Journal of Clinical and Translational Hepatology **2024** vol. 12(4) | 381–388
DOI: 10.14218/JCTH.2023.00378

Original Article

The Relationship between Viral Replication and the Severity of Hepatic Necroinflammatory Damage Changed before HBeAg Loss in Patients with Chronic Hepatitis B Virus Infection

Leijie Wang[1,2], Jian Wang[3], Kunyu Zhao[1], Lina Jiang[4], Xinxin Zhang[5], Jingming Zhao[4], Jie Li[3*] and Fengmin Lu[2*]

[1]Department of Microbiology and Immunology, College of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan, China; [2]Department of Microbiology & Infectious Disease Center, School of Basic Medical Sciences, Peking University, Beijing, China; [3]Department of Infectious Diseases, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, Jiangsu, China; [4]Department of Pathology and Hepatology, The Fifth Medical Center of PLA General Hospital, Beijing, China; [5]Department of Infectious Diseases, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Received: 21 August 2023 | **Revised:** 31 December 2023 | **Accepted:** 1 January 2024 | **Published online:** 27 February 2024

Abstract

Background and Aims: Disease progression of chronic hepatitis B virus (HBV) infection is driven by the interactions between viral replication and the host immune response against the infection. This study aimed to clarify the relationship between HBV replication and hepatic inflammation during disease progression. ***Methods:*** Two cross-sectional, one validation cohort, and meta-analyses were used to explore the relationship between HBV replication and liver inflammation. Spearman analysis, multiple linear regression, and logistic regression were used to explore the relationship between variables. ***Results:*** In the cross-sectional cohorts A and B including 1,350 chronic hepatitis B patients, Spearman analysis revealed a negative relationship between HBV replication (such as HBV DNA) and liver inflammation (such as ALT) in HBeAg-positive patients with higher HBV DNA $>2\times10^6$ IU/mL (rho=−0.160 and −0.042) which turned to be positive in HBeAg-positive patients with HBV DNA $\leq2\times10^6$ IU/mL (rho=0.278 and 0.260) and HBeAg-negative patients (rho=0.450 and 0.363). After adjustment for sex, age, and anti-HBe, results from logistic regression and multiple linear regression showed the opposite relationship still existed in HBeAg-positive patients with different DNA levels; the opposite relationship in HBeAg-positive patients with different DNA levels was validated in a third cohort; the opposite relationship in patients with different HBeAg status was partially confirmed by meta-analysis (overall R: −0.004 vs 0.481). ***Conclusions:*** These results suggested a negative relationship between viral replication and liver inflammation in HBeAg-positive patients with high HBV DNA, which changed to a positive relationship for those HBeAg-positive patients with DNA less than 2×10^6 IU/mL and HBeAg-negative patients.

Citation of this article: Wang L, Wang J, Zhao K, Jiang L, Zhang X, Zhao J, *et al*. The Relationship between Viral Replication and the Severity of Hepatic Necroinflammatory Damage Change before HBeAg Loss in Patients with Chronic Hepatitis B Virus Infection. J Clin Transl Hepatol 2024;12(4):381–388. doi: 10.14218/JCTH.2023.00378.

Introduction

Chronic hepatitis B virus (HBV) infection is a major cause of chronic liver diseases, ranging from viral hepatitis to end-stage liver diseases, including cirrhosis and liver cancer.[1] Although universal hepatitis B vaccination among newborns and infants has significantly reduced new infections, HBV infections remain a major global health burden, with an estimated 296 million people living with chronic infection.[2]

Hepatitis B e antigen (HBeAg) is a secreted non-structural viral protein that is not involved in HBV replication. However, the presence of HBeAg is necessary for the establishment of chronic HBV infection either through vertical or horizontal transmission due to its inhibitory effect on innate and adaptive host immunity against HBV.[3,4] On the other hand, HBeAg expression decreased/abolished virus strain (1,762/1,764/1,896 mutations) could avoid the host-specific adaptive immune response to HBeAg/HBcAg and become the dominant virus strain, resulting in the persistence of chronic HBV infection,[5] which makes HBeAg usually the first HBV viral protein being lost after the activation of host immunity against HBV.

Patients with chronic hepatitis B are usually divided into four phases by HBeAg status and hepatic inflammation

Keywords: Chronic hepatitis B; HBeAg; HBV DNA; ALT.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; cccDNA, covalently closed circular DNA; GGT, gamma-glutamyl transpeptidase; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IH, immunohistochemistry.

***Correspondence to:** Fengmin Lu, Department of Microbiology & Infectious Disease Center, School of Basic Medical Sciences, Peking University, 38 Xueyuan Road, Haidian District, Beijing 100191, China. ORCID: https://orcid.org/0000-0002-1832-3209. Tel/Fax: +86-10-82805136, E-mail: lu.fengmin@hsc.pku.edu.cn; Jie Li, Department of Infectious Diseases, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, Jiangsu 210000, China; ORCID: https://orcid.org/0000-0003-0973-8645. Tel: +86-15863787910, E-mail: lijier@sina.com

(based on serum ALT and/or liver biopsy): HBeAg-positive infection, HBeAg-positive chronic hepatitis B (CHB), HBeAg-negative chronic infection, and HBeAg-negative CHB.[6] Previous studies have suggested that for patients with HBeAg-positive, active liver inflammation is more likely to cause HBV DNA decrease and HBeAg clearance, while for patients who are HBeAg-negative, active liver inflammation is associated with viral reactivation and persistent liver damage. Therefore, most guidelines emphasize in their recommendation that HBeAg-negative patients with chronic hepatitis B should be given timely antiviral treatment to inhibit viral replication and reduce liver damage.[6]

It is well known that the disease progression of chronic HBV infection is driven by the interactions between the HBV virus and the host immune response against HBV infection, which results in liver immunopathological damage manifested by liver inflammation and fibrosis/cirrhosis. However, the correlation between viral replication and the host immune response has not been fully demonstrated. Accumulating studies have indicated that anti-HBc could be considered a surrogate biomarker for liver inflammation in patients with chronic HBV infection. Our recent study reported that the level of anti-HBc was negatively correlated with intrahepatic covalently closed circular DNA (cccDNA) and serum HBV DNA in HBeAg-positive patients ($r=-0.387$, $P<0.05$; $r=-0.220$, $P<0.05$), but positively in those HBeAg-negative patients ($r=0.419$, $P<0.05$; $r=0.570$, $P<0.05$).[7] However, it is still unclear whether this difference is only related to the presence of HBeAg, and whether this transition occurs before or after HBeAg clearance.

In this study, we evaluated the relationship between the severity of liver inflammatory damage and viral replication in CHB patients in different HBeAg status and HBV DNA levels through the analysis of two independent cross-sectional cohorts, one validation cohort, and meta-analyses. We observed an opposite relationship between viral replication and liver inflammation for patients with different HBeAg status. What's more, an opposite relationship existed between HBV replication and liver inflammation in HBeAg-positive patients with HBV DNA $\leq 2\times10^6$ IU/mL and $>2\times10^6$ IU/mL.

Methods

Clinical cohorts

This study included two independent cohorts and one validation cohort. Cohort A is a retrospective cohort from the Fifth Medical Center of the PLA General Hospital of Beijing, China, with the majority of enrolled patients having been previously reported.[8,9] Briefly, the patients were enrolled from 2010 to 2020, and those with available laboratory evidence of chronic HBV infection (serum HBsAg and/or HBV DNA positive for at least 6 months) were included (approved by the Ethics Committee of the Fifth Medical Center of the PLA General Hospital of Beijing, No. KY-2022-1-4-1). None of the enrolled patients had received antiviral treatment in the past 6 months, and the liver necroinflammatory grade of each patient was evaluated by liver biopsy,[10,11] as previously described. Cohort B is the retrospective cohort from Ruijin Hospital, as previously described.[12] Briefly, the database was retrospectively derived from CHB patients who had undergone liver biopsy. The criteria for enrollment were:1) with chronic HBV infection for more than 6 months; 2) not receiving anti-HBV treatment. 3) with clinical information such as serum ALT, HBV DNA, HBeAg, and anti-HBe available. The exclusion criteria were patients with HIV infection, other viral hepatitis infections (hepatitis A, C, D, and E), clinical or imaging evidence of decompensated liver cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease, autoimmune liver disease, drug-induced liver disease, metabolic or genetic disease, and alcohol consumption. A validation cohort enrolled 2,624 treatment-naïve patients with HBeAg-positive CHB from Nanjing Drum Tower Hospital between 2015 and 2022, including 457 who underwent liver biopsy; some of these patients have been described previously.[13]

The following clinical indices were collected for analysis: ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), liver biopsy-determined liver necroinflammatory grade (G), HBV DNA, HBeAg, and anti-HBe. In addition, quantitative serum HBsAg measurement and hepatic immunohistochemistry (IH) and scoring of HBcAg were available for some patients in cohort A to confirm the relationship between hepatic virus replication and liver injury.

Statistical analysis

The data obtained were analysed with IBM SPSS Statistic 22.0 software (International Business Machines Corporation, New York, USA) and GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, California). Clinical characteristics of the patients are described as the mean ± standard error (SEM) or median (-IQR, +IQR), depending on the data distribution. Differences between sample groups were tested using ANOVA following rank transformation or the t-test, as determined by the distribution of data. Spearman's rank correlation coefficient test, linear regression analysis, and logistic regression were used to describe the association between variables. A two-sided *P*-value <0.05 was considered statistically significant.

The systematic review and meta-analysis aimed to explore the relationship between HBV DNA and ALT in CHB patients with different HBeAg statuses.

Search strategies and selection criteria: Databases including Embase and PubMed were searched for relevant literature using search terms (Supplementary Fig. 1). Literature searches were performed on April 25, 2022. A manual search was also performed as supplemental. Only English language studies were reviewed. Briefly, a study was regarded as eligible for inclusion in the meta-analysis if it provided the R-value between HBV DNA and ALT in CHB patients with a specific HBeAg status. Study screening flows are described in Supplementary Figure 1. 16 studies (HBeAg positive: n=7;[14–20] HBeAg negative: n=14[14,16,17,19,21–29]) were finally included for analysis.

Data extraction and Risk of Bias assessment: The following information was extracted from each study: name of the first author, year of publication, number of patients, baseline HBV DNA, ALT level, and R-value between HBV DNA and ALT. The basic information of studies included in Part 1 is summarized in Supplementary Tables 1 and 2. Two authors (Wang. L and Zhao. K) independently extracted the information from the studies included using the same standardized form. Discordances were resolved by consensus and/or by discussion with a third and senior author. Study quality and risk of bias were assessed using the modified ROBINS-E Tool. This scale consists of 7 domains. The risk of bias was evaluated as follows: low risk of bias, some concerns, high risk of bias, and very high risk of bias (Supplementary Tables 3 and 4).

Statistical methods for meta-analysis: Stata 16.0 (StataCorp, Texas, USA) and RevMan 5.4.1 (The Cochrane Collaboration, Oxford, UK) were used to perform meta-analyses. The R-value was transformed to Fisher's Z to calculate the overall R-value. Forest plots were drawn, and pooled ORs and 95%CIs were calculated. Heterogeneity was evaluated

Table 1. Spearman relationship between HBV DNA and clinical index for liver inflammation

Spearman Rho (*P*-Value)	HBV DNA			
	Cohort A (n=993)		Cohort B (n=357)	
	HBeAg positive (n=649)	HBeAg negative (n=344)	HBeAg positive (n=231)	HBeAg negative (n=126)
ALT (IU/mL)	−0.036 (*P*=0.36)	0.450 (*P*<0.01)	0.144 (*P*=0.029)	0.363 (*P*<0.01)
AST (IU/mL)	0.068 (*P*=0.85)	0.459 (*P*<0.01)	0.059 (*P*=0.370)	0.448 (*P*<0.01)
GGT (IU/mL)	−0.157 (*P*<0.01)	0.208 (*P*<0.01)	−0.249 (*P*<0.01)	0.220 (*P*<0.01)
Liver Necroinflammatory grade	−0.176 (*P*<0.01)	0.434 (*P*<0.01)	−0.260 (*P*<0.01)	0.426 (*P*<0.01)

HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

by the Cochrane Q test and I^2 test. Briefly, I^2>50% was considered as heterogeneity exists. Random-effects meta-analysis was used to pool the results from the included studies.

Results

Clinical characteristics of patients

Two independent cohorts of treatment-naïve patients with chronic HBV infection were enrolled in this cross-sectional clinical study. Cohort A was composed of 993 individuals from the Fifth Medical Center of Chinese PLA General Hospital; Cohort B included 357 individuals from Ruijin Hospital. The clinical characteristics of the two cohorts are summarized in Supplementary Table 5. In cohort A, quantitative serum HBsAg measurements were available in 760 patients, and hepatic HBcAg expression was semi-quantitatively scored by immunohistochemistry in 567 patients. The clinical characteristics of the validation cohort including 2,624 HBeAg-positive individuals are summarized in Supplementary Table 6.

Opposite relationships between HBV replication and the severity of hepatic necroinflammation were observed in HBeAg-positive and -negative individuals

As shown in Table 1, the level of serum HBV DNA correlated negatively with the grade of liver necroinflammation (rho=−0.176, *P*<0.01) and GGT (rho=−0.157, *P*<0.01) in patients with HBeAg-positive status, both in cohort A and in cohort B (rho=−0.260, *P*<0.01; and rho=−0.249, *P*<0.01, respectively), though the coefficient correlations were weak. In contrast, serum HBV DNA levels exhibited a significantly positive correlation with the severity of liver inflammatory grade in both cohort A (rho=0.434, *P*<0.01) and in cohort B (rho=0.426, *P*<0.01), in those HBeAg-negative patients, which was supported by a weak to moderately strong correlation of serum HBV DNA levels with biochemical hepatic inflammatory indicators including ALT, AST and GGT. Moreover, the above opposite correlations between patients' serum HBV DNA level and ALT were partially confirmed by meta-analysis (Supplementary Fig. 2, Supplementary Tables 1 and 2). HBV DNA did exhibit a moderate positive correlation with ALT (n=13, r=0.481, 95% CI: 0.286, 0.638, I^2=97.0%), in HBeAg-negative patients (Supplementary Figs. 2A and 3), though forest plots of the meta-analysis showed no correlation between HBV DNA and ALT (n=7, r=−0.004, 95% CI: −0.083, 0.075, I^2=40.8%) in HBeAg-positive patients (Supplementary Fig. 2B).

In addition to HBV DNA, hepatic HBcAg may also reflect the transcriptional activity of hepatic HBV cccDNA. To confirm the above findings, immunohistochemistry (IH) staining and scoring of intrahepatic HBcAg were performed for 569 patients in cohort A, including 329 HBeAg-positive and 240 HBeAg-negative patients. As expected, the HBcAg expression level in the liver tissue of HBeAg-positive patients was significantly higher than that in those HBeAg-negative patients [0.527 (0.071, 1.061) vs. 0.008 (0.001, 0.039), *P<0.01*]. Among the 79.9% (263/329) HBeAg-positive patients accompanied by positive hepatic HBcAg staining. Spearman analysis revealed a marginal negative correlation between the in-situ expression intensity of hepatic HBcAg (semi-quantitated by IH scores) and the liver necroinflammatory grade (Scheuer grade) (r=−0.208, *P*=0.026). Such a tendency was also demonstrated by the decrease in the hepatic HBcAg-positive rate, along with increased severity of liver necro-inflammatory activities (G0–1: 83.85%, G2: 75.98%, and G3–4: 68.89%) (Table 2). Concordantly, the expression intensity of HBcAg in liver tissues of G3–4 patients was significantly lower than that of G2 (*P*=0.014) and G0–1 (*P*=0.001) in the HBeAg-positive patients. In contrast, in HBeAg-negative patients, the hepatic HBcAg-positive staining rate was found to correlate positively with the increased severity of liver necroinflammation, which was supported by the increased rate of hepatic HBcAg-positive staining from 3.86% to 21.27% and then to 46.15%, respectively in parallel with the severity of liver necroinflammatory grade from G0–1 to G2 and G3–4 (Table 2), though only 9.58% HBeAg-negative patients accompanied by positive hepatic HBcAg.

Table 2. Hepatic HBcAg expression in HBeAg-positive or HBeAg-negative patients with different liver necroinflammatory grades

Liver necroinflammation grade/Hepatic HBcAg positive percentage and HBcAg IH scores	G0–1	G2	G3–4
HBeAg-positive	83.85% (n=135/161)	76.98% (n=97/126)	68.89% (n=31/45)
	11.87 (8.49, 13.41)	10.96 (5.01, 13.54)	8.54 (0.00, 11.59)
HBeAg-negative	3.86% (n=7/181)	21.27% (n=10/47)	46.15% (n=6/13)
	0.30 (0.18, 0.43)	1.98 (1.38, 2.58)	3.06 (2.01, 4.11)

HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; IH, immunohistochemistry.

Negative relationship between HBV replication and severity of liver inflammation only present in HBeAg-positive patients with moderate to high serum HBV DNA levels

The results above to some extent reminded us that there could be efficient control of HBV DNA replication by the host immune system against HBV infection in the early phase of immune activation (IA) in the HBeAg-positive stage. First, a threshold of HBV DNA at 2×10^6 IU/mL was explored and determined by Figure 1 and the serial Spearman test. Then, those HBeAg-positive patients were sub-grouped into HBV DNA high (HBeAg+/DNA high) and low (HBeAg+/DNA low) groups, accordingly. The clinical characteristics of HBeAg-positive patients with different HBV DNA levels for cohort A, cohort B, and Nanjing cohort were summarized in Supplementary Table 6. HBeAg-positive patients with lower HBV DNA levels in cohorts A and B were accompanied by higher ALT compared with patients in the validation cohort. As shown in Table 3, the relationship between viral replication and liver inflammation exhibited the most obvious difference in HBV DNA $>2\times10^6$ IU/mL HBeAg+/DNA high group. Such HBeAg+/DNA high patients exhibited a marginal negative relationship of HBV DNA level with ALT (rho=−0.160, $P<0.001$), AST (rho=−0.188, $P<0.001$), and the severity of liver necroinflammation (rho=−0.211, $P<0.001$), respectively in cohort A. However, in HBeAg-positive patients with lower HBV DNA levels, this negative correlation weakened or disappeared, and even changed to a positive correlation, similar to HBeAg-negative patients (Table 1). Consistently, data from cohort B and the Nanjing cohort exhibited similar tendencies (Table 3). Next, when stratified by liver fibrosis stage or anti-HBe, similar tendencies still existed, though not obvious in cohort B patients with significant fibrosis stage (S≥2) (Supplementary Tables 7 and 8). Besides, we also used public data of CHB patients to perform a similar analysis.[30] Clinical data of 124 CHB patients were available in this article.[30] Among them, 94 patients had complete information for HBV DNA level and HBeAg status. As shown in Supplementary Tables 9 and 10, similar tendencies were also observed in these data.

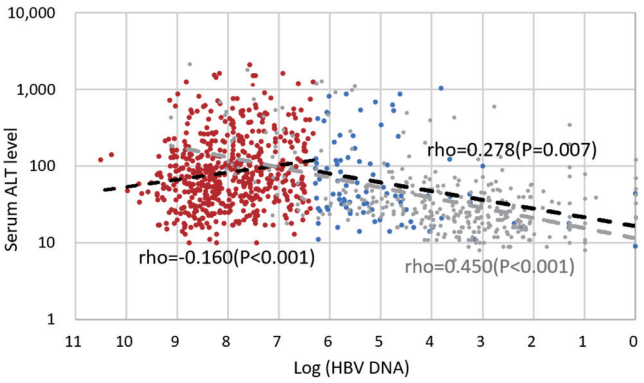

Fig. 1. Scatter plots between serum HBV DNA and ALT of CHB patients with different HBV DNA levels and HBeAg status in cohort A. Note: Red dots represented HBeAg positive patients with HBV DNA $>2\times10^6$ IU/mL; Blue dots represented HBeAg positive CHB patients with HBV DNA $\leq2\times10^6$ IU/mL; Gray dots represented patients with HBeAg negative patients. HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; CHB, chronic hepatitis B.

Furthermore, with the use of linear regression adjusting for age, sex and anti-HBe, the results (Table 4) showed that for HBeAg positive patients with HBV DNA $>2\times10^6$ IU/mL subgroup, patients with active liver inflammation accompanied by a lower HBV DNA level (−0.177 log HBV DNA for ALT elevated, −0.204 log HBV DNA for AST elevated, −0.229 log HBV DNA for GGT elevated and −0.335 log HBV DNA for liver necroinflammation ≥G2) in cohort A. Whereas, for HBeAg positive patients with HBV DNA $\leq2\times10^6$ IU/mL, subgroup with active liver inflammation accompanied by a higher HBV DNA level (0.504 log HBV DNA for ALT elevated, 0.657 log HBV DNA for AST elevated, 0.290 log HBV DNA for GGT elevated and 0.252 log HBV DNA for liver necroinflammation

Table 3. Spearman relationship between serum HBV DNA and ALT, AST, GGT and liver necroinflammatory grade for HBeAg-positive CHB with different HBV DNA levels

Spearman Rho (*P* value) between	HBV DNA and	DNA $>2\times10^6$ IU/mL subgroup	DNA $\leq2\times10^6$ IU/mL subgroup
Cohort A		(n=555)	(n=94)
	ALT	−0.160 (<0.001)	0.278 (0.007)
	AST	−0.188 (<0.001)	0.328 (0.001)
	GGT	−0.141 (0.001)	0.083 (0.429)
	Liver necroinflammatory grade	−0.211 (<0.001)	0.005 (0.958)
Cohort B		(n=152)	(n=79)
	ALT	−0.042 (0.612)	0.260 (0.021)
	AST	−0.169 (0.038)	0.279 (0.013)
	GGT	−0.334 (<0.001)	0.074 (0.539)
	Liver necroinflammatory grade	−0.305 (<0.001)	−0.296 (0.008)
Independent validation Nanjing Cohort		(n=2,041)	(n=583)
	ALT	−0.181 (<0.001)	0.201 (<0.001)
	AST	−0.236 (<0.001)	0.215 (<0.001)
	GGT	−0.194 (<0.001)	0.104 (0.012)
	Liver necroinflammatory grade	−0.158 (0.004) *	0.011 (0.903)#

*n=329; #n=128. HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

Table 4. Association of HBV DNA change with liver inflammation indicators in HBeAg positive patients with different HBV DNA levels (cohort A)

Variables	*Change in log HBV DNA (IU/mL)*							
	HBV DNA >2×10^6 IU/mL subgroup				HBV DNA ≤2×10^6 IU/mL subgroup			
	β*	SE	T value	*P* value	β*	SE	T value	*P* value
ALT>2×ULN	−0.177	0.068	−2.613	0.009	0.504	0.246	2.051	0.043*
AST>1.5×ULN	−0.204	0.069	−2.973	0.003	0.657	0.242	2.712	0.008
GGT>1×ULN	−0.229	0.086	−2.663	0.008	0.290	0.265	1.094	0.277
Liver necroinflammation grade ≥G2	−0.335	0.066	−6.103	<0.001	0.252	0.244	1.035	0.304

The cut-off value was selected according to data distribution. * Adjusted for baseline characteristics including age, sex, and anti-HBe. Ref, reference; β, regression β coefficients; SE, standard error; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ULN, the upper limit of normal.

Table 5. Binary logistic regression analysis between HBV DNA and liver inflammation indicator in HBeAg-positive patients with different HBV DNA levels (cohort A)

Variables	Liver inflammation indicators	OR (95% CI) *	*P* value	β
HBV DNA >2×10^6 IU/mL subgroup				
HBV DNA increased (>1×10^8 IU/mL)	ALT (>2×ULN)	0.572 (0.403–0.813)	0.002	−0.558
	AST (>1.5×ULN)	0.564 (0.396–0.806)	0.002	−0.572
	GGT (>ULN)	0.678 (0.435–1.057)	0.086	−0.388
	Liver necroinflammatory grade (≥G2)	0.459 (0.323–0.651)	<0.001	−0.779
HBV DNA ≤2×10^6 IU/mL subgroup				
HBV DNA increased (>3×10^5 IU/mL)	ALT (>2×ULN)	2.991 (1.133–7.895)	0.027	1.096
	AST (>1.5×ULN)	4.708 (1.680–13.189)	0.003	1.549
	GGT (>ULN)	2.258 (0.840–6.068)	0.106	0.814
	Liver necroinflammatory grade (≥G2)	1.494 (0.622–3.591)	0.369	0.402

The cut-off value was selected according to data distribution. * Adjusted for baseline characteristics including age, sex, and anti-HBe. OR, Odd Ratio; β, β coefficients; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ULN, the upper limit of normal.

≥G2) in cohort A. Binary logistic regression analysis revealed that active liver inflammation was a protective factor for higher HBV DNA in HBeAg positive patients with >2×10^6 IU/mL subgroup, but a risk factor for higher HBV DNA in HBeAg positive patients with ≤2×10^6 IU/mL subgroup (Table 5) in the cohort A. Data from cohort B exhibited similar tendencies (Supplementary Tables 11 and 12).

Moreover, analysis was also performed among a subgroup of patients with available hepatic HBcAg IH score or quantitative serum HBsAg data. Weak to moderate negative correlations of hepatic HBcAg with ALT, AST, and liver necroinflammation grade were observed in those HBeAg+/DNA high patients, but not in HBeAg+/DNA low patients with HBV DNA ≤2×10^6 IU/mL (Table 6). Moderate negative correlations of serum HBsAg with ALT, AST, and liver necroinflammation grade were observed in HBeAg+/DNA high patients but became weakened in HBeAg+/DNA low patients in both cohort A and the Nanjing cohort (Supplementary Table 13).

The results above reveal a statistically significant negative relationship between the level of HBV DNA and the severity of liver inflammation only in HBeAg-positive patients with higher HBV DNA levels (with HBV DNA >2×10^6 IU/mL in this study). However, in HBeAg positive patients with lower HBV DNA levels, this negative correlation weakened or disappeared, and even changed to a positive correlation, similar to in those HBeAg negative patients.

Table 6. Spearman relationship between serum HBsAg/Hepatic HBcAg and clinical index for liver injury for HBeAg positive patients with different HBV DNA levels in cohort A

Spearman Rho (*P* value) between Different variables	HBV DNA >2×10^6 IU/mL (n=286)		HBV DNA ≤2×10^6 IU/mL (n=43)	
	Serum HBsAg	Hepatic HBcAg	Serum HBsAg	Hepatic HBcAg
ALT	−0.325 (0.000)	−0.290 (0.000)	−0.248 (0.109)	0.006 (0.972)
AST	−0.402 (0.000)	−0.279 (0.000)	−0.231 (0.135)	0.018 (0.908)
GGT	−0.278 (0.000)	−0.243 (0.000)	−0.075 (0.635)	−0.083 (0.596)
Liver necroinflammatory grade	−0.403 (0.000)	−0.236 (0.000)	−0.011 (0.942)	0.117 (0.455)

HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

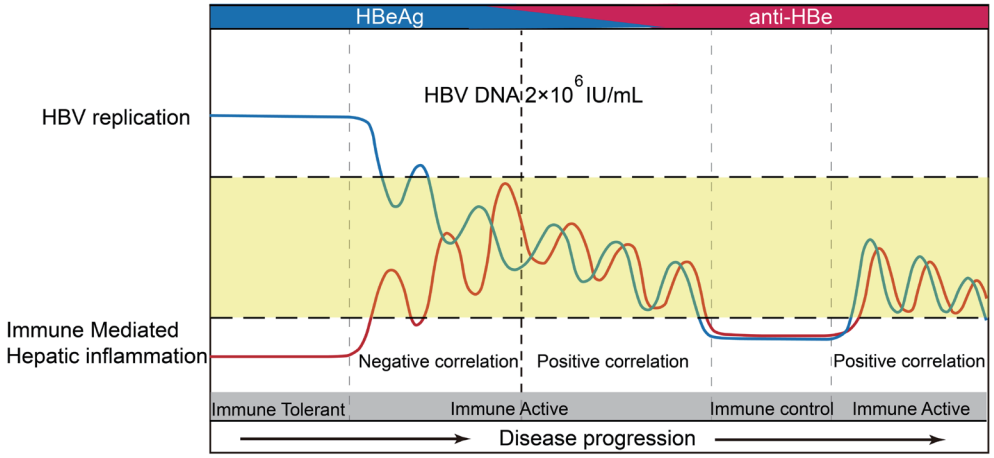

Fig. 2. Schematic diagram of the relationship between viral replication and the severity of hepatic necroinflammation of CHB in disease progression. HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; CHB, chronic hepatitis B.

Discussion

In this study, we explore the relationship between serum HBV DNA and hepatic necroinflammatory damage, especially for patients with different HBeAg status and HBV DNA levels. In general, in both cohort A and cohort B, weak but statistically significant negative correlations between serum HBV DNA level and the severity of hepatic necroinflammation were observed in HBeAg-positive patients. In contrast, such a negative correlation converted to a moderate positive with the loss of HBeAg. In line with these, similar correlation patterns between HBV DNA and biochemical indicators of liver injury were also observed. These phenomena were partially confirmed by meta-analysis. Unexpectedly, we observed the disappearance or reversal of the negative correlation between viral replication activity and most indicators of liver inflammation damage even in HBeAg-positive patients with HBV DNA ≤2×10^6 IU/mL, which were confirmed by the validation cohort composed of 2,624 HBeAg-positive patients, including 457 patients with liver biopsy available.

The key finding of our study demonstrates that a marginal negative relationship between HBV DNA and liver damage mainly exists in HBeAg+/DNA-high (herein in this study, >2×10^6 IU/mL) patients. Noticeably, the relationship between HBV DNA and liver damage had already become positive in HBeAg+/DNA-low (≤2×10^6 IU/mL) patients, similar to that observed in HBeAg-negative patients. A schematic figure was drawn to illustrate these "opposite relationships" observed in this study (Fig. 2). It demonstrated that patients in the yellow zone with moderate HBV DNA levels, especially for those with HBV DNA surrounding the threshold 2×10^6 IU/mL, suffer from more active inflammation and probably possess a higher risk of disease progression, with/without HBeAg loss. In line with this, recent studies reported an increased risk of HCC development for CHB patients with moderate (10^4–10^8 IU/mL) viral replication levels compared to those with HBV DNA >10^8 IU/mL or <10^4 IU/mL, particularly for those with HBV DNA levels between 10^6–10^7 IU/mL.[31,32] Thus, we think that a more aggressive treatment strategy should be recommended for patients with moderate HBV DNA levels regardless of HBeAg status (especially for patients with HBV DNA around 2×10^6 IU/mL).

Notably, in our study, most HBeAg-positive patients with HBV DNA >2×10^6 IU/mL were not in the "real" immune tolerance status, only 11.17% of patients in cohort A and 17.76% of patients in cohort B showed no indication of liver damage, characterized by normal ALT, AST, GGT, and no to mild liver necroinflammation (G=0/1). Thus, a negative correlation between HBV DNA level and the severity of hepatic inflammatory damage was observed in these patients. Unfortunately, antiviral treatment is rarely initiated in HBeAg-positive patients with HBV DNA >2×10^6 IU/mL, who are usually assumed to be in the immune-tolerance phase as their HBV DNA >1×10^6 IU/mL.[6,33] Based on our observations, we sought to determine whether therapeutic efficacy may be improved if antiviral treatment is launched in HBeAg-positive patients with HBV DNA around 2×10^6 IU/mL, supporting the suggestion to "treat-all" individuals with chronic HBV infection

The characteristics of "the negative relationship of active virus replication and hepatic inflammation" and "the positive relationship of virus replication and hepatic inflammation" observed in our studies seemed to constitute two patterns of ALT flares, "good flares" and "bad flares", as previously described.[34,35] The former is usually related to a decrease in HBV DNA and probably leads to the loss of HBeAg and even HBsAg and is therefore considered "good" host immune-induced inflammation. The latter is always relevant to persistent liver injury without efficient control of HBV replication and has been considered a virus-induced injury.[36] Based on our current study, a "good flare" could be expected to occur in HBeAg-positive patients with higher HBV DNA levels (>2×10^6 IU/mL). On the opposite, a "bad flare" would occur most likely in HBeAg-negative patients. A potential explanation for this phenomenon could be that in HBeAg-positive patients, especially those with high HBV DNA levels, almost all hepatocytes are accompanied by HBV infection and active transcription.[37,38] Thus, even nonspecific immune-induced hepatocyte injury would result in the number of a reduction in HBV-infected hepatocytes, which ultimately reduces the HBV DNA level. While in HBeAg-negative patients, activation of the host's immune response induced by HBV replication might cause more serious immunopathological damage, than the clearance of cccDNA-positive hepatocytes, because usually only a few hepatocytes were cccDNA transcriptionally active as indicated by the extremely lower rate of HBcAg positive hepatocytes in previous and current studies.[38]

Although this study provides insight into the relationship between viral replication and the severity of liver inflammatory damage in CHB patients with different virological characteristics, there are still some limitations. First, this was a

retrospective study. To address this, we expanded the sample size through meta-analysis to minimize the impact of study bias. Second, due to the shortcomings of retrospective and cross-sectional studies, detection and collection of the immune indicators of CHB patients, especially HBV-specific immune data were not available. Thus, we use liver damage as the indirect indicator to reflect the host's immune response in this study. Though most likely the severity of liver damage is mediated by the host's immune response against HBV, hepatic damage is the outcome but not the direct indicator of the host's immune response against HBV infection. We expect that a prospective, large-scale, follow-up cohort study with multiple immune index detections (especially HBV-specific immunity) will be conducted in the future to investigate the causes of this phenomenon.

In summary, our study reports distinguishable relationship patterns between serum HBV DNA and liver damage, in patients with different HBeAg statuses and/or HBV DNA levels. The relationship between viral replication and the severity of hepatic necroinflammatory damage changes before the loss of HBeAg in patients with chronic hepatitis B virus infection.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82072280) and Beijing Natural Science Foundation (No. 7212063).

Conflict of interest

FL has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2013, JL has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2024, XZ has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication

Author contributions

FL and LW designed and revised the paper; LW analysed data, wrote the paper, and made figures; KZ analysed data; JW and XZ collected data and analysed the data; LJ revised the paper and analysed data. JL and JZ contributed to the study design and manuscript revision. All authors read and approved the final manuscript

Ethical statement

This study was approved by the Ethics Committee of the Fifth Medical Center of the PLA General Hospital of Beijing (No. KY-2022-1-4-1). All procedures were conducted according to the principles of the Declaration of Helsinki. Written informed consent was waived.

Data sharing statement

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

References

[1] Polaris Observatory C. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3(6):383–403. doi:10.1016/S2468-1253(18)30056-6, PMID:29599078.
[2] Cao G, Jing W, Liu J, Liu M. Countdown on hepatitis B elimination by 2030: the global burden of liver disease related to hepatitis B and association with socioeconomic status. Hepatol Int 2022;16(6):1282–1296. doi:10.1007/s12072-022-10410-y, PMID:36048317.
[3] Tsai KN, Ou JJ. Hepatitis B virus e antigen and viral persistence. Curr Opin Virol 2021;51:158–163. doi:10.1016/j.coviro.2021.10.003, PMID:34717215.
[4] Tian Y, Kuo CF, Akbari O, Ou JH. Maternal-Derived Hepatitis B Virus e Antigen Alters Macrophage Function in Offspring to Drive Viral Persistence after Vertical Transmission. Immunity 2016;44(5):1204–1214. doi:10.1016/j.immuni.2016.04.008, PMID:27156385.
[5] Lau DTY, Ganova-Raeva L, Wang J, Mogul D, Chung RT, Lisker-Melman M, *et al*. Precore and Basal Core Promoter Hepatitis B Virus (HBV) Variants Are Present From a Young Age and Differ Across HBV Genotypes. Hepatology 2021;73(5):1637–1651. doi:10.1002/hep.31506, PMID:32860463.
[6] European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67(2):370–398. doi:10.1016/j.jhep.2017.03.021, PMID:28427875.
[7] Liao H, Li L, Zheng WV, Zou J, Yu G, Si L, *et al*. Characteristics of HBV Novel Serum Markers across Distinct Phases in Treatment-Naive Chronic HBV-Infected Patients. Dis Markers 2022;2022:4133283. doi:10.1155/2022/4133283, PMID:35872701.
[8] Yao M, Wang L, You H, Wang J, Liao H, Yang D, *et al*. Serum GP73 combined AST and GGT reflects moderate to severe liver inflammation in chronic hepatitis B. Clin Chim Acta 2019;493:92–97. doi:10.1016/j.cca.2019.02.019, PMID:30796901.
[9] Wang LJ, Li MW, Liu YN, Chen XM, Zhao JM, Liu SH, *et al*. Natural history and disease progression of chronic hepatitis B virus infection. Beijing Da Xue Xue Bao Yi Xue Ban 2022;54(5):920–6. doi:10.19723/j.issn.1671-167X.2022.05.019, PMID:36241234.
[10] The programme for prevention and control of viral hepatitis. Zhong Hua Nei Ke Za Zhi 2001;40(1):62–68. doi:10.3760/j.issn:0578-1426.2001.01.034.
[11] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991;13(3):372–374. doi:10.1016/0168-8278(91)90084-o, PMID:1808228.
[12] Wang L, Zhu M, Cao L, Yao M, Lu Y, Wen X, *et al*. Liver Stiffness Measurement Can Reflect the Active Liver Necroinflammation in Population with Chronic Liver Disease: A Real-world Evidence Study. J Clin Transl Hepatol 2019;7(4):313–321. doi:10.14218/JCTH.2019.00040, PMID:31915600.
[13] Wang J, Ding W, Liu J, Liu Y, Yan X, Xia J, *et al*. Association of Coexistent Hepatitis B Surface Antigen and Antibody With Severe Liver Fibrosis and Cirrhosis in Treatment-Naive Patients With Chronic Hepatitis B. JAMA Netw Open 2022;5(6):e2216485. doi:10.1001/jamanetworkopen.2022.16485, PMID:35696167.
[14] Schwarz KB, Lombardero M, Di Bisceglie AM, Murray KF, Rosenthal P, Ling SC, *et al*. Phenotypes of Chronic Hepatitis B in Children From a Large North American Cohort. J Pediatr Gastroenterol Nutr 2019;69(5):588–594. doi:10.1097/mpg.0000000000002467, PMID:31436702.
[15] Demirören K, Kocamaz H, Doğan Y. The importance of the serum quantitative levels of hepatitis B surface antigen and hepatitis B e antigen in children with chronic hepatitis B. Turk J Gastroenterol 2015;26(1):36–41. doi:10.5152/tjg.2015.4636, PMID:25698269.
[16] Larsson SB, Eilard A, Malmström S, Hannoun C, Dhillon AP, Norkrans G, *et al*. HBsAg quantification for identification of liver disease in chronic hepatitis B virus carriers. Liver Int 2014;34(7):e238–245. doi:10.1111/liv.12345, PMID:24118747.
[17] Elbir P, Ozgunes N, Elbir TZ, Yalcin D, Aydin O, Ergen P. Evaluation of the relationship between HBV DNA and liver histology in naive chronic hepatitis B patients. Hepatol Int 2014;8(1):S74. doi:10.1007/s12072-014-9519-7.
[18] Mao QG, Pan JS, Fang KN, Zhang RM, Hong QY, Song MN, *et al*. Precise prediction model and simplified scoring system for sustained combined response to interferon-α. World J Gastroenterol 2010;16(27):3465–3471. doi:10.3748/wjg.v16.i27.3465, PMID:20632453.
[19] Shao J, Wei L, Wang H, Sun Y, Zhang LF, Li J, *et al*. Relationship between hepatitis B virus DNA levels and liver histology in patients with chronic hepatitis B. World J Gastroenterol 2007;13(14):2104–2107. doi:10.3748/wjg.v13.i14.2104, PMID:17465456.
[20] Keun Chun Y, Youn Kim J, Jung Woo H, Myung Oh S, Kang I, Ha J, *et al*. No significant correlation exists between core promoter mutations, viral replication, and liver damage in chronic hepatitis B infection. Hepatology 2000;32(5):1154–1162. doi:10.1053/jhep.2000.19623, PMID:11050069.
[21] Esmaeelzadeh A, Saadatnia H, Memar B, Amirmajdi EM, Ganji A, Goshayeshi L, *et al*. Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative hepatitis B patients. Gastroenterol Hepatol Bed Bench 2017;10(1):39–43. PMID:28331563.
[22] Hou Y, Xu P, Lou X, Liang D, Zhang M, Zhang Z, *et al*. Serum decoy receptor 3 is a useful predictor for the active status of chronic hepatitis B in hepatitis B e antigen-negative patients. Tohoku J Exp Med 2013;230(4):227–232. doi:10.1620/tjem.230.227, PMID:23925044.
[23] Wu DL, Xu GH, Lu SM, Ma BL, Miao NZ, Liu XB, *et al*. Age versus clinical virological characteristics in chronic hepatitis B virus infection: a case series study in China. Eur J Gastroenterol Hepatol 2012;24(4):406–413. doi:10.1097/MEG.0b013e32834fbf35, PMID:22273989.
[24] Croagh CMN, Bell SJ, Locarnini S, Desmond PV. Assessment of chronic hepatitis B: The importance of hepatitis B virus DNA testing. Intern Med J 2012;42(2):170–175. doi:10.1111/j.1445-5994.2011.02435.x, PMID:21241443.
[25] Manesis EK, Papatheodoridis GV, Tiniakos DG, Hadziyannis ES, Agelopoulou OP, Syminelaki T, *et al*. Hepatitis B surface antigen: relation to hepatitis B replication parameters in HBeAg-negative chronic hepatitis B. J Hepatol 2011;55(1):61–68. doi:10.1016/j.jhep.2010.10.027, PMID:21145875.
[26] Ijaz B, Ahmad W, Javed FT, Gull S, Hassan S. Revised cutoff values of

ALT and HBV DNA level can better differentiate HBeAg (-) chronic inactive HBV patients from active carriers. Virol J 2011;8:86. doi:10.1186/1743-422X-8-86, PMID:21352567.
[27] Wong GLH, Wong VWS, Choi PCL, Chan AWH, Chim AML, Yiu KKL, *et al*. Evaluation of alanine transaminase and hepatitis B Virus DNA to predict liver cirrhosis in hepatitis B e antigen-negative chronic hepatitis B using transient elastography. Am J Gastroenterol 2008;103(12):3071–3781. doi:10.1111/j.1572-0241.2008.02157.x, PMID:19086958.
[28] Satapathy SK, Garg S, Chauhan R, Malhotra V, Sakhuja P, Sharma BC, *et al*. Profile of chronic hepatitis B virus in children in India: experience with 116 children. J Gastroenterol Hepatol 2006;21(7):1170–1176. doi:10.1111/j.1440-1746.2006.04382.x, PMID:16824071.
[29] Kim KH, Na IH, Cha JM, Cho YK, Park SY, Kim HP, *et al*. Serum ALT and HBV DNA levels in patients with HBeAg-negative chronic hepatitis B. Taehan Kan Hakhoe chi 2003;9(4):284–292. PMID:14695695.
[30] Wang M, Gong Q, Zhang J, Chen L, Zhang Z, Lu L, *et al*. Characterization of gene expression profiles in HBV-related liver fibrosis patients and identification of ITGBL1 as a key regulator of fibrogenesis. Sci Rep 2017;7:43446. doi:10.1038/srep43446, PMID:28262670.
[31] Kim GA, Han S, Choi GH, Choi J, Lim YS. Moderate levels of serum hepatitis B virus DNA are associated with the highest risk of hepatocellular carcinoma in chronic hepatitis B patients. Aliment Pharmacol Ther 2020;51(11):1169–1179. doi:10.1111/apt.15725, PMID:32291781.
[32] Chun HS, Lee M. Risk of hepatocellular carcinoma in untreated patients with chronic hepatitis B: Independent of HBeAg status? Clin Mol Hepatol 2021;27(3):448–450. doi:10.3350/cmh.2021.0130, PMID:34157831.
[33] Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10(1):1–98. doi:10.1007/s12072-015-9675-4, PMID:26563120.
[34] Ghany MG, Feld JJ, Chang KM, Chan HLY, Lok ASF, Visvanathan K, *et al*. Serum alanine aminotransferase flares in chronic hepatitis B infection: the good and the bad. Lancet Gastroenterol Hepatology 2020;5(4):406–417. doi:10.1016/S2468-1253(19)30344-9, PMID:32057301.
[35] Chang ML, Liaw YF. Hepatitis B Flare in Hepatitis B e Antigen-Negative Patients: A Complicated Cascade of Innate and Adaptive Immune Responses. Int J Mol Sci 2022;23(3):1552. doi:10.3390/ijms23031552, PMID:35163476.
[36] You H, Wu X, Ou X, Ma H, Wang Q, Liu T, *et al*. Two patterns of alanine aminotransferase increase to predict long-term viral response in chronic hepatitis B patients: virus- or host-induced? Antivir Ther 2011;16(3):299–307. doi:10.3851/IMP1758, PMID:21555812.
[37] Balagopal A, Grudda T, Ribeiro RM, Saad YS, Hwang HS, Quinn J, *et al*. Single hepatocytes show persistence and transcriptional inactivity of hepatitis B. JCI Insight 2020;5(19):e140584. doi:10.1172/jci.insight.140584, PMID:33004689.
[38] Montanari NR, Ramirez R, Aggarwal A, van Buuren N, Doukas M, Moon C, *et al*. Multi-parametric analysis of human livers reveals variation in intrahepatic inflammation across phases of chronic hepatitis B infection. J Hepatol 2022;77(2):332–343. doi:10.1016/j.jhep.2022.02.016, PMID:35218813.