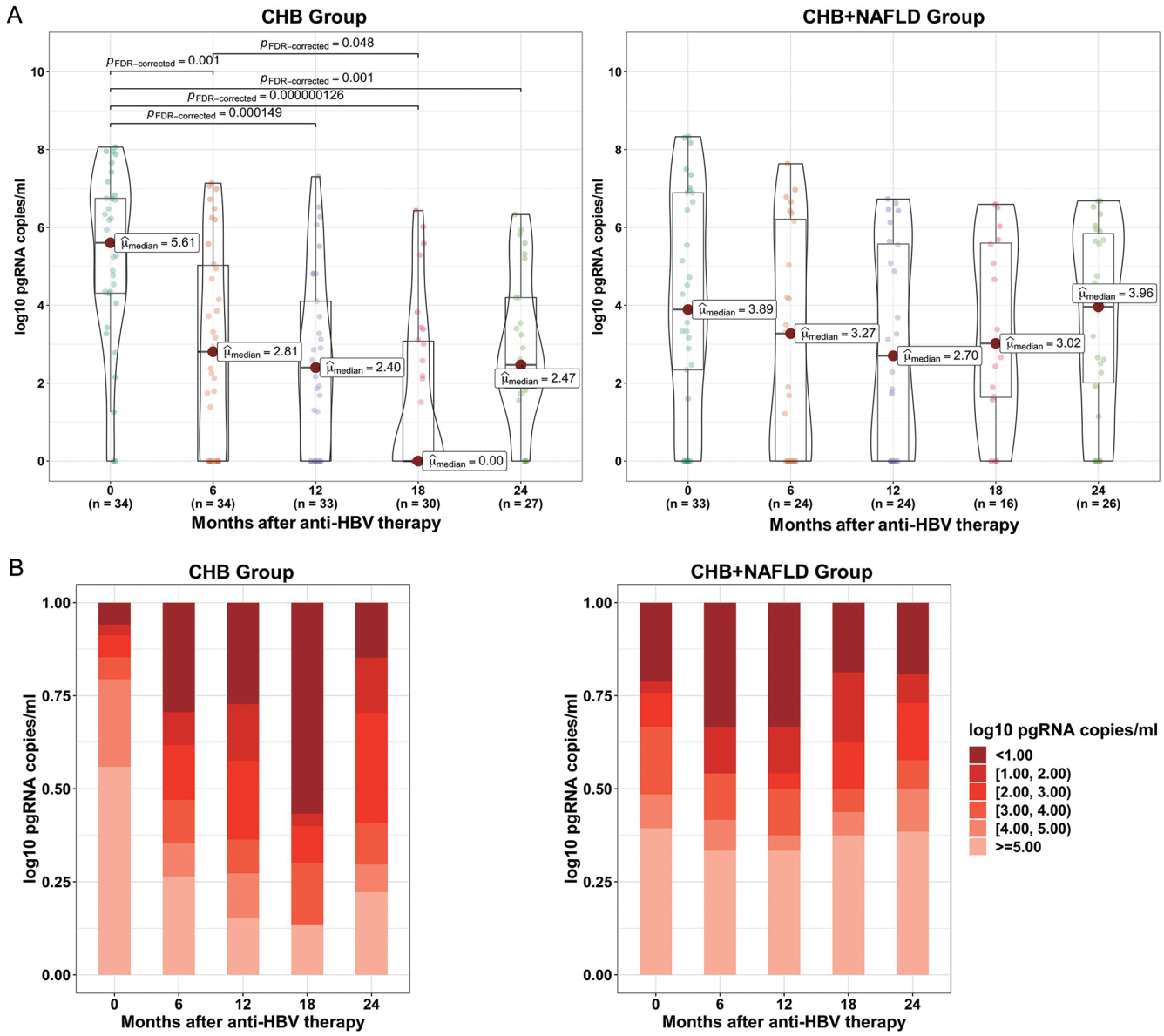

Fig. 3. Dynamic changes of HBV pregenomic RNA in HBV-infected patients with and without concomitant NAFLD. (A) Distribution of pregenomic RNA levels at each time point. (B) Stratification of pregenomic RNA levels at each time point. CHB, chronic hepatitis B; NAFLD, nonalcoholic fatty liver disease; pregenomic RNA, pgRNA.

the Study of Liver Disease and European Association for the Study of the Liver suggest that treatment should be directed at achieving undetectable serum HBV DNA accompanied by persistent normal serum ALT level in the short term.[27,28] Identification of factors that affect the process of CVR and persistent normal levels of liver enzymes is a key imperative. Our study elucidated that hepatic steatosis can restrain the decrease in HBV DNA and pgRNA levels, prolong the median time to achieve CVR from 7 to 11 months, and subsequently increase the cumulative incidence of LLV by 25.6% at or after 1 year of treatment. In addition, we found that hepatic steatosis was associated with higher ALT, AST, and GGT levels and lower normalization rates of those indices among on-treatment patients. Lung-Yi *et al*.[29] demonstrated that fibrosis progression is still possible in virological quiescent CHB with persistent severe steatosis. Our results provide supplementary interpretations to prior findings and implied that treatment strategy of patients with superimposed NAFLD is still a noteworthy issue. Even after the achievement of undetectable HBV DNA during NA treatment, control of severe steatosis may still be an important surrogate therapeutic endpoint.

We observed positive relationship between HBV DNA level and NAFLD during follow-up among on-treatment patients with comparable HBV DNA levels at baseline. However, several previous studies have found a negative correlation between HBV DNA level and NAFLD. That may be attributable to the inhibition of saturated fatty acids, which serve as a potential ligand for TLR4 and activated TLR4 signaling pathway to accelerate the mechanism of inhibiting HBV replication.[30] However, the negative correlation was mainly manifested among treatment-naïve patients, with compara-

ble VL among on-treatment patients.[3] This was consistent with the less significant decline of VL observed in patients with concomitant NAFLD in our study.

Longitudinal monitoring of pgRNA in our study illustrated the adverse impact of NAFLD on the virological response of CHB. The high proportions of $\log_{10}$ pgRNA ≥5 along with 24 months therapy reflected a high cccDNA activity, predicting poor antiviral efficacy and post-NA cessation virological relapse, which manifested as higher incidence of LLV and delayed CVR in patients with concomitant NAFLD. The results imply that CHB patients with NAFLD may require more efficient therapeutic strategy, shorter follow-up intervals, and prolonged duration of antiviral therapy. Additionally, we observed a slight increase in pgRNA levels at 24 months in both groups. Further study is required to clarify whether this was rational and to determine the status of virological response at and after the time points.

Apart from the association between NAFLD and therapeutic response, we identified other traditional risk factors for treatment failure in this study, namely, high levels of HBV DNA, leukocyte count, and HBeAg positivity at baseline. We also found that a higher grade of liver necroinflammation was associated with better virological response. This finding is consistent with prior studies in which higher baseline ALT levels were associated with much better response to Interferon-alpha therapy at 12 months.[31] Compared with the ALT level, the grade of inflammation in liver biopsy is a more accurate marker of host immunologic injury, which assists in the clearance of virus within the hepatocytes and cessation of hepatocyte cell death and normalization of liver enzymes.

With respect to the relationship between liver steatosis and fibrosis, although some cross-sectional analyses had found a positive correlation between them,[14,16] we did not observe a higher proportion of significant or advanced fibrosis in the concomitant NAFLD group at baseline. The burned-out fat in advanced fibrosis and small number of patients may have affected the results,[32] which deserves further clarification by a larger, longitudinal study. Some other limitations of this study should be acknowledged. First, data in this study were collected with prospective and retrospective method. Data pertaining to some variables, such as waist circumference, were not fully available. Thus, we used obesity as an alternative to waist circumference to evaluate the incidence of MetS. Second, serum samples were not collected regularly and sample size was limited owing to retrospectively enrolled patients and/or their occasional visits to other clinical centers. The fluctuation between 18 and 24 months in patients with CHB alone could not be interpretated rationally. Lastly, the number of enrolled patients and the duration of follow-up were limited due to rigorous inclusion criteria (such as HBV DNA LLOQ 20 IU/mL). Delayed CVR and less common events, such as HBeAg seroconversion or HBsAg seroconversion, could not be observed and compared sufficiently in our study. Further studies with a larger cohort and longer duration of follow-up are required to confirm our findings.

Conclusions

In CHB patients receiving initial antiviral therapy, NAFLD was not only significantly associated with higher levels of HBV DNA, pgRNA, and liver enzymes, but also higher incidence of LLV and longer duration to achieve first CVR.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Made substantial contributions to the conception and design of the study, acquisition, analysis and drafting the manuscript (SZ, XZ), contributed to the acquisition of data and/or collection of serum samples (HJ, YD, CD, MH), coordinated the editing of the manuscript (LL, XY), made substantial contributions to the conception, design and guide of the study (YN, J S). All authors read and approved the final manuscript.

Ethical statement

This study was approved by the Ethics Committee of the Third Hospital of Hebei Medical University (K2019-014-2) and Ethics Committee of the Henan Provincial People's Hospital (2020-184), and was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent for use of their data or blood samples (anonymously) for research purposes.

Data sharing statement

Data used for this manuscript are available on request from the corresponding author at nanyuemin@163.com upon request.

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