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SHORT COMMUNICATION

Elevated hepatitis B virus RNA levels in HBeAg-positive patients with low-level viraemia or previous low-level viraemia

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Abstract

The understanding of viral transcription and replication activity in HBeAg-positive chronic hepatitis B (CHB) patients with low-level viraemia (LLV) or previous low-level viraemia (pre-LLV) remains unclear. Our aim was to evaluate and compare circulating hepatitis B virus (HBV) RNA levels in these patient groups with those achieving maintained virological response (MVR). This cross-sectional study included 147 patients: 43 in the LLV group, 25 in the pre-LLV group and 79 in the MVR group. Serum HBV RNA levels were assessed using specific RNA target capture combined with simultaneous amplification and testing method. Propensity score matching (PSM) was used to balance baseline characteristics between groups. Median HBV RNA levels were 6.9 copies/mL in the LLV group, 6.1 copies/mL in the pre-LLV group and 3.8 copies/mL in the MVR group. After PSM, significantly higher HBV RNA levels were observed in the LLV group compared to the MVR group ($p < .001$), and the pre-LLV group also showed higher HBV RNA levels than the MVR group ($p < .001$). Both LLV and pre-LLV HBeAg-positive CHB patients exhibited elevated circulating HBV RNA levels compared to those achieving MVR.

KEYWORDS
chronic hepatitis B, hepatitis B virus RNA, low-level viraemia, maintained virological response

1 | INTRODUCTION

Low-level viraemia (LLV) indicates a partial control of hepatitis B virus (HBV) and is associated with a less favourable prognosis.[1–3] Addressing strategies to mitigate LLV and effectively manage patients with LLV undergoing first-line nucleos(t)ide analogues (NAs) holds paramount clinical significance. However, discrepancies in guideline recommendations persist due to limited clinical evidence availability. Conducting a prospective trial to assess long-term outcomes in LLV patients presents significant feasibility challenges, necessitating urgent identification of laboratory markers that can effectively demonstrate intervention benefits in individuals with LLV and those with a history of prior low-level viraemia (pre-LLV patients) who have now achieved complete viral suppression.

Recently, circulating HBV RNA, a transcriptional product of covalently closed circular DNA (cccDNA), has emerged as a reliable laboratory indicator reflecting virus transcription and replication activity. This study aimed to investigate whether higher levels of circulating HBV RNA are observed in hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients with LLV or pre-LLV, compared to those achieving maintained virological response (MVR).

Abbreviations: cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; IQR, interquartile range; LLQ, lower limit of quantification; LLV, low-level viraemia; MVR, maintained virological response; NAs, nucleos(t)ide analogues; PCR, polymerase chain reaction; pgRNA, pregenomic RNA; pre-LLV, previous low-level viraemia; PSM, propensity score matching; S29, ribosomal protein S29; scWAT, subcutaneous white adipose tissue; TAC, transverse aortic constriction; TAF, tenofovir alafenamide fumarate

Tao Li and Yan Chen contributed equally to this work

2 | PATIENTS AND METHODS

A cross-sectional study was conducted at the Second Hospital of Shandong University between 1 April 2023 and 31 August 2023. HBeAg-positive CHB patients who had been treated with NAs for more than 48 weeks with good adherence to medical treatment were included. Patients co-infected with hepatitis C virus or human immunodeficiency virus and those with decompensated clinical status were excluded. The study procedures adhered to the principles outlined in the Helsinki Declaration and received approval from the Ethical Committee of the Hospital (No. KYLL-2023-431). Informed consent was obtained from all participants.

Patients were categorized into three groups: LLV, pre-LLV and MVR. LLV was defined as having at least two episodes of serum HBV DNA levels between 20 and 2000 IU/mL in CHB patients undergoing first-line NAs (entecavir, tenofovir or tenofovir alafenamide fumarate [TAF]) treatment for a minimum of 48 weeks. Pre-LLV referred to a history of LLV in CHB patients who subsequently achieved undetectable serum HBV DNA (<20 IU/mL) at the time of enrolment. MVR was defined as reaching undetectable serum HBV DNA (<20 IU/mL) after using first-line NAs for less than 48 weeks, with sustained viral suppression during follow-up.

Serum HBV DNA was determined by real-time polymerase chain reaction (PCR) assay using Roche COBAS TaqMan (Basel, Switzerland, lower limit of quantification [LLQ] 20 IU/mL). Serum HBV RNA levels were assessed using a specific RNA target capture coupled with simultaneous amplification and testing method (Rendu Biotechnology, Shanghai, China, LLQ 100 copies/mL). The detailed procedure of SAT refers to the description of a previous study.[4] Propensity score matching (PSM) analyses were applied to minimize the impact of potential confounding factors between the MVR group and the LLV or pre-LLV groups. The match tolerance between two participants was set as 0.2. Covariates used for PSM included age, gender, duration of antiviral therapy, strategies of antiviral therapy, HBsAg and HBeAg. Statistical analyses were performed using IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA).

3 | RESULTS

A total of 147 HBeAg-positive CHB patients were enrolled, including 43 patients in the LLV group, 25 patients in the pre-LLV group and 79 patients in the MVR group. The mean age of the patients was 46.5 years, with a majority being male (94 patients, 63.9%). Patients received treatment with entecavir, tenofovir or TAF for a median duration of 58.0 months (interquartile range [IQR] 33.0–84.0). Among these, 36 patients, primarily from the LLV and pre-LLV groups, were administered a combination of entecavir and tenofovir/TAF. Additionally, 40 patients, with single application of entecavir exceeding half of the total antiviral duration, were categorized as the entecavir group, while the remaining 107 patients were classified as the tenofovir/TAF group. The median HBV RNA levels in the three groups were as follows: 6.9 copies/mL in the LLV group, 6.1 copies/mL in the pre-LLV group and 3.8 copies/mL in the MVR group (Table 1).

When compared to the MVR group, patients in the LLV group exhibited a shorter duration of antiviral therapy and higher levels of HBsAg, HBeAg quantification and HBV RNA. Similarly, patients in the pre-LLV group also showed elevated levels of HBeAg quantification and HBV RNA (Table 1). PSM analyses were conducted to align the baseline characteristics between groups. In the MVR versus LLV comparison, PSM resulted in 21 matched pairs, showing comparable confounding variables such as age, gender, antiviral therapy duration, treatment strategies, HBsAg and HBeAg between the two groups. Notably, the HBV RNA levels were significantly higher in the LLV group compared to the MVR group ($p < .001$). A similar analysis between the MVR and pre-LLV groups using PSM produced 22 matched pairs, indicating similar confounding factors while observing significantly higher HBV RNA levels in the pre-LLV group compared to the MVR group ($p < .001$).

A sensitivity analysis was conducted by excluding participants with liver cirrhosis. Similarly, in comparison to the MVR group, patients in both the LLV group and pre-LLV group were characterized with analogous primary confounding factors after PSM and presented notably elevated levels of HBV RNA.

4 | DISCUSSION

The current study has elucidated the presence of elevated circulating HBV RNA levels in HBeAg-positive CHB patients with LLV or pre-LLV when compared to those achieving MVR. This observation signifies heightened viral transcription and replication activity in LLV patients and has laid the foundation for the evaluation of interventions. To the best of our knowledge, this study represents the inaugural investigation involving HBV RNA levels in CHB patients with a previous history of LLV.

Viral replication plays a crucial role in the progression of chronic liver disease. Effective suppression of HBV has proven highly beneficial in halting disease progression, reducing the risk of hepatic decompensation and HCC.[5] However, due to the competitive inhibition mechanism of NAs on HBV DNA replication, incomplete viral suppression, defined as LLV or partial virological response, offsets some clinical benefits.

Prior studies have highlighted an increased risk of liver-related adverse events and/or HCC in CHB patients with LLV.[2,3] However, conflicting findings exist regarding the predictive value of LLV for adverse outcomes, with some studies suggesting no significant association when patients exhibit good treatment adherence.[6] Furthermore, there is a lack of research concerning the prognosis of patients with pre-LLV.

The core element of HBV infection, cccDNA, serves as a template for pregenomic RNA (pgRNA) production. PgRNA, in turn, contributes to the generation of virus-like particles and is detectable in

TABLE 1 Clinical characteristics of the enrolled patients.

Variables	Total ($n=147$)	MVR ($n=79$)	LLV ($n=43$)	p^*	Pre-LLV ($n=25$)	p^{**}
Age, years	46.5 ± 10.9	46.9 ± 10.1	45.3 ± 12.6	.440	47.2 ± 10.4	.920
Sex, *n* (%)				.397		.140
Female	53 (36.1)	28 (35.4)	12 (27.9)		13 (52.0)	
Male	94 (63.9)	51 (64.6)	31 (72.1)		12 (48.0)	
Antiviral strategies, *n* (%)				.667		.797
Entecavir	40 (27.2)	21 (26.6)	13 (30.2)		6 (24.0)	
TFV	107 (72.8)	58 (73.4)	30 (69.8)		19 (76.0)	
Cirrhosis, *n* (%)				.626		1.000
No	123 (83.7)	67 (84.8)	35 (81.4)		21 (84.0)	
Yes	24 (16.3)	12 (15.2)	8 (18.6)		4 (16.0)	
Antiviral duration, months	58.0 (33.0, 84.0)	70.0 (43.0, 102.0)	35.0 (20.0, 60.0)	<.001	63.0 (34.5, 71.5)	.067
ALT, U/L	21.0 (15.0, 30.0)	20.0 (14.0, 27.0)	24.0 (16.0, 35.0)	.012	20.0 (13.5, 32.5)	.900
AST, U/L	20.0 (17.0, 24.0)	20.0 (17.0, 23.0)	22.0 (17.0, 26.0)	.096	18.0 (16.0, 23.5)	.262
TBIL, μmol/L	13.6 (9.8, 18.1)	12.4 (9.3, 17.7)	13.6 (10.3, 18.0)	.266	14.1 (10.2, 22.0)	.235
HBeAg, S/Co	18.3 (5.2, 221.3)	6.5 (3.2, 16.7)	342.3 (85.2, 777.3)	<.001	81.8 (17.8, 293.1)	<.001
HBsAg, IU/mL	3175.8 (1241.0, 7261.7)	2657.7 (1174.9, 5068.6)	6772.7 (2999.1, 20035.1)	<.001	4929.4 (1059.4, 7652.2)	.116
lgHBV DNA, IU/mL	0.0 (0.0, 1.4)	0.0 (0.0, 0.0)	1.8 (1.4, 2.1)	<.001	0.0 (0.0, 0.0)	1.000
lgHBV RNA, copies/mL	5.0 (3.6, 6.5)	3.8 (3.1, 4.6)	6.9 (6.2, 7.2)	<.001	6.1 (5.5, 6.6)	<.001

Note: Continuous variables were presented as either mean ± standard deviation (SD) or median and interquartile range.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B Virus; LLV, low-level viraemia; MVR, maintained virological response; pre-LLV, a history of previous low-level viremia; TBIL, total bilirubin; TFV, tenofovir or tenofovir alafenamide fumarate.

*p values between the MVR versus LLV groups. **p values between the MVR versus pre-LLV groups.

blood serum as 'circulating HBV RNA'.[7] Therefore, HBV RNA levels directly reflect the dynamic activity of the virus. Our study revealed significantly elevated HBV RNA levels in LLV and pre-LLV patients compared to those with MVR, highlighting heightened viral activity in these patients.

Despite garnering attention, the optimal management approach for LLV patients remains undetermined. Current strategies, including NAs add-on therapy, switching to another potent NAs, or increase of entecavir dosage, primarily focus on enhancing competitive inhibition of HBV DNA replication without directly affecting HBV RNA levels.[8] Interferon therapy may emerge as a promising tool for suppressing circulating HBV RNA. Assessing the impact of various intervention approaches on HBV RNA is crucial due to the scarcity of evidence regarding the long-term prognosis of LLV patients post-intervention.

It is important to note that the cross-sectional study design and limited sample size are significant limitations of this study. In conclusion, both HBeAg-positive CHB patients with LLV and pre-LLV exhibit elevated circulating HBV RNA levels, indicating heightened viral activity and a potential risk of disease progression in these patient cohorts.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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