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ORIGINAL ARTICLE

High-normal alanine aminotransferase is an indicator for liver histopathology in HBeAg-negative chronic hepatitis B

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Abstract

Objective We aimed to assess liver histological changes of HBeAg-negative chronic hepatitis B (CHB) patients with normal ALT, and determined the association between significant liver injury and age, ALT, and HBV DNA levels.

Methods We retrospectively examined 327 patients who underwent liver biopsy from 2009 to 2018. Significant liver histological change is defined as liver necroinflammation ≥ G2 and/or liver fibrosis ≥ F2.

Results The proportion of patients with significant liver necroinflammation or fibrosis in the high-normal ALT group (ALT > 20 U/L) was higher than that in the low-normal ALT group (ALT ≤ 20 U/L) (44.6% vs 26.5%, 61.0% vs 41.7%, $p < 0.01$); also the proportion in the group with HBV DNA ≥ 2000 IU/mL was significantly higher than that in the group with HBV DNA < 2000 IU/mL (58.5% vs 27.1%, 67.9% vs 46.2%, $p < 0.01$). There was no significant difference in hepatic histopathology between < 40 and ≥ 40 years groups. Among 221 patients with normal ALT and low HBV DNA levels (< 2000 IU/mL), 27.1% of them had significant liver necroinflammation and 46.2% had significant liver fibrosis. The multiple logistic regression analysis showed that ALT > 20 U/L and HBV DNA ≥ 2000 IU/mL were independently associated with significant liver histopathology ($p < 0.01$).

Conclusion HBeAg-negative CHB patients with normal ALT and low HBV DNA level (< 2000 IU/mL) were suggested to perform liver biopsy or noninvasive methods for histopathology assessment, then to be determined for antiviral therapy. ALT > 20 U/L and HBV DNA ≥ 2000 IU/mL are good independently predictive factors for evaluating significant liver histopathology for HBeAg-negative CHB patients with normal ALT.

Clinical Trials Registration Chinese Clinical Trial Registry (ChiCTR-IOR-14005474).

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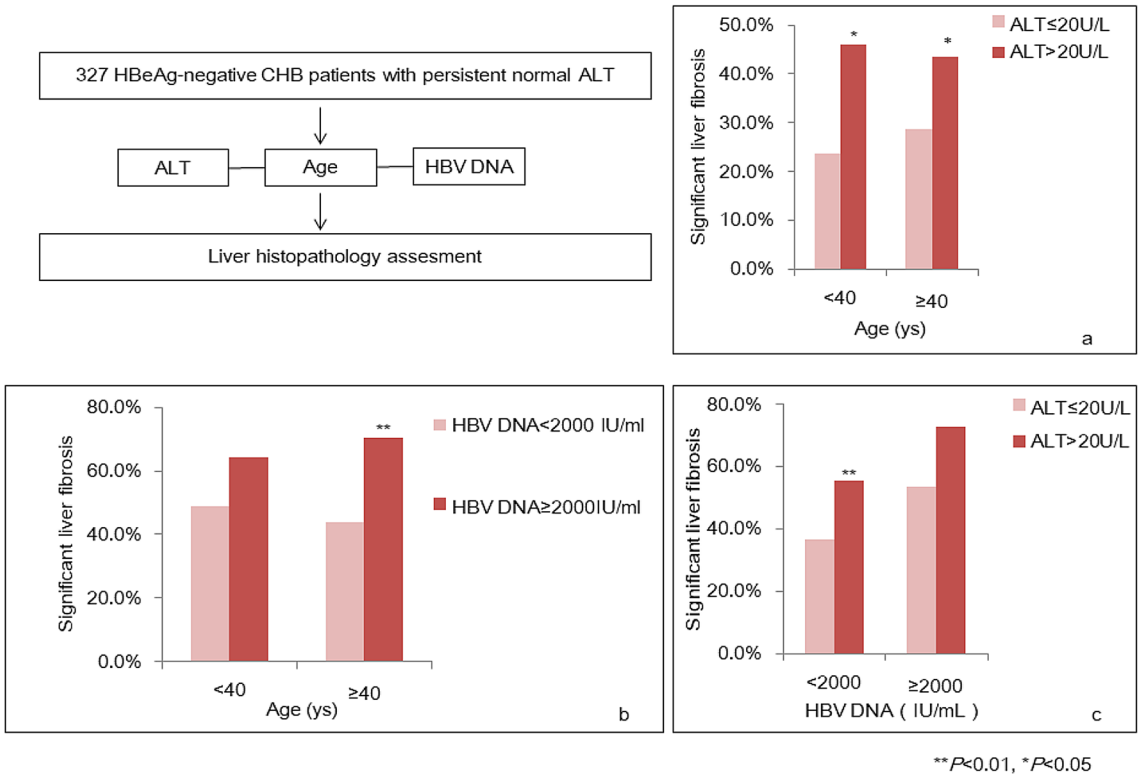
Graphic Abstract



Keywords Chronic hepatitis B (CHB) patient · HBeAg negative · Inactive carrier · Liver biopsy · Histological assessment · Normal alanine aminotransferase (ALT) · Low-normal ALT · HBV DNA level · Age · Antiviral therapy

Introduction

Chronic hepatitis B virus (HBV) infection is a considerable problem affecting the world public health. According to the World Health Organization (WHO) report [1], there were about 257 million people infected with HBV chronically in 2015 around the world. In the past 3 decades, China has made a great progress in preventing mother-to-child transmission by high vaccination coverage [2]. It is estimated that the present prevalence of HBsAg in the general population in China is 5%–6%, and approximately 70 million people infected with chronic HBV, of which about 20–30 million are chronic hepatitis B (CHB) patients [3]. Owing to the largest population in China, eliminating the threat of hepatitis B in 2030 for the remaining 10 years is still a huge challenge [4].

The natural history of CHB is commonly divided into several phases: immune tolerant, immune clearance, inactive carrier, and reactive phase, which is based on several laboratory markers including hepatitis B e antigen (HBeAg), hepatitis B surface antigen (HBsAg), serum alanine aminotransferase (ALT), and HBV DNA, as well as the disease activity [5]. HBeAg-negative patients account for the largest proportion of worldwide chronic HBV infection [6]. HBeAg-negative CHB patients with persistent normal ALT and low HBV DNA levels (< 2000 IU/mL) are usually defined as inactive carriers (low replicative chronic HBV infection), having no or minimal liver injuries. Therefore, antiviral treatment for these patients is generally not recommended by most international practice guidelines [7–10]; instead, it is essential to monitor HBV DNA and ALT levels regularly for long periods (at least 1 year). However, recently, more evidences have shown that HBeAg-negative patients with persistent normal ALT levels have significant liver histological changes [11, 12], even patients with low viral load (HBV DNA < 2000 IU/mL), which indicates that inactive carriers may have risk of disease progression such as cirrhosis and hepatocellular carcinoma (HCC). Besides, a retrospective study found that 70.8% of treatment naïve CHB patients with HCC were HBeAg-negative patients, and more than 20.0% of patients had low HBV DNA levels and normal ALT levels [13]. Therefore, it is imperative to identify true inactive carriers.

In this study, we sought to investigate the prevalence of significant liver histopathology in HBeAg-negative CHB patients with normal ALT, and determine association between significant liver histopathology and age, ALT levels, and HBV DNA levels.

Materials and methods

Patients

A total of 2063 patients who underwent liver biopsy in Guangdong Provincial Hospital of Traditional Chinese Medicine, between April 2009 and December 2018, were retrospectively analyzed. For this study, the inclusion criteria included: HBsAg positive for more than 6 months, HBeAg negative, and normal ALT (≤ the upper limit of normal, ULN, 40 U/L) for at least 6 months, and all patients had liver biopsy pathological diagnosis results. The exclusion criteria were as follows: patients had HBV with HIV (human immunodeficiency virus), HCV (hepatitis C virus) or HDV (hepatitis D virus) co-infection, as well as infection with EBV and CMV, or evidence of schistosomiasis liver disease and Wilson disease, patients had received antiviral therapy, patients with alcohol liver disease (alcohol consumption ≥ 40 g/day for male and ≥ 20 g/day for female), and patients had liver damage induced by other causes (non-alcoholic fatty liver, drugs, autoimmune hepatitis, etc.). As shown in Fig. 1, finally, 327 patients were included in this study.

Laboratory tests

According to archived electronic medical records, the ALT and AST (aspartate aminotransferase) levels, as well as serum HBV DNA levels were collected, and the ULN of ALT and AST for male and female are both 40 U/L. HBV immune markers including HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were also collected. The ALT and AST levels were tested with a fully automatic biochemical detector (Roche Cobas 6000, Roche Corporation, Basel, Switzerland). HBV immune markers were detected with enzyme-linked immunosorbent assay (ELISA) kit (Roche e 601 instrument and Roche reagent). Serum HBV DNA was measured by real-time quantitative fluorescent polymerase

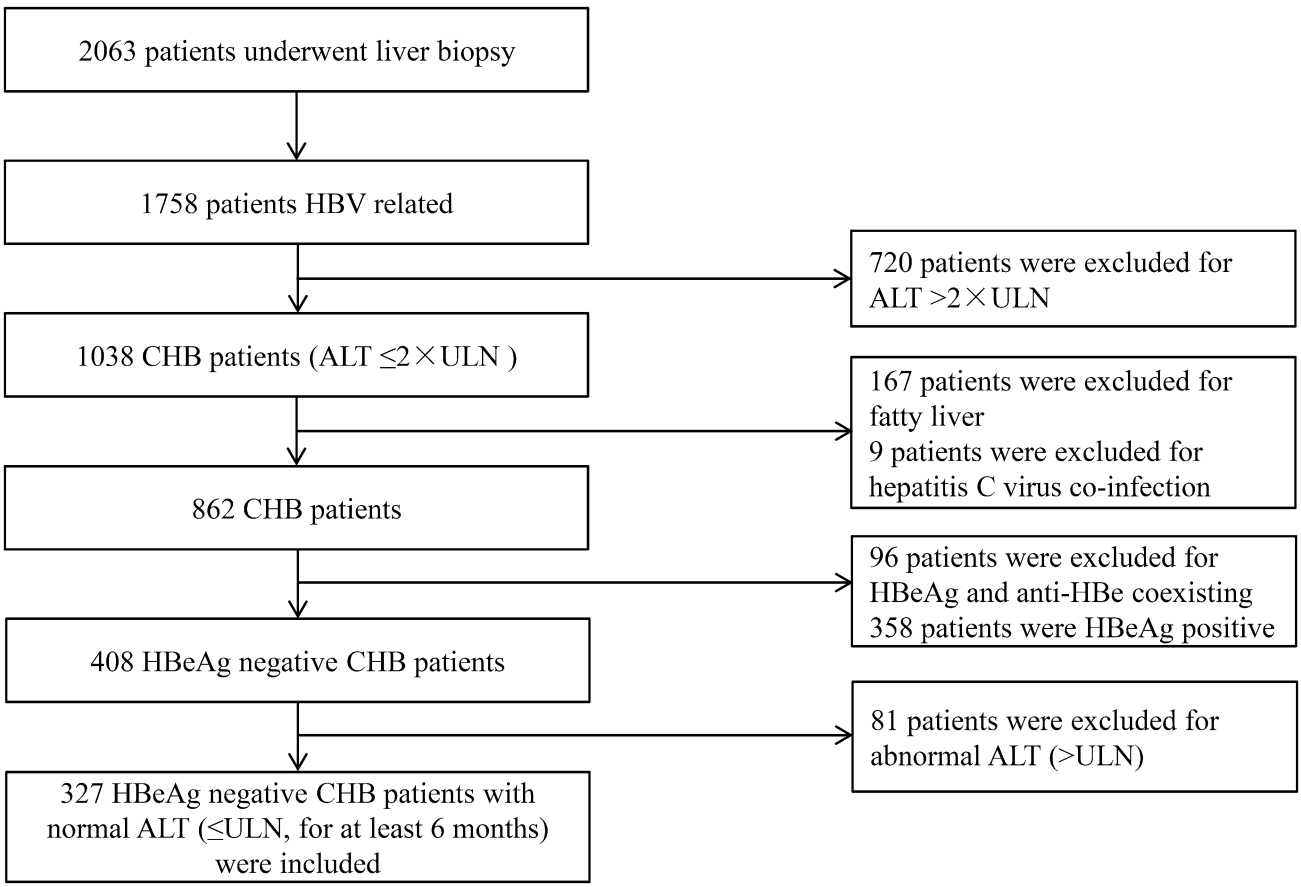


Fig. 1 Flow diagram of study patients and reason of exclusion. *HBV* hepatitis B virus; *ULN* upper limit of normal, 40 U/L; *CHB* chronic hepatitis B; *ALT* alanine aminotransferase

chain reaction (qPCR) method using ABI 7300 (Applied Biosystems Inc, NYC, New York, USA), with the lower detection limit of 100 IU/mL (Da An Gene Co, Ltd. of Sun Yat-sen University, Daan reagent). All parameters were tested at hospital clinics.

Liver biopsy

The liver tissue was obtained by percutaneous liver puncture with a 16-gauge disposable needle. All liver tissues were immediately fixed with 10% formalin and then paraffin embedded. The paraffin embedded sample were sectioned, and then stained with hematoxylin and eosin, and Masson stained for pathological analysis. Liver necroinflammation and fibrosis were evaluated according to the recommended standards of Chinese guidelines of the CHB prevention and treatment (2019 version). The METAVIR scoring system was adopted to evaluate liver necroinflammation and fibrosis. Histological grading of liver necroinflammation is classified into G0 ~ G4, and fibrosis was staged from F0 to F4. Significant liver histological changes were defined as necroinflammation grade ≥ 2 (≥ G2) and/or fibrosis stage ≥ 2 (≥ F2). Liver samples were assessed by experienced pathologists who were blinded to clinical and biochemical data.

Statistical analysis

The statistical analysis was performed using SPSS ver. 26.0 (SPSS, Chicago, IL). Quantitative variables with non-normal distribution were expressed as the middle and range, which were compared by Mann–Whitney U test. Categorical variables were demonstrated with frequency and percentage, which were compared by Chi-squared test. Spearman correlation analysis was used to explore association between liver histopathology and clinical factors. Multiple logistic regression analysis was performed to determine predictors for evaluating significant liver histological changes. All significance tests were two-tailed, and a p value of < 0.05 was considered to be statistically significant.

Results

Patient baseline characteristics

The baseline characteristics of the study patients are depicted in Table 1. Among 327 HBeAg-negative CHB patients, 132 patients (40.4%) had a low-normal ALT (≤ 20 U/L) and 195 patients (59.6%) had a high-normal ALT (> 20 U/L). There was no significant difference in age between low- and high-normal ALT groups. The proportion of male patients was significantly higher in the high-normal ALT group than that in the low-normal ALT group (75.4% *vs* 45.5%, $p < 0.001$). And the high-normal ALT group had a significantly higher BMI, HBV DNA, and GGT levels compared to the low-normal ALT group (23.4 vs 21.7 kg/m^2, $p < 0.001$; 3.1 vs 2.8 log IU/mL, $p = 0.02$; 32.0 vs 25.5 U/L, $p < 0.001$). Besides, the proportion of significant liver necroinflammation and fibrosis in the

Table 1 Baseline characteristics of 327 HBeAg-negative chronic hepatitis B patients

Baseline characteristics	Total	ALT ≤ 20 U/L	ALT > 20 U/L	*p* value
Num. of cases	327	132	195	
Age (years)	40 (15–64)	40 (24–63)	41 (15–64)	0.666
Male, *n* (%)	207 (63.3)	60 (45.5)	147 (75.4)	0.000
BMI(kg/m^2)	22.6 (16.6–31.5)	21.7 (16.6–30.4)	23.4 (17.1–31.5)	0.000
ALT (U/L)	22 (5–40)	15 (5–20)	28 (21–40)	0.000
AST (U/L)	21 (5–70)	18 (9–42)	23 (5–70)	0.000
HBV DNA (log IU/mL)	3.0 (2.0–7.0)	2.8 (2.0–6.6)	3.1 (2.0–7.0)	0.020
HBsAg (IU/mL)	5433.2 (1.3–11,740.0)	5684.5 (2.7–11,740.0)	6368.0 (1.3–10,645.0)	0.638
GGT (U/L)	30.0 (2–162)	25.5 (7.0–107.0)	32.0 (2.0–162.0)	0.000
PLT (10^9/L)	194 (58–387)	194 (65–387)	188 (58–380)	0.403
FIB-4	0.96 (0.2–7.05)	0.96 (0.34–7.05)	0.98 (0.2–4.44)	0.542
Necroinflammation n (%)				
G0	0 (0)	0 (0)	0 (0)	
G1	205 (62.7)	97 (73.5)	108 (55.4)	
≥ G2	122 (37.3)	35 (26.5)	87 (44.6)	0.001
Fibrosis *n* (%)				
F0	37 (11.3)	19 (14.4)	18 (9.2)	
F1	116 (35.5)	58 (43.9)	58 (29.7)	
≥ F2	174 (53.2)	55 (41.7)	119 (61.0)	0.001

Table 2 Distribution of liver necroinflammation and fibrosis in different age and ALT groups (%)

Histological grading	< 40 years (144)		*p* value	≥ 40 years (183)		*p* value
	ALT ≤ 20 U/L (59)	ALT > 20 U/L (85)		ALT ≤ 20 U/L (73)	ALT > 20 U/L (110)	
G0	0 (0)	0 (0)		0 (0)	0 (0)	
G1	45 (76.3)	46 (54.1)		52 (71.2)	62 (56.4)	
≥ G2	14 (23.7)	39 (45.9)	0.007	21 (28.8)	48 (43.6)	0.042
F0	7 (11.9)	7 (8.2)		12 (16.4)	11 (10.0)	
F1	29 (49.2)	24 (28.2)		29 (39.7)	34 (30.9)	
≥ F2	23 (39.0)	54 (63.5)	0.004	32 (43.8)	65 (59.1)	0.043

Table 3 Distribution of liver necroinflammation and fibrosis in different age and HBV DNA groups (%)

Histological grading	< 40 years (144)		*p* value	≥ 40 years (183)		*p* value
	< 2000 IU/mL (102)	≥ 2000 IU/mL (42)		< 2000 IU/mL (119)	≥ 2000 IU/mL (64)	
G0	0 (0)	0 (0)		0 (0)	0 (0)	
G1	73 (71.6)	18 (42.9)		88 (73.9)	26 (40.6)	
≥ G2	29 (28.4)	24 (57.1)	0.001	31 (26.1)	38 (59.4)	0.000
F0	12 (11.8)	2 (4.8)		18 (15.1)	5 (7.8)	
F1	40 (39.2)	13 (31.0)		49 (41.2)	14 (21.9)	
≥ F2	50 (49.0)	27 (64.3)	0.095	52 (43.7)	45 (70.3)	0.001

high-normal ALT group was significantly higher than that in the low-normal ALT group (44.6% *vs* 26.5%; 61.0% *vs* 41.7%, $p = 0.001$).

Age subgroup liver histopathology comparison

There was no significant difference in liver histological grading between patient groups of < 40 years and ≥ 40 years (Supplementary Table 1). Patients were further categorized by ALT levels, as shown in Table 2. The high-normal ALT group had a significantly higher proportion of significant liver necroinflammation and fibrosis than the low-normal ALT group whether age was < 40 years (≥ G2: 45.9% *vs* 23.7%, $p = 0.007$; F2: 63.5% *vs* 39.0%, $p = 0.004$) or ≥ 40 years (≥ G2: 43.6% *vs* 28.8%, $p = 0.042$; ≥ F2: 59.1% *vs* 43.8%, $p = 0.043$). Patients were further sorted by age and HBV DNA levels, as shown in Table 3, finding that whether age was < 40 or ≥ 40 years, and the proportion of significant liver necroinflammation was significantly higher in patients with HBV DNA ≥ 2000 IU/mL than that in < 2000 IU/mL (57.1% *vs* 28.4%, $p = 0.001$, 59.4% *vs* 26.1%, $p < 0.001$). Meanwhile, when age of patients was ≥ 40 years, the proportion of significant liver fibrosis was significantly higher in patients with HBV DNA ≥ 2000 IU/mL than that in group with HBV DNA < 2000 IU/mL (70.3% *vs* 43.7%, $p = 0.001$).

Table 4 Distribution of liver necroinflammation and fibrosis in different HBV DNA groups (%)

Histological grading	< 2000 IU/mL (221)	≥ 2000 IU/mL (106)	*p* value
G0	0 (0)	0 (0)	
G1	161 (72.9)	44 (41.5)	
≥ G2	60 (27.1)	62 (58.5)	0.000
F0	30 (13.6)	7 (6.6)	
F1	89 (40.3)	27 (25.5)	
≥ F2	102 (46.2)	72 (67.9)	0.000

HBV DNA subgroup liver histopathology comparison

The liver histopathology of patient was also analyzed according to different HBV DNA levels, and the proportion of significant liver necroinflammation and fibrosis in patients with HBV DNA ≥ 2000 IU/mL was significantly higher than that in HBV DNA < 2000 IU/mL group (58.5% *vs* 27.1%, 67.9% *vs* 46.2%, $p < 0.001$), as shown in Table 4. When comparing the low- and high-normal ALT groups, discovering that the proportion of significant liver necroinflammation

and fibrosis in the high-normal ALT group was significantly higher than that in the low-normal ALT group, while HBV DNA level was < 2000 IU/mL (≥ G2: 33.6% *vs* 19.2%, $p = 0.017$; ≥ F2: 54.1% *vs* 36.4%, $p = 0.009$). And when HBV DNA level was ≥ 2000 IU/mL, there was no significant difference in liver histological changes between the low- and high-normal ALT groups, as shown in Table 5. As shown in Table 6, patients with significant liver histopathology (≥ G2 and/or ≥ F2) had higher AST, GGT, and FIB-4 levels, but lower PLT level when compared to patients with hepatic histopathology G0/G1 and/or F0/F1.

Predictors of significant liver histological changes

Univariate analysis and multivariate logistic regression analysis were performed to examine age, sex, ALT, and HBV DNA levels as potential independent predictors of significant liver histopathology. As described in Supplementary Table 2, Spearman correlation analysis revealed that there was a close positive correlation between liver necroinflammation and ALT ($r = 0.165$, $p = 0.003$), and HBV DNA levels ($r = 0.294$, $p < 0.001$). And ALT ($r = 0.172$, $p = 0.002$) and HBV DNA ($r = 0.276$, $p < 0.001$) levels were also closely positive correlated with liver fibrosis. However, association between age and liver histopathology was not found, as well as sex. Age, sex, ALT, and HBV DNA were further analyzed by multiple logistic regression analysis to search for predictors of significant liver histological changes (Table 7). ALT > 20 U/L (OR = 2.579, 95% CI, 1.499–4.437, $p = 0.001$; OR = 2.033, 95% CI, 1.254–3.298, $p = 0.004$, respectively) was an independent predictor for significant liver necroinflammation and fibrosis, and as well as HBV DNA ≥ 2000 IU/mL (OR = 3.544, 95% CI, 2.145–5.856, $p < 0.001$; OR = 2.307, 95% CI, 1.406–3.786, $p = 0.001$, respectively). At the same time, we observed that female (OR = 2.106, 95% CI, 1.232–3.602, $p = 0.007$) is also a predictor of significant liver necroinflammation but not of liver fibrosis.

Discussion

HBeAg-negative CHB patients with persistent normal ALT levels and HBV DNA < 2000 IU/mL are commonly known as "inactive carriers". European Association for the Study of the Liver (EASL) redefined patients at this phase as HBeAg-negative chronic HBV infection [8], and point out that HBV DNA levels in some patients at this phase can be between 2000–20,000 IU/mL. Liver injury is uncommon among inactive carriers; a systematic review assessed the

Table 5 Distribution of liver necroinflammation and fibrosis in different HBV DNA and ALT groups (%)

Histological grading	< 2000 IU/mL (221)		*p* value	≥ 2000 IU/mL (106)		*p* value
	≤ 20 U/L (99)	> 20 U/L (122)		≤ 20 U/L (33)	> 20 U/L (73)	
G0	0 (0)	0 (0)		0 (0)	0 (0)	
G1	80 (80.8)	81 (66.4)		17 (51.5)	27 (37.0)	
≥ G2	19 (19.2)	41 (33.6)	0.017	16 (48.5)	46 (63.0)	0.160
F0	16 (16.2)	14 (11.5)		3 (9.1)	4 (5.5)	
F1	47 (47.5)	42 (34.4)		11 (33.3)	16 (21.9)	
≥ F2	36 (36.4)	66 (54.1)	0.009	19 (57.6)	53 (72.6)	0.125

Table 6 Baseline characteristics of 221 patients with normal ALT and HBV DNA < 2000 IU/mL

Characteristics	Total	G0/G1 and/or F0/F1	≥ G2 and/or ≥ F2	*p* value
Num. of cases	221	107	114	
Age (years)	40 (15–64)	41 (15–62)	39.5 (21–64)	0.237
Male, *n* (%)	141 (63.8)	69 (64.5)	72 (63.2)	0.837
BMI (kg/m^2)	22.8 (16.6–31.5)	23.0 (17.1–31.5)	22.6 (16.6–30.1)	0.434
ALT (U/L)	21 (5–40)	20 (7–40)	22 (5–40)	0.306
AST (U/L)	20 (5–70)	19 (5–31)	21 (10–70)	0.001
HBV DNA (log IU/mL)	2.7 (2.0–3.3)	2.7 (2.0–3.3)	2.7 (2.0–3.3)	0.106
HBsAg (IU/mL)	5470.5 (1.3–11,740.0)	5528.0 (1.3–11,440.0)	6368.0 (2.9–11,740.0)	0.862
GGT (U/L)	45 (7–162)	38.0 (9.0–162.0)	53.5 (7.0–117.0)	0.008
PLT (10^9/L)	202 (73–387)	211 (100–387)	183.5 (73–317)	0.000
FIB-4	0.92 (0.2–6.22)	0.86 (0.20–2.03)	0.98 (0.39–6.22)	0.006

Table 7 Multiple logistic regression analysis for predictors of significant liver necroinflammation and fibrosis

	Significant necroinflammation			Significant fibrosis		
	OR	95% CI	*p* value	OR	95%CI	*p* value
Age						
< 40 years	1.000			1.000		
≥ 40 years	0.904	0.555–1.473	0.843	0.926	0.587–1.461	0.742
Sex						
Male	1.000					
Female	2.106	1.232–3.602	0.007	1.003	0.612–1.643	0.991
ALT						
≤ 20 U/L	1.000			1.000		
> 20 U/L	2.579	1.499–4.437	0.001	2.033	1.254–3.298	0.004
HBV DNA						
< 2000 IU/mL	1.000			1.000		
≥ 2000 IU/mL	3.544	2.145–5.856	0.000	2.307	1.406–3.786	0.001

extent of liver histological changes in inactive carriers [14], discovering that significant liver fibrosis was rare with an estimated rate of 10%. However, our results showed that 27.1% of patients with normal ALT and low HBV DNA levels (< 2000 IU/mL) had liver necroinflammation ≥ G2 and 46.2% had liver fibrosis ≥ F2. A study from Zhejiang, China found that 38.2% of inactive carriers had significant liver disease [15]. And a previous study from Guangzhou, China also showed that 30.9% of inactive carriers had significant liver fibrosis [16], which was similar to our results. In addition, a study from Brazil found 40.7% of inactive carriers had advanced liver histological changes [17], whereas ALT levels of patients included were ≤ 2 × ULN. Therefore, our study demonstrated that a considerable proportion of HBeAg-negative patients with normal ALT levels and low HBV DNA level (< 2000 IU/mL) have significant liver histological changes, which imply that "inactive carriers" may have active liver disease. According to Chinese guidelines**,** HBeAg-negative chronic hepatitis B patients with persistently normal ALT and positive HBV DNA level should be treated if they have significant liver necroinflammation and fibrosis (≥ G2 and/or ≥ F2) [10]. Therefore, we should pay more attention to find true inactive carriers correctly and optimize the management of HBeAg-negative patients with persistent normal ALT.

In the light of international guidelines such as the American Association for the Study of Liver Diseases (AASLD) and the Asia–Pacific Association for the Study of the Liver (APASL), as well as Chinese guidelines (2019), antiviral treatment is not recommended for HBeAg-negative patients with normal ALT and HBV DNA < 2000 IU/mL [7, 9, 10]. However, more studies had reported using only ALT and HBV DNA levels to define inactive carriers might misclassify a fair proportion of gray area patients with significant liver histopathology. There is an urgent need for liver biopsy and antiviral treatment may be benefit for patients with active disease compared to true inactive carriers. Nevertheless, it is not practical to perform liver biopsy regularly in the clinical management of HBeAg-negative patients with normal ALT levels [18], although liver biopsy is considered as the gold standard to assess liver histopathology [19]. It is prospective to utilize the predictors of liver histopathology, commonly used age, ALT, and HBV DNA levels. APASL guidelines recommend that ALT level is divided into high- (0.5–1 × ULN) and low-normal (≤ 0.5 × ULN) ALT [9], so we evaluated the liver histological grade of all patients categorized into different ALT (low- and high-normal ALT), HBV DNA, and age groups, and determined the predictors of significant liver histopathology.

In our study, we found that the proportion of HBeAg-negative CHB patients with significant liver fibrosis in the high-normal ALT group was significantly higher than the low-normal ALT group (61.0% *vs* 41.7%, $p < 0.01$), which was also observed in the age groups of < 40 and ≥ 40 years. In the same way, this can be also seen in patients with HBV DNA levels at < 2000 IU/mL. Our results suggested an urgent need for liver biopsy in the high-normal ALT group compared to the low-normal ALT group. Several researchers suggested to revise the ULN of ALT. Prati et al. had recommended that the ULN for ALT should be decreased to 30 U/L for men and 19 U/L for women [20], Lee et al. also proposed reducing the ULN of ALT to 35 U/L for men and 25 U/L for women [21], posing a challenge to the traditional ULN at 40 U/L. Similar to our results, the previous studies also had the evidence to revise the ULN of ALT by discovering that high-normal ALT patients had more significant liver histopathology [22]. Gui et al [23] found a significant higher prevalence of liver histopathology in the high-normal ALT group of CHB patients (39.2% of patients were HBeAg-negative) than in the low-normal ALT group (40.0% *vs* 16.6%, $p < 0.01$), suggesting that a higher proportion of patients with high-normal ALT may have risk of disease progression.

We also compared the HBV DNA groups of < 2000 IU/mL and ≥ 2000 IU/mL in all HBeAg-negative patients, finding that the proportion of significant liver necroinflammation and fibrosis in group with HBV DNA ≥ 2000 IU/mL was significantly higher than that in < 2000 IU/mL (58.5% *vs* 27.1%, 67.9% *vs* 46.2%, $p < 0.01$). According to the guidelines, HBV DNA < 2000 IU/mL is considered as a factor to discriminate inactive carriers from HBeAg-negative CHB patients. A study focused on same type of patients like our study and found that CHB patients with HBV DNA ≥ 2000 IU/mL had a higher proportion to progress into advanced liver disease than patients with HBV DNA < 2000 IU/mL ($p = 0.018$) [24]. Another study found that untreated HBeAg-negative patients with normal ALT and HBV DNA ≥ 2000 IU/mL had significant higher risk of HCC and death/transplantation than treated active patients [25], which indicated that patients with HBV DNA ≥ 2000 IU/mL were at a risk of developing advanced liver disease.

Previous research showed that HBV DNA is an independent predictive factor for evaluating liver histopathology in HBeAg-negative CHB patients [26], and elevated HBV DNA levels in HBeAg-negative CHB patients associated with an increased risk of HCC [27]. In this study, we observed that ALT and HBV DNA levels were positively correlated with significant liver histopathology. Furthermore, the multiple logistic regression analysis was used to evaluate clinical factors to predict significant liver histopathology. Interestingly, we found that ALT > 20U/L and HBV DNA ≥ 2000 IU/mL were both good factors independently associated with significant liver histopathology of HBeAg-negative CHB patients with normal ALT. This was in consistent with results of our grouped analysis.

Our results also showed female as a predictor of significant liver necroinflammation. Several studies found that male patients were at a risk of active liver disease. A recent study reported that male and HBeAg-negative hepatitis were risk factors of liver fibrosis [28]. Another previous study showed that male sex (OR = 1.82, 95% CI, 1.10–3.01, $p = 0.019$) was an independent factor associated with a high ALT level [29]. Several studies reported that the ULNs of ALT for male and female were different [30–33]. They suggested 30 U/L for male and 19 U/L for female. Therefore, ALT > 20 U/L might be normal for male but abnormal for female in our study. It explains why female patients had a higher proportion of significant liver histopathology (62.5% *vs* 38.8%, $p = 0.004$, Supplementary Table 3), although the proportion of ALT > 20 U/L was higher in male. Therefore, it is better to carry out more studies to investigate the relationship between gender and liver histopathology among HBeAg-negative CHB patients with normal ALT, and gender could also be taken into consideration in evaluating ULN of ALT levels.

In a previous study, increasing age was found as an independent predictor of significant liver fibrosis in HBeAg-negative CHB patients with persistent normal ALT [34]. However, our results showed that age had no association with significant liver necroinflammation and fibrosis. As recommended by the AASLD guidelines, patients with age > 40 years were associated with a higher likelihood of significant histological disease [7]; while the patients with age < 40 years in our study had a fair proportion at 53.5% of significant liver fibrosis, which suggested that even patients with age < 40 years should call the need for closer monitoring.

In consequence, the management of HBeAg-negative CHB patients with normal ALT should be considered prudently. Questions about how to evaluate liver histopathology in patients at inactive carrier's state, and how to manage HBeAg-negative CHB patients with normal ALT and low viral load but significant liver damage and the long term cost-effect of antiviral therapy for them should be further explored [35]. Other than continuously stringent monitoring, more researchers suggested individualized care for better management of inactive carriers. Novel clinical parameters and noninvasive methods to better evaluate the extent of liver damage should also be further investigated.

In conclusion, our study demonstrated that a considerable proportion of HBeAg-negative CHB patients with normal ALT had significant liver histological changes, and even 46.2% of patients with normal ALT and low HBV DNA levels (< 2000 IU/mL) had significant liver fibrosis. ALT > 20 U/L and HBV DNA ≥ 2000 IU/mL are both good independently predictive factors for evaluating significant liver histopathology. For HBeAg-negative CHB patients, normal ALT and low viral load may not be sufficient for confirming no liver injuries, so we recommend performing liver biopsy or noninvasive methods (such as transient elastography examinations) for the estimation of the extent of fibrosis, and to treat the patients who have significant liver histopathology.

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Data availability Data will be available according to request.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Consent to participate (ethics) The informed consents of patients have been obtained. The FDF files of signed informed consents have been attached.

Consent to publish (ethics) We have the consent to publish this study.

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