Original Article

Elevated Hepatitis B virus RNA levels in hepatocellular carcinoma patients compared to cirrhotic individuals: A propensity score matched analysis

Yuying Wang, Juanli Wu, Yushuang Zhang, Lei Wang, Tao Li

Department of Infectious Disease and Hepatology, The Second Hospital of Shandong University, Jinan, China

Abstract

Background: To delineate the levels of serum Hepatitis B virus (HBV) RNA in patients with HBV-related hepatocellular carcinoma (HCC) and study comparisons with those of individuals afflicted with cirrhosis.

Methods: Adult patients diagnosed with HBV-related cirrhosis or HCC (initial diagnosis) were enrolled in the cross-sectional study. Serum HBV DNA level was quantified through a real-time polymerase chain reaction assay with a lower limit of quantification (LLQ) of 20 IU/ml. Additionally, serum HBV RNA was quantified employing RNA real-time fluorescence thermostatic amplification detection technology with LLQ of 100 copies/ml. Propensity score matching (PSM) was conducted to ensure balance in between-group confounders.

Results: A total of 187 patients (47 with HCC and 140 with cirrhosis) were recruited, among whom 140 (74.9%) had undergone antiviral therapy prior to their inclusion, with varying durations. Serum HBV RNA was detectable in 89.4% of HCC patients at the time of carcinoma diagnosis. After PSM, individuals with HCC exhibited significantly elevated levels of serum HBV DNA and HBV RNA compared to those with cirrhosis (median lgHBV RNA 3.1 vs 2.0 copies/ml, $P = 0.001$). Subgroup analysis, including 38 patients who exhibited ultrasensitive HBV DNA negativity, revealed similar results (median lgHBV RNA 3.0 vs 0.0 copies/ml, $P < 0.001$).

Conclusions: Serum HBV RNA levels were significantly higher in HBV-related HCC patients compared to cirrhotic patients. The presence of serum HBV RNA positivity or elevated levels was associated with the onset of HCC.

Keywords: Cirrhosis, Hepatitis B virus, Hepatitis B virus RNA, hepatocellular carcinoma

Address for correspondence: Dr. Tao Li, No. 247, Beiyuan Street, Jinan City, Shandong Province, China.
E-mail: litaojr@sdu.edu.cn
ORCID ID: 0000-0002-2548-5476

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INTRODUCTION

Hepatocellular carcinoma (HCC) remains a significant disease posing a threat to human health.[1,2] Although nonviral elements, such as metabolic factors, are gaining prominence in hepatocellular carcinogenesis, chronic Hepatitis B virus (HBV) infection remains a major causative agent of HCC, especially in the Asia-Pacific region.[3] The mechanisms underlying HBV-induced HCC are intricate and multifaceted. HBV has the capacity to instigate HCC through the induction of chronic inflammation, integration-induced chromosomal instability within the host, and modulation of cellular pathways

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linked to tumorigenesis, among other pathways.[4-7] Since approximately 2 decades ago, the advent of potent nucleos(t)ide analogs (NAs) has markedly enhanced the prognosis of patients with chronic Hepatitis B (CHB).[8] The control of HBV DNA is a pivotal factor in reducing the risk of HCC, and it stands as a primary antiviral treatment objective recommended by current guidelines.[9-11] However, even among patients with well-controlled HBV DNA, occurrences of HCC can still be observed.[12] Hence, there is an ongoing clinical necessity for novel markers to indicate the risk of HCC development and to identify high-risk populations for HCC screening.

Recently, studies have indicated that the primary form of HBV RNA detected in the serum of CHB patients is the 3.5-kb pregenomic RNA (pgRNA), mainly originating from the direct transcription of covalently closed circular DNA (cccDNA).[13,14] HBV RNA has been recognized as a dependable surrogate for reflecting viral transcription and replication activity within hepatocytes.[8,13] Moreover, it emerges as a novel indicator to evaluate the response to viral control following the attainment of serum HBV DNA negativity in individuals with CHB.[15-17] Meanwhile, research has demonstrated that HBV pgRNA promotes the proliferation, stemness, and tumorigenicity of cells through the pgRNA-IGF2BP3 axis, thereby promoting the development of HBV-associated HCC.[18] Theoretically, elevated levels of serum HBV RNA signify heightened viral replication, which may significantly contribute to the development of HCC and indicate a higher risk of carcinoma incidence in clinical practice.[13,19] However, there have been limited studies delineating the burden of HBV RNA in HCC patients, and the potential relationship between the two remains far from being fully clarified.

In this study, we aimed to delineate the levels of serum HBV RNA in patients with HBV-related HCC and conducted comparisons with those of individuals afflicted with cirrhosis. These findings will offer novel insights into comprehending the correlation between serum HBV RNA and HCC, thus providing valuable leads for future etiological investigations.

PATIENTS AND METHODS

Eligible patients visiting the Second Hospital of Shandong University between October 2022 and July 2023 were enrolled in the cross-sectional study. The inclusion criteria comprised adult patients (age ≥18 years) diagnosed with HBV-related cirrhosis or HCC (initial diagnosis). There were no restrictions on the presence of cirrhosis among HCC patients. The diagnosis of cirrhosis followed one of the following criteria: histological evidence of cirrhosis from liver biopsy, imaging studies indicating cirrhosis and/or portal hypertension, endoscopic findings of gastroesophageal varices, liver stiffness measurements consistent with cirrhosis, or biochemical evidence such as decreased albumin levels (<35 g/L) and/or prolonged prothrombin time by more than 3 s compared to controls. Additionally, a complete blood count showing platelet counts $<100 \times 10^9$/L was also considered, with other causes excluded.[11] The diagnosis of HCC was based on[1] a tumor with a maximum diameter of ≥2 cm showing typical HCC characteristics such as arterial hypervascularity and venous or delayed phase washout on ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI) or[2] nodules of 1–2 cm confirmed by two consistent imaging techniques. The conclusive diagnosis was the result of a thorough evaluation carried out by a seasoned clinician. There were no constraints regarding pre-enrolment long-term NA therapy. Exclusion criteria were set as follows: 1. Patients with additional hepatophilic viral infections; 2. Individuals diagnosed with HBV-associated HCC for over 3 months or experiencing recurrent HCC; 3. Patients with concurrent malignant tumors originating from other organs; 4. Daily alcohol consumption exceeding the equivalent of 40 g of alcohol for males or 20 g for females for a duration of at least 5 years. Demographic data and laboratory examination results of the participants were gathered for subsequent analysis. The study was approved by the hospital's Ethics Committee, and informed consent was acquired from all patients.

Serum samples were collected from patients either during their consultation or on the second day of hospitalization. These samples were meticulously stored at -80°C until the time of testing. The quantification of serum HBV DNA levels was conducted through a real-time polymerase chain reaction (PCR) assay, utilizing the Roche COBAS TaqMan system (Basel, Switzerland, lower limit of quantification [LLQ] 20 IU/ml). Additionally, serum HBV RNA was quantified employing RNA real-time fluorescence thermostatic amplification detection technology (SAT) (Rendu Biotechnology, Shanghai, China, LLQ 100 copies/ml).

Statistical analyses

Continuous variables were presented as either mean ± standard deviation or median [interquartile range (IQR)]. Comparisons between groups were carried out using either the Student *t*-test or the Mann–Whitney U-test, depending on appropriateness. Categorical variables were expressed as counts and percentages. Group comparisons for

categorical variables were performed using the Chi-square test or Fisher's exact test, as suitable for the data. To ensure balance in between-group confounders, propensity score matching (PSM) was conducted with a matching tolerance of 0.05. Statistical significance was established at a *P* value of less than 0.05. The above statistical analyses were conducted using R Statistical Software (Version 4.2.2, http://www.R-project.org, The R Foundation) and Free Statistics analysis platform (Version 1.8, Beijing, China).

RESULTS

Clinical characteristics of the recruited patients

A total of 187 patients meeting the prescribed criteria were recruited for this study. This cohort comprised 47 patients afflicted with HBV-associated HCC and 140 patients diagnosed with Hepatitis B-induced cirrhosis. The median age of the patients was 53.6 years. The majority of the patients were male (140 patients, 74.9%), and a predominant portion of the cases were categorized as Child-Pugh grade A (157 patients, 84.0%). Out of the 187 patients included, 140 (74.9%) had undergone antiviral therapy prior to their inclusion, with varying durations. The antiviral agents employed encompassed tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), entecavir (ETV), and tenofovir amibufenamide (TMF). Additionally, certain patients had previously undergone treatment with adefovir (11 patients), lamivudine (10 patients), and telbivudine (5 patients). The administration of these antiviral agents ranged from a minimum of 3 months to a maximum of 13 years. Presently, all patients have transitioned to first-line antiviral medications. The clinical characteristics of the recruited patients are delineated in Table 1.

The median AFP of the included 47 HCC patients was 112.0 (IQR 9.4–1110.5). The median maximum diameter of the tumor was 4.5 cm (IQR 2.6–10.1 cm). Of the 47 HCC patients, 33 had a single tumor. Furthermore, 34 patients were characterized as having early to middle stage liver cancer [China Liver Cancer Staging (CNLC) stage Ia-IIIa], while 13 cases were classified as advanced HCC (CNLC stage IIIb and IV). We also calculated the AST to Platelet Ratio Index (APRI) to represent the fibrosis stage for the HCC group, with a median score of 0.63 (IQR 0.41–1.26).

Upon enrolment, 31 out of the 47 patients diagnosed with HCC had not previously undergone regular antiviral therapy. Fourteen patients had received first-line antiviral therapy for over a year prior to enrolment, and 11 patients had achieved undetectable serum HBV-DNA. Serum HBV RNA was discernible in 89.4% of HCC patients at the

Table 1: Clinical characteristics of the recruited patients

Variables	Cirrhosis (*n*=140)	HCC (*n*=47)	*P*
Age, Mean±SD	52.5±11.0	56.7±10.7	0.023
Sex, *n* (%)			0.138
male	101 (72.1)	39 (83)	
female	39 (27.9)	8 (17)	
Child-Pugh classification, *n* (%)			0.097
A	122 (87.1)	35 (74.5)	
B	13 (9.3)	8 (17)	
C	5 (3.6)	4 (8.5)	
Antiviral duration, *n* (%)			<0.001
<12 months	35 (25)	33 (70.2)	
≥12 months	105 (75)	14 (29.8)	
HBsAg status, *n* (%)			0.165
<100 IU/ml	48 (34.3)	11 (23.4)	
≥100 IU/ml	92 (65.7)	36 (76.6)	
HBeAg status, *n* (%)			0.078
negative	106 (75.7)	28 (62.2)	
positive	34 (24.3)	17 (37.8)	
APRI, Median (IQR)	0.54 (0.32, 0.94)	0.63 (0.41, 1.26)	0.033
PLT (10^9/L), Median (IQR)	126.0 (76.0, 173.0)	156.0 (96.5, 211.5)	0.059
ALT, Median (IQR)	24.0 (18.0, 33.0)	29.0 (20.0, 38.5)	0.050
GGT, Median (IQR)	28.0 (18.0, 40.2)	69.0 (32.0, 143.0)	<0.001
albumin, Median (IQR)	46.0 (40.9, 48.2)	40.9 (34.0, 44.5)	<0.001
TB, Median (IQR)	18.6 (13.4, 29.7)	18.1 (13.5, 26.1)	0.789
creatinine, Median (IQR)	68.5 (57.0, 79.0)	65.0 (55.0, 72.0)	0.151
INR, Median (IQR)	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.030
lgHBV DNA, Median (IQR)	0.0 (0.0, 1.3)	2.6 (1.5, 4.8)	<0.001
lgHBV RNA, Median (IQR)	2.6 (2.0, 3.5)	3.1 (2.2, 4.5)	0.008

HCC, hepatocellular carcinoma; SD, standard deviation; IQR, interquartile range; HBsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B e antigen; APRI, AST to Platelet Ratio Index; PLT, platelet count; ALT, alanine aminotransferase; GGT, glutamyl transpeptidase; TB, total bilirubin; INR, International normalized ratio; HBV, Hepatitis B virus

juncture of carcinoma diagnosis. In comparison to patients diagnosed with cirrhosis, those with HCC exhibited an advanced age, a shorter duration of antiviral treatment, elevated levels of serum HBV DNA, HBV RNA, and glutamyl transpeptidase (GGT), and diminished levels of albumin and international normalized ratio (INR) [Table 1]. The fibrosis stage of patients in the two groups also differed, as indicated by the varying APRI scores [Table 1].

Comparative analysis of HBV RNA levels in HCC and cirrhotic patients using PSM

PSM was employed to mitigate the impact of confounding variables in both the HCC and cirrhosis groups. The variables used for matching comprised age, gender, duration of uninterrupted antiviral treatment (<12 months vs ≥12 months), HBsAg status (<100 IU/ml vs ≥100 IU/ml), and HBeAg status (positive vs negative). A total of 72 cases (36 pairs) were successfully matched, ensuring comparability of the aforementioned variables between the two groups [Table 2]. The study revealed that individuals with HCC exhibited markedly elevated levels of serum HBV DNA and HBV RNA in comparison to those with cirrhosis. This difference was statistically significant (median 2.4 vs 0.0 IU/ml, $P < 0.001$ for lgHBV DNA, and median 3.1 vs 2.0 copies/ml, $P = 0.001$ for lgHBV RNA) [Table 2 and Figure 1].

Subgroup analysis of matched patients with serum HBV DNA negativity

Out of the 72 successfully matched patients, 38 exhibited ultrasensitive HBV DNA negativity. This group included nine patients diagnosed with HCC and 29 patients diagnosed with cirrhosis. Within the subset of patients achieving undetectable HBV DNA, those in the HCC group demonstrated significantly higher serum HBV RNA levels compared to their counterparts in the cirrhosis group (median lgHBV RNA 3.0 vs 0.0 copies/ml, $P < 0.001$). Notably, there was no statistically significant difference between the two groups concerning age, gender, Child-Pugh classification, duration of antiviral treatment, APRI, HBsAg status, HBeAg status, and HBV DNA, as elaborated in Table 3.

We also performed the receiver operating characteristic curve analysis and determined an area under the receiver operating characteristic curve of 0.881 for the lgHBV RNA level in assessing HCC risk. The identified threshold for lgHBV RNA was 2.19 copies/ml.

DISCUSSION

Upon analyzing serum HBV RNA levels in CHB patients who progressed to HCC and comparing them with those

Table 2: Clinical characteristics in propensity score matching patients

Variables	Cirrhosis (*n*=36)	HCC (*n*=36)	*p*
Age, Mean±SD	55.6±11.9	55.0±10.0	0.798
Sex, *n* (%)			0.173
male	33 (91.7)	29 (80.6)	
female	3 (8.3)	7 (19.4)	
Child-Pugh classification, *n* (%)			0.678
A	31 (86.1)	28 (77.8)	
B	3 (8.3)	5 (13.9)	
C	2 (5.6)	3 (8.3)	
Antiviral duration, *n* (%)			0.633
<12 months	20 (55.6)	22 (61.1)	
≥12 months	16 (44.4)	14 (38.9)	
HBsAg status, *n* (%)			0.083
<100 IU/ml	16 (44.4)	9 (25)	
≥100 IU/ml	20 (55.6)	27 (75)	
HBeAg status, *n* (%)			0.102
negative	30 (83.3)	24 (66.7)	
positive	6 (16.7)	12 (33.3)	
APRI, Median (IQR)	0.58 (0.33, 1.02)	0.66 (0.47, 1.42)	0.398
PLT (10^9/L), Median (IQR)	120.0 (73.5, 156.8)	157.5 (100.5, 198.2)	0.067
ALT, Median (IQR)	21.5 (17.0, 35.6)	29.0 (20.0, 38.0)	0.171
GGT, Median (IQR)	35.5 (26.8, 46.8)	51.5 (29.2, 136.8)	0.028
albumin, Median (IQR)	44.7 (37.9, 47.3)	42.2 (36.2, 45.1)	0.120
TB, Median (IQR)	18.2 (13.2, 30.2)	16.9 (12.6, 26.1)	0.510
creatinine, Median (IQR)	71.0 (61.0, 79.0)	65.5 (55.5, 71.2)	0.087
INR, Median (IQR)	1.1 (1.1, 1.2)	1.1 (1.0, 1.2)	0.098
lgHBV DNA, Median (IQR)	0.0 (0.0, 1.3)	2.4 (1.3, 4.8)	<0.001
lgHBV RNA, Median (IQR)	2.0 (0.0, 2.7)	3.1 (2.2, 4.4)	0.001

HCC, hepatocellular carcinoma; SD, standard deviation; IQR, interquartile range; HBsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B e antigen; APRI, AST to Platelet Ratio Index; PLT, platelet count; ALT, alanine aminotransferase; GGT, glutamyl transpeptidase; TB, total bilirubin; INR, International normalized ratio; HBV, Hepatitis B virus

Table 3: Clinical characteristics of the 38 patients exhibited ultrasensitive HBV DNA negativity in subgroup analysis

Variables	Cirrhosis (*n*=29)	HCC (*n*=9)	*P*
Age, Mean±SD	56.4±12.0	59.3±12.8	0.529
Sex, *n* (%)			0.075
male	27 (93.1)	6 (66.7)	
female	2 (6.9)	3 (33.3)	
Child-Pugh classification, *n* (%)			0.233
A	27 (93.1)	7 (77.8)	
B	2 (6.9)	2 (22.2)	
Antiviral duration, *n* (%)			1.000
<12 months	13 (44.8)	4 (44.4)	
≥12 months	16 (55.2)	5 (55.6)	
HBsAg status, *n* (%)			0.052
<100 IU/ml	15 (51.7)	1 (11.1)	
≥100 IU/ml	14 (48.3)	8 (88.9)	
HBeAg status, *n* (%)			0.322
negative	25 (86.2)	6 (66.7)	
positive	4 (13.8)	3 (33.3)	
APRI, Median (IQR)	0.55 (0.34, 0.96)	0.50 (0.36, 1.23)	0.850
PLT (10^9/L), Median (IQR)	121.0 (74.0, 156.0)	104.0 (90.0, 153.0)	0.837
ALT, Median (IQR)	20.0 (17.0, 34.0)	28.0 (22.0, 29.0)	0.302
GGT, Median (IQR)	33.0 (27.0, 46.0)	30.0 (24.0, 38.0)	0.429
albumin, Median (IQR)	44.6 (40.3, 48.3)	41.9 (38.7, 44.0)	0.198
TB, Median (IQR)	18.6 (13.9, 29.6)	25.1 (15.4, 28.8)	0.959
creatinine, Median (IQR)	71.2 (62.5, 76.8)	66.0 (51.0, 82.0)	0.504
INR, Median (IQR)	1.1 (1.1, 1.2)	1.1 (1.0, 1.3)	0.557
lgHBV RNA, Median (IQR)	0.0 (0.0, 2.0)	3.0 (2.8, 3.8)	<0.001

HCC, hepatocellular carcinoma; SD, standard deviation; IQR, interquartile range; HBsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B e antigen; APRI, AST to Platelet Ratio Index; PLT, platelet count; ALT, alanine aminotransferase; GGT, glutamyl transpeptidase; TB, total bilirubin; INR, International normalized ratio; HBV, Hepatitis B virus

in patients with cirrhosis, the present study revealed that the detection rate and levels of serum HBV RNA were significantly higher in patients with HCC. Specifically, among the 11 patients with HCC who achieved negative serum HBV DNA, a considerable portion—9 patients—still exhibited detectable serum HBV RNA, predominantly at elevated levels, which were markedly higher than those observed within the cirrhosis-afflicted group.

The stable pool of cccDNA within hepatocytes is widely acknowledged as a pivotal element contributing to the challenge of achieving comprehensive viral eradication and the progression of cirrhosis and HCC in patients chronically infected with HBV.[8,20,21] However, the detection of intracellular cccDNA within hepatocytes is constrained by the invasive nature of liver puncture biopsy, rendering it less feasible for widespread adoption in clinical settings.[22] A dependable marker for gauging the transcriptional activity of intracellular cccDNA in hepatocytes is the 3.5 kb HBV pgRNA directly transcribed from cccDNA. In this study, our investigation was centered on serum HBV RNA. Prior investigations have demonstrated that the HBV RNA identified in the serum encompasses viral particle-like entities, truncated pgRNA at the 3'end, various pgRNA splice variants, and HBx transcripts. Notably, pgRNA stands as the predominant constituent in this context.[13,19,23] Consequently, serum HBV RNA holds promise as a novel indicator for gauging the response to viral activity within hepatocytes.

In accordance with the aforementioned mechanisms, the heightened levels of HBV RNA observed in individuals affected by HCC, as delineated in this present study, signify heightened viral replication activity either prior to or concurrent with the onset of carcinoma. Furthermore, this elevation in HBV RNA levels suggests an intensified state of hepatic inflammation and hepatocellular fibrosis, potentially constituting a pivotal etiological factor in the development of HCC. A few precedent studies have delved into the concentrations of HBV RNA within patients afflicted by HCC and cirrhosis. Lin *et al*.[24] conducted a comparative analysis of serum HBV RNA levels in a cohort of 100 cirrhotic patients and 100 HCC patients previously treated with NAs. Their findings indicated an absence of statistically significant disparity between the two groups. Nonetheless, it is imperative to acknowledge the palpable heterogeneity inherent in the two cohorts and the elevated lower limit of detection for serum HBV RNA (established at 1000 copies/ml), potentially obscuring the distinctions in serum HBV RNA levels between these two distinct patient populations. Enhancing upon the aforementioned research, the present study employed PSM methodology to equalize potential confounding variables among patients

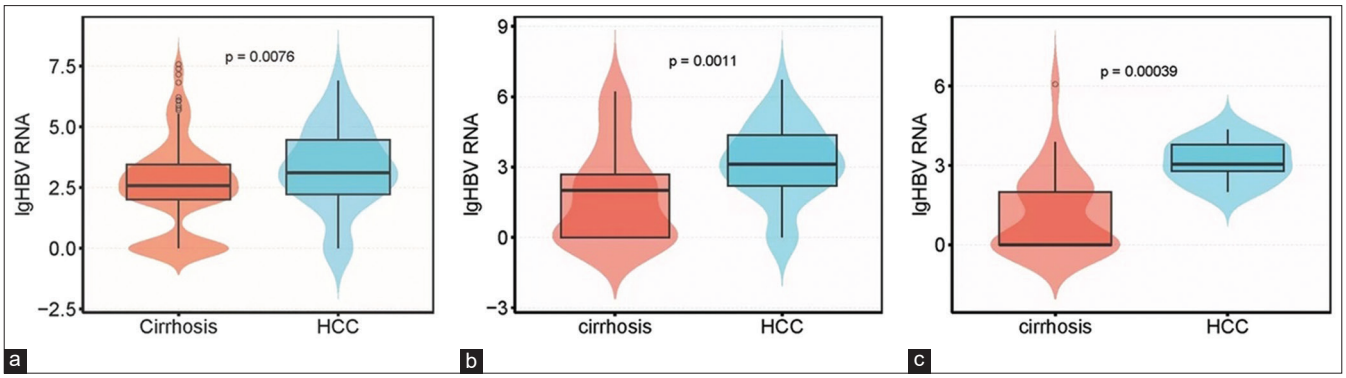

Figure 1: Hepatitis B virus RNA levels in hepatocellular carcinoma patients compared to cirrhotic individuals. a: Comparison of lgHBV RNA before PSM; b: Comparison of lgHBV RNA after PSM; c: Comparison of lgHBV RNA in subgroup analysis

with HCC and cirrhosis, thereby bolstering the credibility of the conclusions.

According to current guidelines, the objective of therapeutic intervention is to achieve optimal and enduring suppression of HBV replication, mitigate liver necroinflammation and fibrosis, and protract and mitigate the incidence of liver failure, decompensation, cirrhosis, HCC, and other associated complications.[9-11] Presently, clinicians encounter considerable challenges in achieving a clinical cure, with maintained virological response being the typically contemplated fundamental treatment objective. However, from the conclusions of this study, it is evident that long-term maintenance of HBV DNA negativity is insufficient in significantly reducing the incidence of HCC. We conducted a subgroup analysis on patients who attained a state of serum HBV DNA negativity, revealing that the majority of individuals with HCC displayed significantly elevated serum HBV RNA levels. Hence, elevated levels of serum HBV RNA persist as a common occurrence, even in cases displaying a complete response in terms of serum HBV DNA, and they maintain an association with the progression of HCC. Consequently, in the administration of antiviral therapy for individuals with CHB, the endeavor toward achieving a complete response in serum HBV RNA becomes imperative. This approach aims to mitigate adverse outcomes in patients with CHB, thereby extending survival and enhancing long-term prognosis. The conclusions of several studies corroborate the aforementioned propositions. In a prospective cohort study involving 2,794 patients with CHB undergoing initial antiviral therapy, the 5-year cumulative incidence of HCC was notably elevated in patients who tested positive for baseline HBV RNA compared to those who tested negative.[25] Additionally, a case-control study encompassing 52 patients with HCC and 52 patients without HCC established an association between serum positivity for both HBV DNA and HBV RNA and the development of HCC.[26]

Moreover, serum HBV RNA exhibits associations not only with the onset of HCC but also with the severity and long-term prognosis of the disease. The quantification of serum HBV RNA levels may facilitate clinical differentiation between high-risk and low-risk liver cancer patients, allowing for refined stratification and improved disease management, ultimately enhancing overall prognosis. In a retrospective cohort study involving 136 patients with Hepatitis B-related HCC who underwent hepatectomy, elevated preoperative serum HBV RNA levels were markedly linked to lower tumor differentiation, heightened liver necroinflammation, fibrosis, and cirrhosis and displayed a strong correlation with delayed tumor recurrence.[18]

Based on the current evidence, we also postulate the existence of a distinct oncogenic mechanism specific to HBV RNA. On one facet, certain investigations have demonstrated that pgRNA can heighten the stemness of tumor cells through its interaction with IGF2BP3, potentially fostering the genesis of HBV-associated HCC. Furthermore, pgRNA serves as a sponge for miR-let-7e-5p, resulting in the upregulation of IGF2BP3 expression at the post-transcriptional level, thereby promoting proliferation, stemness, and tumorigenesis of HCC cells.[18] Additionally, the transcription of cccDNA into HBV RNA necessitates the degradation of Smc5 and Smc6 proteins, pivotal in maintaining chromosome structure and inhibiting transcription from cccDNA to RNA. Hence, the presence of HBV RNA signifies the degradation of Smc5/6, which is intricately linked to DNA repair. Consequently, the accumulation of HBV RNA could potentially instigate carcinogenesis. Furthermore, HBV RNA may potentially instigate the development of HCC by binding to host miRNAs, including miR-122, miR-199a-3p, miR-15a, and let-7 family miRNAs.[27]

In the context of this study, individuals diagnosed with CHB are encouraged to persist in their efforts to attain

HBV RNA negativity, even subsequent to achieving a complete response in serum HBV DNA. This strategic approach aims to mitigate the likelihood of HCC, especially within high-risk groups such as patients with cirrhosis. In clinical practice, individuals demonstrating persistent or elevated levels of serum HBV RNA should exercise heightened vigilance, warranting an increase in the frequency of HCC screenings to fortify defenses against this malignancy. Patients with sustained elevation in serum HBV RNA levels may benefit from a treatment regimen involving a combination of two NAs or adjunct interferon therapy. Moreover, emerging antiviral agents targeting the HBV RNA production pathway, such as core inhibitors or capsid assembly modifiers (CAMs), as well as drugs directed at cccDNA, hold promise for this demographic and merit further in-depth investigation.[28,29]

Our study offers several notable strengths. Primarily, we analyzed serum HBV RNA levels at the onset of HCC in CHB patients undergoing new first-line NA therapy. This approach contrasts with most other studies that predominantly relied on previously archived sera. Second, our study was conducted precisely at the point of HCC development, enabling us to demonstrate that patients with a gradual progression of carcinoma still display elevated serum HBV RNA levels during this critical period. This discovery not only addresses a research gap but also furnishes novel evidence.

Nonetheless, this study has its limitations. First, due to practical constraints, HCC cannot always be detected and diagnosed at the initial presentation. Consequently, we relied on the serum HBV RNA level at the time of carcinoma detection, which may not fully represent the serum HBV RNA level at the actual onset of the disease. Second, employing a cross-sectional design, the study could not provide sufficient etiological insights into the association between serum HBV RNA and HCC. Third, the small sample size hindered a more detailed analysis between various stages of HCC and different pathological types with distinct degrees of differentiation. Fourth, we used only the APRI to represent the patients' fibrosis stage. We did not use other noninvasive fibrosis markers (e.g. transient elastography) or pathological verification, which means we cannot entirely exclude the impact of the absence of cirrhosis on HBV RNA levels between the HCC and cirrhosis groups. Prospective cohort studies involving larger samples are necessary to gain more comprehensive insights.

In conclusion, serum HBV RNA levels were significantly higher in HBV-related HCC patients compared to cirrhotic patients. The presence of serum HBV RNA positivity or elevated levels was associated with the onset of HCC. Thus, reducing the serum HBV RNA level or achieving a negative status holds promise in providing significant benefits for the long-term prognosis of these patients.

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Conflicts of interest

There are no conflicts of interest.

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