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The Levels of Serum HBV Pre-Genomic RNA and Its Associated Factors Among HBV-Infected Patients: A Retrospective Cohort Study in Hangzhou, Zhejiang, China

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Introduction: This study aimed to explore serum HBV pre-genomic RNA (pgRNA) levels and its associated factors among HBV-infected patients in the real world.

Methods: This retrospective cohort study was conducted from May 10, 2023, to January 15, 2024. Univariate logistic analysis for positive serum HBV pgRNA was performed first, and variables with statistical significance were included in a multivariate logistic model. A decreasing trend of serum HBV pgRNA and HBV DNA levels was also detected first by univariate logistic regression and then by multivariate logistic regression.

Results: 482 patients were included in our analysis at baseline, and 191 patients were followed up. Multivariate logistic regression revealed that positive HBV DNA (AOR: 2.63, 95% CI: 1.46–4.75, P=0.001), ≥1000 hBsAg (AOR: 2.29, 95% CI: 1.08–4.89, P=0.03), positive HBeAg (AOR: 28.26, 95% CI: 15.2–52.55, P<0.001), and ALP (AOR: 1.01, 95% CI: 1.001–1.02, P=0.03) were positively correlated with positive HBV pgRNA at baseline. Two independent multivariate logistic regression models were constructed for the decreasing trend of serum HBV pgRNA and HBV DNA for the 191 follow-up patients. Results showed that the decreasing trend of HBV pgRNA was positively correlated with positive baseline HBV DNA (AOR: 4.60, 95% CI: 1.84–11.51, P=0.001), baseline HBsAg ≥1000 IU/mL (AOR: 8.74, 95% CI: 1.09–70.10, P=0.04), and HDL (AOR: 5.01, 95% CI: 1.28–19.66, P=0.02). The decreasing trend of HBV DNA was positively correlated with positive baseline HBV pgRNA (AOR: 3.80, 95% CI: 2.00–8.83, P<0.001) and AST (AOR: 1.06, 95% CI: 1.03–1.08, P<0.001).

Conclusion: Our study revealed that HBV DNA, HBsAg, HBeAg, and ALP were significantly correlated with positive HBV pgRNA at baseline. The baseline HBV DNA, HBsAg, and HDL were significantly correlated with decreasing levels of HBV pgRNA. A decreasing trend of HBV DNA significantly correlated with patients' baseline HBV pgRNA and AST.

Keywords: chronic hepatitis B, HBsAg, HBV pgRNA, HBV DNA

Introduction

Hepatitis B virus (HBV) infection is a major global public health issue that is prevalent in African and Asian countries. A study in 2019[1] revealed that the global prevalence of hepatitis B is approximately 4.1%, with approximately 316 million infected individuals worldwide. At present, chronic HBV infection remains the main cause of liver cancer death worldwide, and patients infected with HBV have a significantly increased risk of developing liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).[2] China has the highest burden of HBV infection in the world. The World Health Organization estimates that China has 87 million chronic hepatitis B (CHB) virus infections,[3] and the

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number of deaths from HBV-related liver diseases in China accounts for more than 30% of the global HBV mortality rate.[4]

The covalently closed circular DNA (cccDNA) of HBV in liver cells serves as the most important template for HBV pgRNA coding, initiating viral replication and being a key factor in sustained infection and recurrence. However, the current treatment regime has a small impact on erasing cccDNA, and the long-term existence of cccDNA makes it difficult to reach the "HBV functional cure"[5] goal. The level of cccDNA is the best prognostic indicator for antiviral therapy,[6] but its clinical application has been limited by its uneven distribution[7,8] in liver tissue and the harmfulness of invasive testing. In contrast, HBV pgRNA is not only a direct transcript of cccDNA but can also encode HBV polymerase, and the polymerase can convert RNA into rcDNA and subsequently repair rcDNA to form HBV cccDNA. HBV DNA below the detection limit only indicates inhibition of the reverse transcription process, while cccDNA may still be expressed at low levels.[9,10] In theory, a cccDNA in liver cells can lead to recurrence after nucleos(t)ide analogues (NAs) discontinuation.[10] Therefore, in addition to HBV DNA, cccDNA must be tracked to evaluate the prognosis of HBV patients, and HBV pgRNA is a good indicator of cccDNA. A review[11] revealed that serum HBV pgRNA has three applications: monitoring cccDNA transcriptional activity, predicting relapses after NAs discontinuation, and predicting HBV antigen seroconversion/loss.

HBV DNA in CHB patients can quickly turn negative after NAs treatment, and HBV pgRNA can continue to evaluate the therapeutic effect on patients and monitor the transcription activity of cccDNA. The main purpose for CHB patients is to achieve a functional cure. Patients with negative HBV RNA or small values are more likely to achieve HBsAg loss, especially in (1) patients using both NAs and interferon therapy; (2) appropriate patients withdraw NAs; and (3) pregnant women after giving birth (a dominant group). Therefore, observing the characteristics of patients with HBV RNA decline during the follow-up process is of clinical significance.

Currently, many studies have focused on the host and viral factors associated with HBV pgRNA, such as HBeAg status, HBV DNA, age, and patient immunity. HBeAg status,[12,13] HBV DNA,[14,15] and Th2 immunity[16] were positively associated with HBV pgRNA, but age[17,18] and Th1 immunity[16] had negative effects on HBV pgRNA. A multivariate linear regression study[19] revealed that age was an independent factor of the magnitude of the change in HBV RNA concentration. Still, the small sample size and small number of variables limited its use.

This study aimed to explore the levels of HBV RNA and its associated factors among HBV-infected patients in the real world. Moreover, the decreasing trends in the levels of HBV RNA and HBV DNA and their influencing factors were also studied in our research.

Materials and Methods

Study Subjects

We conducted a retrospective cohort study from May 10, 2023, to January 15, 2024. All the subjects were HBV-infected patients who visited the inpatient and outpatient departments of Hangzhou Xixi Hospital. The inclusion criteria were as follows: (1) ≥ 18 years of age; (2) HBsAg positivity for > 6 months, and a diagnosis of CHB, cirrhosis, or liver cancer based on the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (version 2022).[20] The exclusion criteria were (1) pregnant or lactating women; (2) concomitant presence of other viral infections, such as HIV, HCV, or HDV, or the presence of autoimmune liver disease; (3) incomplete blood test results; and (4) no clear HBV treatment information. To understand the changes in HBV pgRNA in patients under treatment or untreated conditions and their influencing factors, we excluded four individuals who took medication during the follow-up process. The flowchart of this study is shown in Figure 1. This research was approved by the Ethics Committee of Hangzhou Xixi Hospital (ethics 2024 Research No. 028). Written informed consent was not required due to the retrospective nature of this study. All the data used in this study were anonymized.

Detection of Biochemical and Virological Indicators

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), high-density lipoprotein (HDL), low-density lipoprotein (LDL), gamma-glutamyl transpeptidase (GGT), total bilirubin (TBIL),

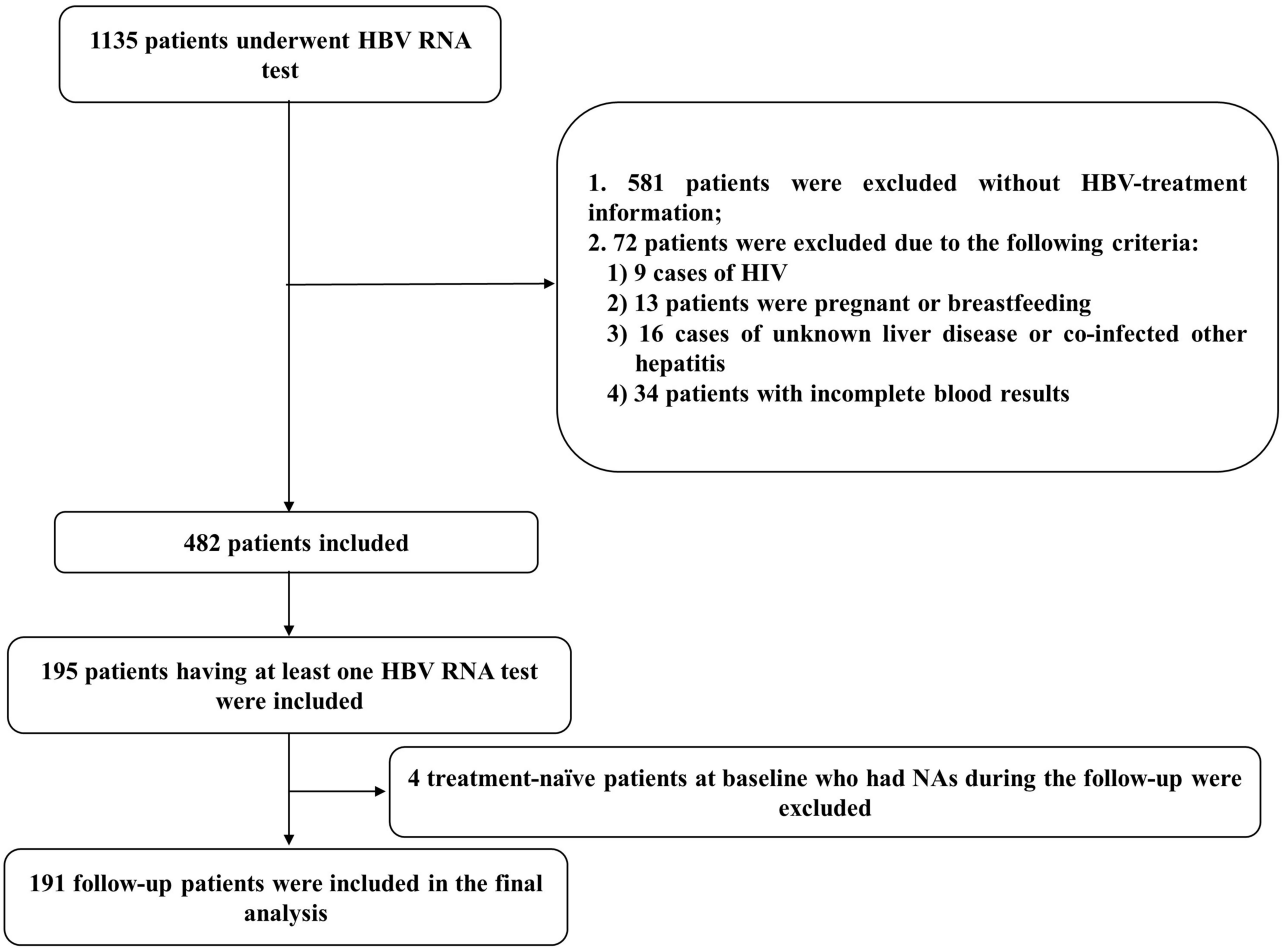

Figure 1 Flowchart of the HBV-infected patient enrollment.

blood urea nitrogen (BUN), creatinine (CREA), uric acid (URIC), total cholesterol (TC), triglyceride (TG), and estimated glomerular filtration rate (eGFR) were detected using a Beckman Coulter AU5831 automatic biochemical analyzer in the laboratory.

The detection of the hepatitis B surface antigen (HBsAg) and E antigen (HBeAg) was completed on an Abbott Alinity I fully automated chemiluminescent immunoassay analyzer (USA). For the samples with HBsAg greater than 250 IU/mL, the machine was used to dilute the samples 500 times and calculate the surface antigen value. A HBsAg concentration < 0.05 IU/mL was considered negative, and a HBeAg concentration < 1 S/CO was considered negative. The detection of HBV DNA was completed using the Sansure Biotech hepatitis B virus nucleic acid quantitative detection kit, and patients were negative for HBV DNA < 10 IU/mL. Serum HBV pgRNA was detected using Hotgen Biotech (Beijing, China), which has a low limit of detection (LLOD) of 100 copies/mL and a linear detection range of 10^3–10^8 copies/mL.[21]

The magnitude of change in HBV pgRNA was calculated as the second HBV pgRNA result minus the baseline HBV pgRNA result, and the value of HBV pgRNA under LLOD was estimated at 50 copies/mL. A decreasing trend in HBV pgRNA levels was defined as a negative change magnitude in HBV pgRNA. The decreasing trend of the HBV DNA levels was calculated in the same way.

FibroTouch Assessment

The FibroTouch (Wuxi HISKY Medical Technologies) liver stiffness measurement (LSM) value and ultrasound attenuation parameter (UAP) were measured for 10 consecutive effective tests, and the median was used as the final result. All operations were completed by a well-trained doctor. The FibroTouch results one month before and after the first HBV pgRNA detection

were included in the analysis. According to Chinese guidelines and the manufacturer's recommendations,[22] the grading criteria for steatosis were normal (UAP≤240 dB/m), mild steatosis (240<UAP≤265 dB/m), moderate steatosis (265<UAP≤295 dB/m), and severe steatosis (UAP>295 dB/m), and the grading criteria for fibrosis were F0–F1 (normal) (LSM<6.7 kPa), F2 (mild hepatic fibrosis) (6.7≤LSM<9.8 kPa), and F3 (progressive hepatic fibrosis) (LSM≥9.8 kPa).

Statistical Analysis

All the statistical analyses were conducted with R software (version 4.0.2, R Development Core Team 2020). Skewed data are presented as medians and interquartile ranges (IQRs). Chi-squared tests for categorical variables and Kruskal–Wallis tests for skewed continuous variables were used to assess group differences. Univariate logistic analysis for positive HBV pgRNA was performed first, and variables with statistically significant results from the univariate analysis were then included in a multivariate logistic model. A decreasing trend in the levels of HBV pgRNA and HBsAg was also detected first by univariate logistic regression and then by multivariate logistic regression. Two-sided p values <0.05 were considered statistically significant in our study.

Results

Clinical Characteristics of Patients

482 patients were included in our analysis at baseline, and Table 1 summarizes their characteristics. The patients had a median age of 47 (38–56) years, 355 (73.7%) were male, 408 (84.6%) were treatment-experienced, 358 (74.3%) were HBV DNA negative, and 370 (76.8%) were HBeAg negative. A total of 158 (32.8%) patients did not have liver fibrosis, 87 (18%) had F2, 55 (11.4%) had F3, 189 (39.2%) had no hepatic steatosis, 50 (10.4%) had mild steatosis, 35 (7.3%) had moderate steatosis, and 26 (5.4%) had severe steatosis (Table 1).

Table 1 Characteristics of HBV-Infected Patients Among Negative HBVRNA and Positive HBVRNA Groups

Variables	Total (n = 482)	Negative HBVRNA (n = 344)	Positive HBVRNA (n = 138)	P
Age, Median (Q1, Q3)	47 (38, 56)	49 (40, 58)	43 (36, 54)	< 0.001
Gender, n (%)				0.037
Male	355 (73.7)	263 (76.5)	92 (66.7)	
Female	127 (26.3)	81 (23.5)	46 (33.3)	
Diagnosis, n (%)				0.881
CHB	370 (76.8)	266 (77.3)	104 (75.4)	
Cirrhosis	91 (18.9)	63 (18.3)	28 (20.3)	
HCC	21 (4.4)	15 (4.4)	6 (4.3)	
BMI, n (%)				0.296
<18.5	12 (2.5)	8 (2.3)	4 (2.9)	
18.5–24	162 (33.6)	109 (31.7)	53 (38.4)	
≥24	123 (25.5)	95 (27.6)	28 (20.3)	
Missing	185 (38.4)	132 (38.4)	53 (38.4)	
LSM (kPa), n (%)				0.981
F0-F1 (<6.7 kPa)	158 (32.8)	111 (32.3)	47 (34.1)	
F2 (6.7–9.8 kPa)	87 (18)	62 (18)	25 (18.1)	
F3 (≥9.8 kPa)	55 (11.4)	40 (11.6)	15 (10.9)	
Missing	182 (37.8)	131 (38.1)	51 (37)	
UAP (dB/m), n (%)				0.484
Normal (≤240)	189 (39.2)	128 (37.2)	61 (44.2)	
Mild steatosis (240–265)	50 (10.4)	39 (11.3)	11 (8)	
Moderate steatosis (265–295)	35 (7.3)	25 (7.3)	10 (7.2)	
Severe steatosis (≥295)	26 (5.4)	21 (6.1)	5 (3.6)	
Missing	182 (37.8)	131 (38.1)	51 (37)	

(*Continued*)

Table 1 (Continued).

Variables	Total (n = 482)	Negative HBVRNA (n = 344)	Positive HBVRNA (n = 138)	P
Treatment, n (%)				0.303
No	74 (15.4)	57 (16.6)	17 (12.3)	
Yes	408 (84.6)	287 (83.4)	121 (87.7)	
HBVDNA, n (%)				< 0.001
Negative	358 (74.3)	280 (81.4)	78 (56.5)	
Positive	124 (25.7)	64 (18.6)	60 (43.5)	
HBsAg (IU/mL), n (%)				< 0.001
0.05–100	124 (25.7)	110 (32)	14 (10.1)	
100–1000	164 (34)	126 (36.6)	38 (27.5)	
≥1000	194 (40.2)	108 (31.4)	86 (62.3)	
HBeAg, n (%)				< 0.001
Negative	370 (76.8)	325 (94.5)	45 (32.6)	
Positive	112 (23.2)	19 (5.5)	93 (67.4)	
ALT, Median (Q1, Q3)	23.5 (17, 36)	22 (17, 35.25)	28 (19, 37.75)	0.011
AST, Median (Q1, Q3)	26 (22, 33)	25 (21, 32)	27 (23, 35)	0.026
ALP, Median (Q1, Q3)	81 (68, 99)	79 (66.75, 97.25)	84.5 (69.25, 101.75)	0.126
HDL, Median (Q1, Q3)	1.12 (0.95, 1.31)	1.12 (0.95, 1.29)	1.12 (0.95, 1.37)	0.675
LDL, Median (Q1, Q3)	2.42 (2.02, 3.01)	2.43 (2.01, 3)	2.4 (2.03, 3.03)	0.853
GGT, Median (Q1, Q3)	25 (18, 43)	26 (18, 42.25)	24 (17, 43)	0.517
eGFR, Median (Q1, Q3)	99 (88, 113)	98 (87, 111)	103 (92, 119)	0.004
TBIL, Median (Q1, Q3)	14.11 (10.48, 18.96)	14.31 (10.99, 19.25)	13.32 (9.77, 18.36)	0.137
DBIL, Median (Q1, Q3)	6 (4.62, 7.74)	6.12 (4.78, 7.9)	5.83 (4.43, 7.49)	0.195
BUN, Median (Q1, Q3)	5 (4.2, 5.9)	5 (4.3, 6)	4.9 (4, 5.7)	0.047
CREA, Median (Q1, Q3)	74 (63, 83)	75 (66, 83)	69 (59, 83)	0.012
URIC, Median (Q1, Q3)	340.6 (274.8, 388.68)	345.2 (281.75, 391.45)	333 (262, 381)	0.144
TG, Median (Q1, Q3)	1.12 (0.81, 1.61)	1.13 (0.83, 1.69)	1.05 (0.75, 1.43)	0.056
TC, Median (Q1, Q3)	4.14 (3.59, 4.71)	4.15 (3.57, 4.7)	4.12 (3.63, 4.73)	0.764

Abbreviations: BMI, Body mass index; UAP, ultrasound attenuation parameter; LSM, Liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GGT, gamma-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; TBIL, total bilirubin; DBIL, direct bilirubin; BUN, blood urea nitrogen; URIC, uric acid; TG, triglyceride; TC, total cholesterol.

There were significant differences in age, gender, HBV DNA status, HBsAg status, HBeAg status, ALT, AST, eGFR, BUN, and CREA between the HBV pgRNA negative and positive groups (P<0.05, Figure 2). However, the two groups had no significant differences in diagnosis, BMI, LSM, UAP, treatment, ALP, HDL, LDL, GGT, TBIL, DBIL, URIC, TG, or TC. Although the positive rate of serum HBV pgRNA was not statistically significant between naïve treatment and treatment groups, the load of serum HBV DNA and HBV pgRNA in the non-treatment group was higher than in the treatment group (P<0.05).

Univariate and Multivariate Logistic Analyses for Positive HBV pgRNA

Among the 482 hBV-infected patients, univariate logistic regression analysis revealed significant differences between positive HBV pgRNA and gender, age, HBV DNA, HBsAg, HBeAg, AST, ALP, and eGFR (P<0.05). Significant variables in univariate logistic regression were included in multivariate logistic regression, and the results showed that positive HBV DNA (AOR: 2.63, 95% CI: 1.46–4.75, P=0.001), ≥1000 hBsAg (AOR: 2.29, 95% CI: 1.08–4.89, P=0.03), positive HBeAg (AOR: 28.26, 95% CI: 15.2–52.55, P<0.001), and ALP (AOR: 1.01, 95% CI: 1.001–1.02, P=0.03) were positively correlated with positive HBV pgRNA (Table 2).

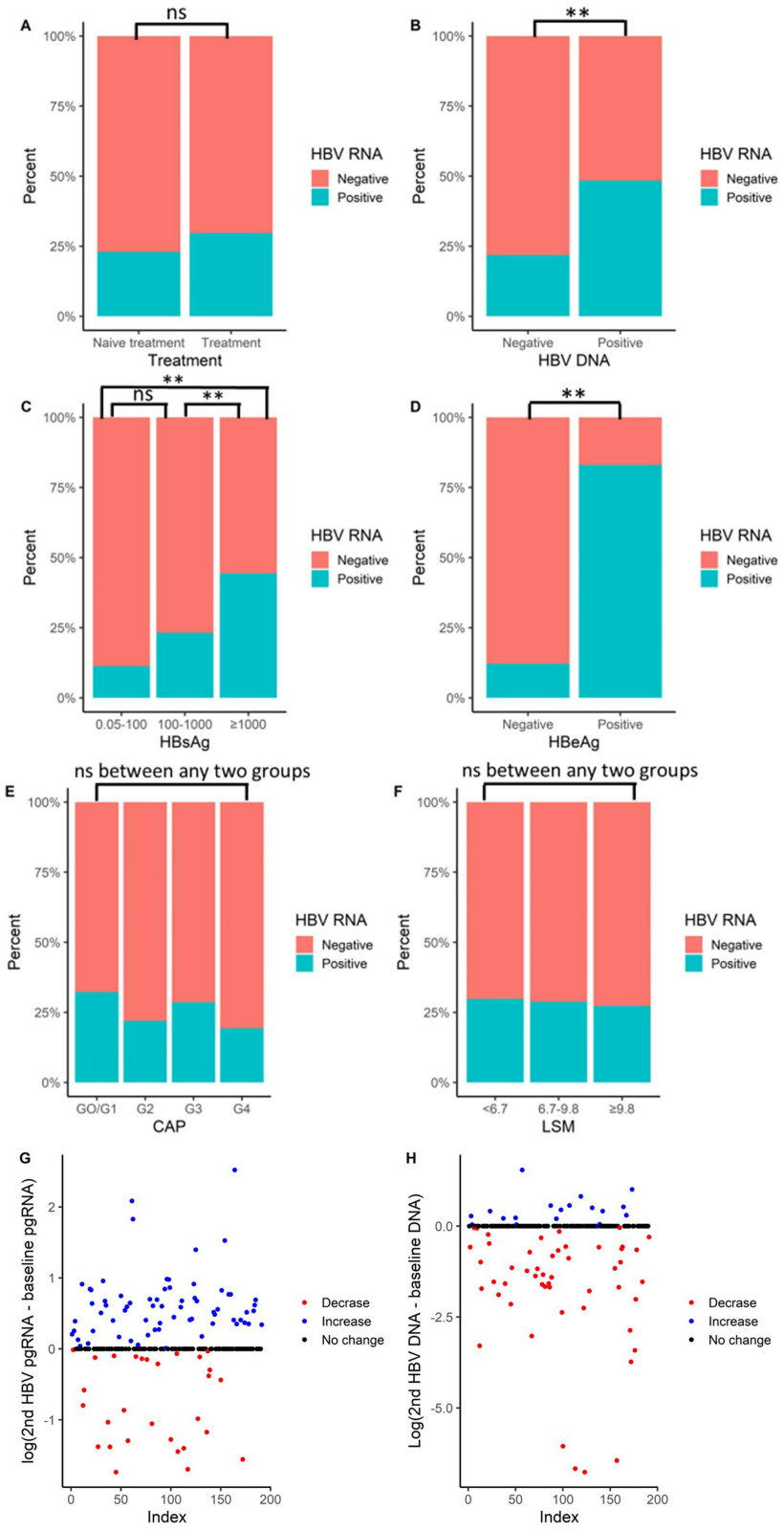

Figure 2 Correlation between HBV RNA and hepatitis B virology, hepatic steatosis, and liver fibrosis among 482 hBV-infected patients. (**A**) Treatment, (**B**) HBV DNA, (**C**) HBsAg, (**D**) HBeAg, (**E**) CAP, (**F**) LSM; (**G**) HBV pgRNA change magnitude; and (**H**) HBV DNA change magnitude. ns: not significant; **P<0.01.

Table 2 Univariate and Multivariate Logistic Regression for Positive HBVRNA Among HBV-Infected Patients

Variables	Univariate			Multivariate		
	OR	95% CI	P	AOR	95% CI	P
Gender						
Male	1.00					
Female	1.62	1.05–2.5	0.028			
Age	0.97	0.95–0.98	<0.001			
Diagnosis						
CHB	1.00					
Cirrhosis	1.14	0.69–1.87	0.62			
HCC	1.02	0.39–2.71	0.96			
BMI, n (%)						
<18.5	1.00					
18.5–24	0.97	0.28–3.37	0.96			
≥24	0.59	0.17–2.10	0.42			
Missing	0.80	0.23–2.78	0.73			
LSM (kPa), n (%)						
F0-F1 (<6.7 kPa)	1.00					
F2 (6.7–9.8 kPa)	0.95	0.54–1.69	0.87			
F3 (≥9.8 kPa)	0.89	0.45–1.76	0.73			
Missing	0.92	0.57–1.47	0.73			
UAP (dB/m), n (%)						
Normal (≤240)	1.00					
Mild steatosis (240–265)	0.59	0.28–1.23	0.16			
Moderate steatosis (265–295)	0.84	0.37–1.86	0.67			
Severe steatosis (≥295)	0.50	0.18–1.39	0.18			
Missing	0.82	0.52–1.27	0.37			
Treatment						
No	1.00					
Yes	1.41	0.79–2.53	0.24			
HBVDNA						
Negative	1.00					
Positive	3.37	2.18–5.19	<0.001	2.63	1.46–4.75	0.001
HBsAg (IU/mL)						
0.05–100	1.00					
100–1000	2.37	1.22–4.60	0.01	1.94	0.89–4.23	0.09
≥1000	6.26	3.35–11.68	<0.001	2.29	1.08–4.89	0.03
HBeAg						
Negative	1.00					
Positive	35.35	19.72–63.37	<0.001	28.26	15.2–52.55	<0.001
ALT	1.01	1–1.01	0.06			
AST	1.01	1–1.02	0.02			
ALP	1.01	1–1.01	0.01	1.01	1.001–1.02	0.03
HDL	0.98	0.51–1.88	0.95			
LDL	1.05	0.8–1.38	0.71			
GGT	1	0.99–1	0.41			
eGFR	1.01	1–1.02	0.002			
TBIL	0.99	0.97–1.02	0.65			
DBIL	1.01	0.98–1.04	0.5			
BUN	1.01	0.9–1.13	0.84			
CREA	1	1–1.01	0.74			

(*Continued*)

Table 2 (Continued).

Variables	Univariate			Multivariate		
	OR	95% CI	P	AOR	95% CI	P
URIC	1	00.99–1.01	0.28			
TG	1.03	0.91–1.17	0.66			
TC	1.09	0.88–1.34	0.43			

Abbreviations: BMI, Body mass index; UAP, ultrasound attenuation parameter; LSM, Liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GGT, gamma-glutamyl transpeptidase; eGFR, estimated glomerular filtration rate; TBIL, total bilirubin; DBIL, direct bilirubin; BUN, blood urea nitrogen; URIC, uric acid; TG, triglyceride; TC, total cholesterol.

Decreasing Trend of HBV pgRNA Levels and Its Associated Factors

482 patients included at baseline were followed up, and 191 patients were successfully included, with a median follow-up time of 147 (115–181) days. Among the 191 patients, 29 (15.2%) patients had a decreasing trend in HBV pgRNA levels, 94 (49.2%) patients had LLOD at baseline and follow-up for HBV pgRNA, and 68 (35.6%) patients had an increasing trend in HBV pgRNA levels (Figure 2G).

A univariate logistic regression model for the decreasing trend of HBV pgRNA levels was constructed, and the significant variables identified by univariate logistic regression were included in the multivariate logistic model. The results showed that positive baseline HBV DNA (AOR: 4.60, 95% CI: 1.84–11.51, P=0.001), baseline HBsAg ≥1000 IU/mL (AOR: 8.74, 95% CI: 1.09–70.10, P=0.04), and HDL (AOR: 5.01, 95% CI: 1.28–19.66, P=0.02) were positively correlated with decreasing trend in HBV pgRNA levels (Table 3).

Decreasing Trend of HBV DNA Levels and Its Associated Factors

Among the 191 follow-up patients, 51 (26.7%) patients had a decreasing trend in HBV DNA, 121 (63.4%) patients had negative results at baseline and follow-up for HBV DNA, and 19 (9.9%) patients had an increasing trend in HBV DNA (Figure 2H).

A univariate logistic regression model for the declining trend of HBV DNA levels was constructed, and the significant variables in the univariate logistic models were included in the multivariate logistic model. The results showed that positive baseline HBV pgRNA (AOR: 3.80, 95% CI: 2.00–8.83, P<0.001) and AST (AOR: 1.06, 95% CI: 1.03–1.08, P<0.001) were positively correlated with a decreasing trend in HBV DNA levels (Table 4).

Table 3 Multivariate Factors Associated with the Decreasing Trend for the Follow-Up HBV RNA Among the HBV-Infected Patients

Variables	Univariate			Multivariate		
	OR	95% CI	P	AOR	95% CI	P
Baseline HBV DNA						
Negative	1.00					
Positive	5.99	2.57–13.97	<0.001	4.60	1.84–11.51	0.001
Baseline HBsAg (IU/mL)						
0.05–100	1.00					
100–1000	3.93	0.46–33.92	0.21	3.97	0.44–35.50	0.22
≥1000	14.43	1.87–111.31	0.01	8.74	1.09–70.10	0.04
HDL	6.13	1.71–21.99	0.005	5.01	1.28–19.66	0.02

Abbreviation: HDL, high-density lipoprotein.

Table 4 Multivariate Factors Associated with the Decreasing Trend for the Follow-Up HBV DNA Among the HBV-Infected Patients

Variables	Univariate			Multivariate		
	OR	95% CI	P	AOR	95% CI	P
Baseline HBV RNA						
Negative	1.00					
Positive	3.62	1.85–7.06	<0.001	3.80	2.00–8.83	<0.001
AST	1.05	1.03–1.08	<0.001	1.06	1.03–1.08	<0.001

Abbreviation: AST, aspartate aminotransferase;

Discussion

482 hBV-infected patients were included at baseline, and 191 patients were successfully followed up in our study. This study aimed to explore the levels of HBV pgRNA and its associated factors in the real world and elucidate the decreasing trends in the levels of HBV pgRNA and HBV DNA and their influencing factors. Our study revealed that HBV DNA, HBsAg, HBeAg, and ALP were significantly correlated with positive HBV pgRNA at baseline. However, hepatic steatosis and liver fibrosis were not significantly different from HBV pgRNA in our study. We also found that baseline HBV DNA, HBsAg, and HDL levels in HBV-infected patients were significantly correlated with a decreasing trend in HBV pgRNA. A decreasing trend in HBV DNA significantly correlated with patients' baseline HBV pgRNA and AST levels.

Positive HBV DNA, positive HBeAg, and high HBsAg level were found to be independent risk factors for positive HBV pgRNA, which is consistent with previous studies.[12,14,15,17,19] Serum HBV DNA comes from the reverse transcription of HBV pgRNA, so a greater amount of HBV DNA also indicates a greater amount of HBV pgRNA.[23] cccDNA can be transcribed into RNA, such as pgRNA, pre-core mRNA, and Pre-S/S mRNA.[23] Pre-core mRNA can directly encode HBeAg, and PreS/S mRNA is the origin of HBsAg. Higher HBeAg and HBsAg can also indicate higher HBV pgRNA. In this study, the proportion of patients who were positive for HBeAg among the HBV pgRNA-positive patients was significantly greater than that among the HBV RNA-negative patients (5.5% vs 67.4%), as were the proportions of patients who were positive for HBV DNA (18.6% vs 43.5%) and HBsAg. Interestingly, we found that ALP, rather than ALT, was significantly correlated with HBV RNA in our analysis. Most of the research that found a positive correlation between HBV RNA and ALT was among treatment-naïve patients,[17,24] which was only 74 (15.4%) in our study, and this positive correlation has not been proven among treatment patients. It can be speculated that in patients with chronic hepatitis B, the high level of HBV pgRNA may be related to the degree of liver inflammation and fibrosis, and liver injury may lead to the release of ALP from the liver to the blood, which may affect the level of ALP. The relationship between HBV DNA and ALP should be explored in future studies.

We found no significant differences in serum HBV pgRNA or hepatic steatosis or liver fibrosis in our study. Interestingly, some studies found a negative relationship between HBV pgRNA and hepatic steatosis or liver fibrosis among HBV-infected patients,[19,24,25] but others found that HBV pgRNA was positively correlated with the histological scores for hepatic steatosis and liver fibrosis among ETV-treated patients[26] and treatment-naïve patients.[27] Future studies are needed to further elucidate the relationships between HBV pgRNA and hepatic steatosis and liver fibrosis, regardless of treatment.

HBV pgRNA can evaluate the therapeutic effect on patients and monitor the transcription activity of cccDNA. Patients with negative HBV RNA or small values are more likely to achieve HBsAg loss. Therefore, it is of clinical significance to observe the characteristics of patients with HBV RNA decline during the follow-up process.We also found that the baseline positive HBV DNA, HBsAg ≥1000 IU/mL, and HDL were significantly correlated with a decreasing trend in the level of HBV pgRNA. One study[28] reported that the decrease in HBV pgRNA can be categorized into a rapid decrease phase and a slow decrease phase. We followed the patients for a median time of 147 days, so a declining trend for HBV pgRNA may occur in the faster phase with relatively high levels of HBV DNA and HBsAg. Although we found that individuals with high levels of HBV DNA and HBsAg were more likely to achieve a decrease in HBV pgRNA,

individuals with low levels of HBV DNA and HBsAg at baseline were more likely to achieve the goal of negative HBV pgRNA.[15,18,28,29] The relationship between HDL and the decreasing trend of HBV pgRNA may be attributed to the anti-inflammatory effect of HDL. Researchers have shown that HDL can bind and neutralize bacterial lipopolysaccharide (LPS) to play an important anti-inflammatory role.[30] There is clear evidence that inflammation significantly changes the structure and function of HDL, which may lead to the pro-inflammatory form of HDL.[31] Therefore, a high HDL level means that the inflammation level is low, and the replication activity of HBV pgRNA is not active, which may be the reason for the rapid decline of HBV pgRNA.

A decreasing trend in HBV DNA was detected, and baseline HBV pgRNA and AST levels were significantly different. From the perspective of the source of HBV DNA, serum HBV DNA comes from the reverse transcription of HBV pgRNA, so a greater amount of HBV DNA also indicates a greater amount of HBV pgRNA.[23] Previous studies[15,32] also revealed that patients with high baseline HBV pgRNA levels were more likely to have a faster decrease in the amount of HBV DNA. We also found that AST was significantly correlated with declining HBV DNA in our study. The flares of aminotransferases anticipate the decrease of HBV DNA which corresponds to liver cell death and reduction of the replication-competent hepatocyte pool.

This study has several limitations. First, we included a single center, Hangzhou Xixi Hospital, which is located in Zhejiang, China, and this study cannot represent the national situation. Future studies should include more hospitals and demonstrate the national level of HBV pgRNA changes and associated factors, especially against the background of expanded treatment of hepatitis B. Second, we found no significant differences in serum HBV pgRNA, hepatic steatosis or liver fibrosis in our study, which may be restricted by the sample size of FibroTouch. Future studies should include more patients infected with HBV via FibroTouch to elucidate the relationship between HBV pgRNA and FibroTouch. Third, other important variables, such as the HBV genotype, HBcrAg, immune status, and HBV BCP/PC mutation status, should be included in future research.

Conclusion

In conclusion, our study revealed that HBV DNA, HBsAg, HBeAg, and ALP were significantly correlated with positive HBV pgRNA at baseline. The baseline HBV DNA, HBsAg, and HDL were significantly correlated with decreasing levels of HBV pgRNA. A decreasing trend of HBV DNA significantly correlated with patients' baseline HBV pgRNA and AST.

Ethics Approval and Consent to Participate

The study protocol followed the ethical standards of the institutional research committee and the ethics guidelines of the 1975 Declaration of Helsinki. The institutional ethics review committee of Hangzhou Xixi Hospital approved the study (Ethics 2024 Research No. 028). Written informed consent was not required due to the retrospective nature of this study. All the data used in this study were anonymized.

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Disclosure

The authors declare that they have no competing interests in this work.

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