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Original Article

Clinical implications of serum hepatitis B virus RNA quantitation in untreated chronic hepatitis B virus-infected patients

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Abstract: Serum hepatitis B virus (HBV) RNA quantitation may be useful for managing untreated chronic HBV-infected patients, but its distribution characteristics and relationship to HBV DNA are unclear. A retrospective cohort including 149 untreated HBV-infected patients was divided into four clinical phenotypes: hepatitis B envelope antigen (HBeAg) positive with normal alanine transaminase (ALT; EPNA) or with elevated ALT (EPEA), HBeAg-negative with normal ALT (ENNA) or with elevated ALT (ENEA). Serum HBV RNA levels were quantified by a high-sensitivity real-time fluorescent quantitative PCR method and liver biopsy was performed in those with undetectable serum HBV DNA or RNA. The detectable serum HBV RNA levels (log_{10} copies/mL) in EPNA, EPEA, ENNA, and ENEA were 6.02±1.48, 6.54±1.27, 2.51±0.78 and 3.54±1.25, respectively. The low level (< 2.0 log_{10} copies/mL) comprised mainly of ENNA phenotype (76.9%), while the high level (> 6.0 log_{10} copies/mL) was HBeAg-positive patients (98.1%). Serum HBV RNA level were significantly correlated with serum HBV DNA and HBsAg in HBeAg-positive phenotypes, but a correlation only with HBV DNA was observed in ENEA patients. Serum HBV DNA and RNA were both independent risk factors associated with elevated ALT in HBeAg-negative patients. Seven serum HBV DNA-undetectable but RNA-detectable patients underwent liver biopsy, showing moderate or severe liver inflammation. Varying serum HBV RNA levels can reflect natural disease phases in untreated HBV-infected patients, indicating that this biomarker could reflect liver inflammation in untreated HBeAg-negative patients as successfully as serum HBV DNA. Serum HBV RNA can complement clinical management strategies when serum HBV DNA is undetectable.

Keywords: HBV RNA, untreated, HBeAg negative, liver inflammation, antiviral initiation

Introduction

Chronic hepatitis B virus (HBV) infection is a global public health problem with an estimated 240 million infections worldwide [1]. In the natural course of chronic HBV infection, prognosis is associated with the dynamic interaction between the host immune response and HBV replication [2]. In untreated European and Asian patients, spontaneous hepatitis B surface antigen (HBsAg) seroclearance can occur, but with annual seroclearance rates of only 0.72% and 0.07%, respectively [3]. Thus, untreated chronic HBV-infected individuals can usually be divided into four main clinical phenotypes: hepatitis B envelope antigen (HBeAg) positive with normal alanine transaminase (ALT; EPNA), HBeAg-positive with elevated ALT (EPEA), HBeAg-negative with normal ALT (ENNA), and HBeAg-negative with elevated ALT (ENEA). It is generally believed that persistent intrahepatic covalently closed circular DNA (cccDNA) is the root cause of chronic infection, HBV replication re-activation, and liver inflammation [4, 5], thus causing the dynamic range of disease prognosis. However, detection of cccDNA relies on invasive liver biopsy. In clinical practice, liver biopsy cannot be widely per-

formed due to the potential for complications in many patients [6]. Thus, a reliable surrogate biomarker that permits long-term monitoring, which is non-invasive and can ideally be measured in the serum, is highly desirable.

Previous studies have confirmed that serum hepatitis B surface antigen (HBsAg) quantitation can reflect the transcription activity of cccDNA [7]; however, the source of HBsAg is not only from cccDNA, but also from viral DNA integrated in the host genome [8]. Therefore, detection of HBsAg for monitoring cccDNA is limited. Although serum HBV DNA can also indicate cccDNA activity [9], nucleos(t)ide analogues (NAs) and/or pegylated-interferon (Peg-IFN) treatment can suppress production of serum HBV DNA to below the lower limit of detection (LLoD) [10, 11], thus rendering serum HBV DNA useless for monitoring cccDNA transcription activity.

Unlike of HBsAg, serum HBV RNA, which mostly consists of 3.5-kb pre-genomic RNA (pgRNA) [12], is only derived from cccDNA transcription. Furthermore, compared with serum HBV DNA, serum HBV RNA cannot be directly suppressed by NAs and can be detected over a longer time during the consecutive antiviral therapies [13]. Based on the characteristics and superiority of serum HBV RNA, recent studies have shown that its circulating and dynamically changing levels are useful for monitoring and predicting curative effects of NAs [13-15] and/or Peg-IFN [16, 17] treatment, as well as the viral rebound [18] and safe drug withdrawal [19]. However, there is a lack of baseline serum RNA distribution data in treatment-naïve chronic HBV infected patients. In the majority of untreated chronic HBV-infected patients, serum HBV DNA can be detected in sera at various levels [20]. Thus, the relationship between serum HBV DNA and RNA, both of which can act as HBV-related virological biomarkers, and the differences in clinical implications remain unclear.

Therefore, in this study, we used a commercial high-sensitivity real-time fluorescent quantitative PCR (qPCR) method to quantify serum HBV RNA and assessed merits of clinical implications in the untreated chronic HBV-infected individuals.

Patients and methods

Patients

We retrospectively analyzed 149 well-documented, treatment-naïve individuals attending Southwest Hospital, Chongqing, China.

The inclusion criteria were: a) HBsAg positive > 6 months; b) HBV monoinfection; c) Normal ALT status followed up ≥12 months at least two time points per year after the first tested time point; d) Serum HBeAg or anti-HBe mono-positive. The exclusion criteria were: a) previously or currently receiving antiviral treatment; b) pregnancy; c) co-infection with another hepatitis virus or HIV; d) the presence of another liver disease (autoimmune, alcoholic, drug, or metabolic liver disease); e) liver transplantation; and f) cirrhosis or hepatocellular carcinoma.

The study protocol was approved by the Ethical Committee of Southwest Hospital and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Liver biopsy

Needle liver biopsies were performed by skilled infectious disease physicians under ultrasonographic guidance in 21 particular nucleic acid detection pattern patients, which were defined as patients with undetectable levels of at least one type of HBV-related nucleic acid. Liver inflammation grade (G) and fibrosis stage (S) were scored independently by two experienced histopathologists, who were blinded to the clinical information, using the Scheuer scoring system [21]. If they failed to reach a consensus, a third histopathologist reviewed the material and made a conclusion.

Standard laboratory assessments

Serum HBV DNA was quantified using the Hepatitis B Virus DNA PCR-Fluorescence Quantitiative Kit (Fosun Long March Medical Science Co., Ltd, Shanghai, China), with a detection range of 5×10^1 IU/mL-1×10^8 IU/mL. For statistical analysis, a result below the LLoD was replaced by a nominal value of 25 IU/mL. An automatic biochemical analyzer was used to test the biochemical indicators, with ALT upper limit of normal (ULN) of 40 U/L. HBsAg, anti-

Table 1. Clinical characteristics of 149 untreated chronic HBV-infected patients

	HBeAg-positive		HBeAg-negative		*P* value
	Normal ALT (EPNA, n=26)	Elevated ALT (EPEA, n=51)	Normal ALT (ENNA, n=32)	Elevated ALT (ENEA, n=40)	
Age (years)	31±11 (14-57)	35±10 (16-57)	36±12 (18-64)	40±9 (24-65)	.005
Gender (M/F)	15/11	35/16	18/14	27/13	/
HBV-DNA (log_{10} IU/mL)	6.40±1.55 (3.48-8.49)	6.89±1.20 (3.56-8.63)	3.44±0.54 (2.30-4.24)[a]	4.87±1.16 (2.56-7.23)[b]	2.215×10^{-19}
HBV-RNA (log_{10} copies/mL)	6.02±1.48 (3.11-7.77)	6.54±1.27 (2.36-8.69)	2.51±0.78 (1.79-4.35)[c]	3.54±1.25 (1.85-6.89)[d]	2.293×10^{-21}
HBsAg (log_{10} IU/mL)	4.10±0.91 (1.71-5.54)	4.20±0.69 (2.35-5.30)	2.70±1.08 (-0.47-4.02)	3.16±0.78 (0.32-4.26)	8.884×10^{-12}
HBeAg (log_{10} s/co)	3.11[e] (0.74-3.21)	3.07[e] (1.94-3.17)	/	/	/
ALT (U/L)	28.8±9.4 (11-39.7)	68.9[e] (53.3-136.7)	27.8±8.3 (10.7-39.8)	56.6[e] (45.6-69.5)	2.653×10^{-23}

All expression data were based on detectable levels. HBeAg, hepatitis B envelope antigen; ALT, alanine transaminase; EPNA, HBeAg-positive with normal ALT; EPEA, HBeAg-positive with elevated ALT; ENNA, HBeAg-negative with normal ALT; ENEA, HBeAg-negative with elevated ALT; M, male; F, female; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; LLoD, lower limit of detection. a. Levels were below LLoD in 10 patients. b. Levels were below LLoD in 3 patients. c. Levels were below LLoD in 11 patients. d. Levels were below LLoD in 3 patients. e. Median (IQR).

HBs quantitation, HBeAg, anti-HBe, and anti-HBc were detected using the Architect i2000SR System (Abbott Laboratories Diagnostics Division, Abbott Park, USA).

Methods for serum HBV RNA quantification

Serum HBV RNA was extracted from 600 μL serum and detected at Guangzhou SupBio Biotechnology and Science Co., Ltd labs by PCR-fluorescent probing with a HBV Pregenomic RNA Detection Kit (SUPBIO, #SUPI-0208, Guangzhou, China). The linear range was 5×10^1 copies/mL-1×10^8 copies/mL, with a LLoD of 15 copies/mL. When the results were within the range of 15-50 copies/mL, testing was repeated to calculate an average; if results were below the LLoD after repetition, we then defined the serum HBV RNA in that sample as undetectable and the value was replaced by a nominal value of 15 copies/mL for statistical analysis. The Supbio-lab was blinded to any clinical information except serum from chronic HBV-infected individuals.

Statistical analysis

Continuous data were expressed as mean ± SD (range) if normally distributed and as median (interquartile range, IQR) if not normally distributed. Pearson's or Spearman's correlations, the chi-square test, Student's t-test, the Mann-Whitney U test, and logistic regression analysis were carried out as appropriate. All significance testing was two-sided and $P < 0.05$ was considered significant. SPSS v.18.0 (IBM, Inc., Armonk, NY, USA) was used for the analyses.

Results

Baseline characteristics

In total, 149 individuals were definitively classified into four clinical phenotypes EPNA, EPEA, ENNA and ENEA in this study. Demographic data and baseline clinical characteristics are shown in **Table 1**. Age in the ENEA group was significantly higher than the other groups (P=0.005), while HBV DNA and HBsAg levels in the HBeAg-positive groups were significantly higher than those in the HBeAg-negative groups ($P=2.215\times10^{-19}$ and 8.884×10^{-12}, respectively). The median (IQR) ALT levels in HBeAg-positive and HBeAg-negative groups were 68.9 U/L (53.3-136.7) and 56.6 U/L (45.6-69.5) (P= 0.009).

Distribution characteristics of serum HBV RNA levels across various clinical phenotypes

There were significant differences in serum HBV RNA levels distribution across the four clinical phenotypes. Serum HBV RNA levels in HBeAg-positive patients were significantly higher than those in HBeAg-negative cases (6.37± 1.36 *VS.* 2.81±1.31, $P=1.196\times10^{-34}$). As shown in **Figure 1A**, the average of serum HBV RNA levels in EPNA and EPEA patients were 6.02±1.48 and 6.54±1.27, respectively, compared with detectable serum HBV RNA levels of 2.51±0.78, 3.54±1.25 in ENNA and ENEA patients, respectively. However, in HBeAg-negative patients, serum HBV RNA levels in the ENEA group were significantly higher than those in the ENNA groups ($P=2.261\times10^{-5}$), but no significant difference was observed between the EPNA and EPEA groups (P=0.143).

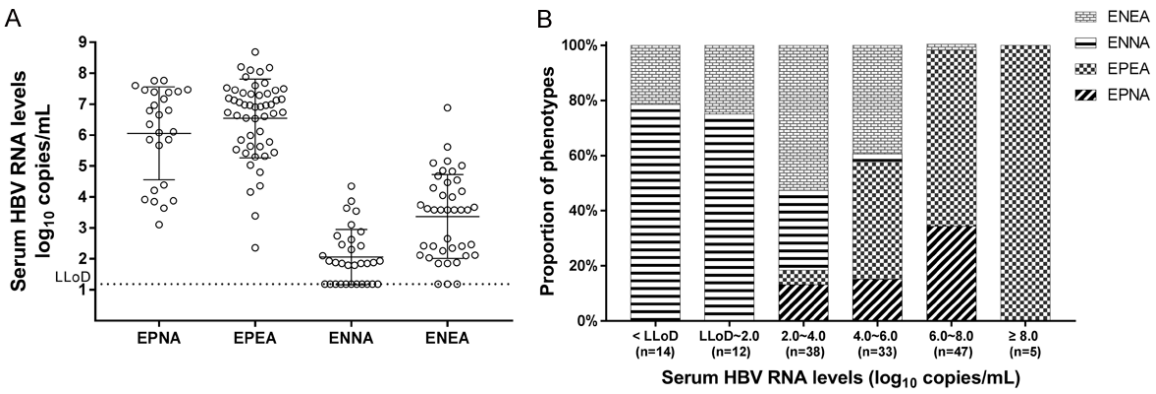

Figure 1. Distribution characteristics of serum HBV RNA levels across various clinical phenotypes. A. Serum HBV RNA levels in various clinical phenotypes. EPNA *vs.* EPEA, ENNA, ENEA, P=0.143, 1.475×10^{-14} and 2.276×10^{-10}, respectively; EPEA *vs.* ENNA, ENEA, $P=3.906\times10^{-29}$ and 2.712×10^{-19}, respectively; ENNA *vs.* ENEA, $P=2.261\times10^{-5}$. B. The proportion of phenotypes in serial serum HBV RNA levels gradients. ENNA phenotype mainly comprised the low level (< 2.0 $\log_{10}$ copies/mL, n=26) serum HBV RNA gradients (20/26, 76.9%); HBeAg-positive phenotypes (EPNA and EPEA) mainly comprised the high level (> 6.0 $\log_{10}$ copies/mL, n=52) serum HBV RNA gradients (51/52, 98.1%). EPNA, HBeAg-positive with normal ALT; EPEA, HBeAg-positive with elevated ALT; ENNA, HBeAg-negative with normal ALT; ENEA: HBeAg-negative with elevated ALT. LLoD, lower limit of detection.

Next, stratified analysis was performed and the proportion of phenotypes in serial serum HBV RNA levels gradients are shown in **Figure 1B**. There was an interesting proration of clinical phenotypes according to the serum HBV RNA gradients. Although the LLoD of serum HBV RNA detection was as low as possible, 19.4% (14/72) HBeAg-negative patients were still below the LLoD, while all HBeAg-positive patients demonstrated positive detection of HBV RNA in peripheral blood. In fact, all cases with low serum HBV RNA levels (< 2.0 $\log_{10}$ copies/mL, including < LLoD and LLoD~2.0 $\log_{10}$ copies/mL, n=26) were for HBeAg-negative patients, mainly of ENNA phenotype (20/26; 76.9%) followed by ENEA phenotype (6/26; 23.1%; $P=1.032\times10^{-4}$). The majority of patients with high serum HBV RNA levels (> 6.0 $\log_{10}$ copies/mL, n=52) were for HBeAg-positive cases (51/52; 98.1%), including both EPNA and EPEA phenotypes, with the exception of only one ENEA patient in this gradient.

Correlations between HBV RNA, HBV DNA and HBsAg

Based on the significant differences in serum HBV RNA distribution between HBeAg-positive and HBeAg-negative phenotypes, Pearson correlation analysis was performed separately. As shown in **Figure 2**, a significantly positive correlation was observed between serum HBV RNA and HBV DNA in EPNA, EPEA (**Figure 2A**) and ENEA (**Figure 2C**) phenotypes (r=0.868, $P=9.480\times10^{-9}$; r=0.687, $P=2.621\times10^{-8}$; r=0.769, $P=6.686\times10^{-9}$, respectively), but not in the ENNA (**Figure 2C**) phenotype (r=0.324; P=0.075). Furthermore, the correlation of serum HBV RNA and HBsAg was different between HBeAg-positive and HBeAg-negative phenotypes. In EPNA and EPEA phenotypes, a significant positive correlation was observed (**Figure 2B**; r=0.777, $P=2.999\times10^{-6}$; r=0.637, $P=4.993\times10^{-7}$, respectively), but this phenomenon was not observed in HBeAg-negative patients in either ENNA (r=0.176, P=0.316) or ENEA (r=0.134, P=0.396) (**Figure 2D**).

Clinical biomarkers associated with elevated ALT in HBeAg-negative patients

Considering that there were significant differences of serum HBV RNA between ENNA and ENEA, we used univariate logistic regression analysis to explore the associated factors for elevated ALT, defined as greater than the ULN of 40 U/L, in HBeAg-negative patients. As shown in **Table 2**, the serum HBV DNA ($\log_{10}$ IU/mL) and HBV RNA levels ($\log_{10}$ copies/mL) were significant risk factors associated with elevated ALT with odds ratios (OR) of 2.650 ($P=6.823\times10^{-5}$) and 2.570 ($P=2.971\times10^{-4}$), respectively. Meanwhile the HBsAg level ($\log_{10}$ IU/mL) was borderline significant (OR=1.717, P=0.053).

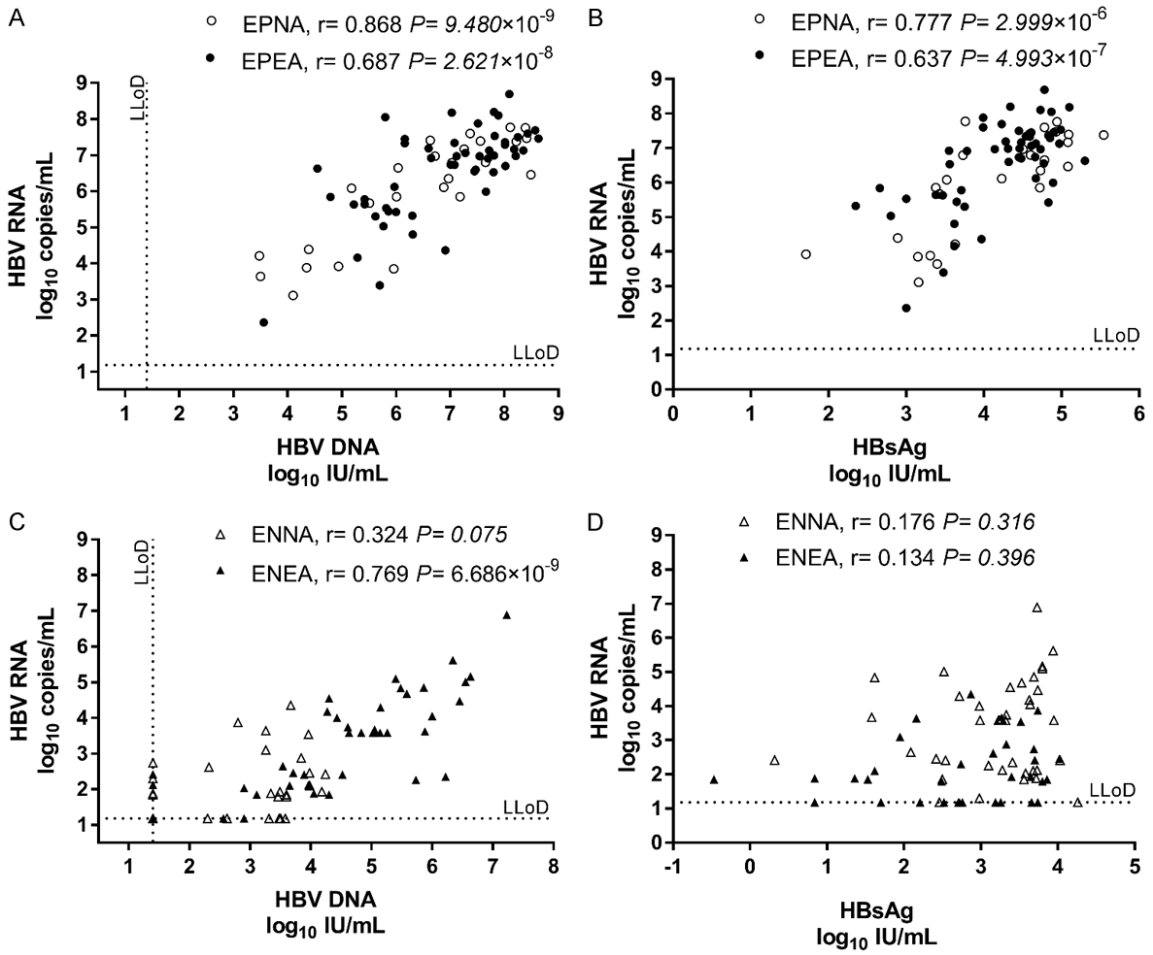

Figure 2. Pearson correlation analysis between HBV RNA , HBV DNA and HBsAg. A. Correlation between serum HBV DNA and RNA in HBeAg-positive patients; B. Correlation between serum HBV HBsAg and RNA in HBeAg-positive patients; C. Correlation between serum HBV DNA and RNA in HBeAg-negative patients; D. Correlation between serum HBV HBsAg and RNA in HBeAg-negative patients. EPNA, HBeAg-positive with normal ALT; EPEA, HBeAg-positive with elevated ALT; ENNA, HBeAg-negative with normal ALT; ENEA: HBeAg-negative with elevated ALT. HBsAg, hepatitis B surface antigen; LLoD, lower limit of detection.

Table 2. Univariate logistic regression analysis for clinical biomarkers associated with elevated ALT in HBeAg-negative patients

HBV-related biomarkers	B	OR (95% CI)	*P*
HBsAg (log_{10} IU/mL)	0.541	1.717 (0.993-2.969)	.053
DNA (log_{10} IU/mL)	0.975	2.650 (1.640-4.282)	6.823×10^{-5}
RNA (log_{10} copies/mL)	0.944	2.570 (1.541-4.286)	2.971×10^{-4}

HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen.

Clinical data of patients with particular nucleic acid detection pattern

We focused on the particular nucleic acid detection pattern patients, which were defined as patients with undetectable levels of at least one type of HBV-related nucleic acid. As shown in **Table 3**, age, HBsAg (log_{10} IU/mL) and ALT (U/L) were not significantly different in these patients. An interesting characteristic in seven DNA-negative, RNA-postive patients was that although serum HBV DNA could not be detected, the liver histopathologic score confirmed that these patients all demonstrated moderate or severe liver inflammation (G≥2) following which, serum HBV RNA was detectable (**Figure 3**). A similar phenomenon also occurred in eight DNA-positive, RNA-negative patients who demonstrated moderate or severe liver inflammation (G≥2) irrespective of ALT level. Patients demonstrating double

Table 3. Clinical data of patients with particular nucleic acid detection pattern

	DNA (-) & RNA (+) (n=7)	DNA (+) & RNA (-) (n=8)	DNA (-) & RNA (-) (n=6)	*P* value
Age (years)	32±7 (23-43)	42±14 (25-64)	31±15 (16-53)	.218
Gender (M/F)	5/2	6/2	4/2	/
HBsAg (log_{10} IU/mL)	1.78±1.54 (-0.47-3.69)	2.89±0.42 (2.46-3.74)	2.66±1.29 (0.84-4.26)	.185
HBV-DNA (log_{10} IU/mL)	/	3.02±0.49 (2.30-3.54)	/	/
HBV-RNA (log_{10} copies/mL)	2.16±0.32 (1.79-2.74)	/	/	/
ALT (U/L)	45.1±24.1 (25.2-83.8)	43.4±14.2 (28.1-69.7)	27.1±15.3 (10.7-52.3)	.181
Elevated ALT, n (%)	3 (42.9%)	2 (25%)	1 (16.6%)	/
Live biopsy				
Grading scores (G) (0/1/2/3/4)	0/0/4/3/0	0/0/5/3/0	1/5/0/0/0	/
Staging scores (S) (0/1/2/3/4)	2/2/2/1/0	0/3/4/1/0	2/4/0/0/0	/

All patients demonstrating particular nucleic acid detection patterns were HBeAg-negative and underwent liver biopsy. M, male; F, female; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase.

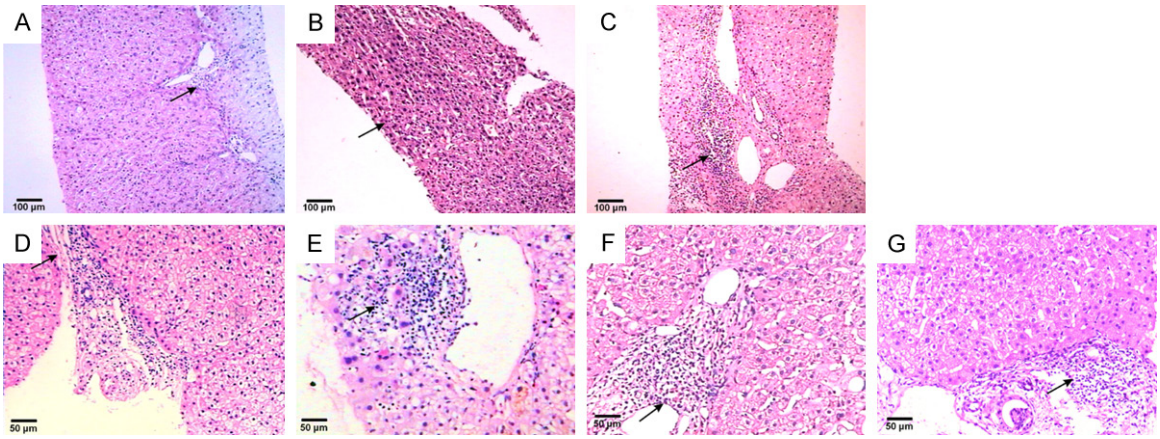

Figure 3. Significant inflammatory and fibrotic liver pathologic changes in seven DNA-undetectable but RNA-detectable patients (H&E staining, A-C: ×100; D-G: ×200). All seven patients demonstrated moderate or severe liver inflammation with/without fibrosis. (A) G2S1, inflammatory cell infiltration in portal area; patient with serum HBV RNA 1.79 log_{10} copies/mL, ALT 25.8 U/L; (B) G2S0, hepatocyte spotty necrosis with inflammatory cell infiltration, patient with serum HBV RNA 2.74 log_{10} copies/mL, ALT 35.0 U/L; (C) G3S2, marked inflammatory cells infiltrate in portal area, patient with serum HBV RNA 1.92 log_{10} copies/mL, ALT 72.7 U/L; (D) G3S3, marked inflammatory cell infiltrate and fibrous septum formation, patient with serum HBV RNA 1.85 log_{10} copies/mL, ALT 26.0 U/L; (E) G3S2, hepatocyte piecemeal necrosis with marked inflammatory cell infiltration, patient with serum HBV RNA 2.12 log_{10} copies/mL, ALT 83.8 U/L; (F) G2S1, inflammatory cell infiltration in portal area, patient with serum HBV RNA 2.42 log_{10} copies/mL, ALT 47.4 U/L; (G) G2S0, inflammatory cell infiltration in portal area, patient with serum HBV RNA 2.30 log_{10} copies/mL, ALT 25.2 U/L. G, inflammation grade; S, fibrosis stage.

negativity for serum viral nucleic acids had no or minor liver inflammation (G≤1).

Discussion

Serum HBV RNA has received much attention in recent years. In contrast to other studies, we did not use nomenclatures of clinical phenotypes derived from the natural history of chronic HBV infection as recommended in the guidelines of AASLD [22]. This was because in clinical practice, not all chronic HBV-infected patients fully meet the criteria following the above-mentioned guidelines, such as DNA and ALT levels. In fact, liver biopsy was needed for histopathologic evaluation and definitive phenotype classification [22, 23]. Thus, patients' phenotypes can usually be classified using the definitions presented herein. While providing serum HBV RNA reference and baseline data for dynamic and sequential follow-up studies, the purpose of the present study was to determine the serum HBV RNA level distribution characteristics in untreated chronic HBV-infected patients with various clinical phenotypes and explore the relationship with serum HBV DNA as well as the clinical implications.

Serum HBV RNA levels were significantly higher in HBeAg-positive patients than that in HBeAg-negative cases, indicating that serum HBeAg-positive status can reflect vigorous cccDNA transcription in the liver, therefore highlighting the need for long-term follow-up. We focused on and presented the clinical phenotypes prorated by serial serum HBV RNA level gradients. An interesting phenomenon was that ENNA status mainly comprised the low level (< 2.0 $\log_{10}$ copies/mL) serum HBV RNA gradients, while on the contrary, in high level (> 6.0 $\log_{10}$ copies/mL) gradients, HBeAg-positive patients (EPNA and EPEA) were the major proportion. This phenomenon indicates that serum HBV RNA levels may be used for distinguishing clinical phenotypes. Meanwhile, a highly sensitivity method for quantitation of serum HBV RNA method is necessary, especially at levels lower than 2.0 ($\log_{10}$ copies/mL).

Apart from the ENNA phenotype, we found a significantly positive correlation between HBV DNA and HBV RNA in EPNA and elevated ALT phenotypes. Liu et al. [24] found that in EPNA patients, the two biomarkers showed a significantly weak correlation (r=0.341), while consistent with our findings, Wang et al. [25] showed a significantly strong correlation (r=0.752 and r=0.868, respectively). However, the correlation coefficients in EPEA also varied between studies; r=0.559 in Liu et al.'s study [24] and r=0.849 in Wang et al.'s study [25], while correlation coefficients in the present cohort fell between these two (r=0.687). Similar results were also shown in the ENEA phenotype, but we did not find a significant correlation in the ENNA phenotype, while other studies demontrated the opposite [24, 25]. Furthermore, the correlations between serum HBV RNA and HBsAg were quite different between these studies [24, 25]. In HBeAg-positive patients, we observed significantly positive correlation in EPNA and EPEA, but other studies did not [24, 25]. The discrepancies between these studies may be due to the differences in cohort enrollment criteria. In addition, another possible reason was that the methodology of serum HBV RNA quantitation were varied [24-27], so an internationally recognized universal method is urgently needed.

Although the elevated ALT is not an absolute indicator for determining liver inflammation status, previous studies have confirmed that the proportion of moderate or severe liver histopathologic inflammation in normal ALT (< 1×ULN) is significantly higher than that in elevated ALT (> 1×ULN) patients [23]. In clinical practice, differentiating between untreated HBeAg-negative individuals being in a stable ENNA status (or named inactive chronic hepatitis B [22]) as compared with those in HBeAg-negative immune reactivation status is of importance for differing management strategies, as the latter is characterized by elevated ALT, moderate-to-severe liver necroinflammation or fibrosis, and a higher risk of end-stage liver disease [22, 28, 29], and therefore requires active antiviral therapy. In this study, we specifically studied the risk factors associated with elevated ALT. Univariate logistic regression analysis showed serum HBV DNA and RNA were both independent risk factors for elevated ALT, thus untreated HBeAg-negative patients with sustained detectable serum HBV-related nucleic acid may need proactive antiviral treatment to delay or reverse liver inflammation and fibrosis.

Next, we analyzed the clinical data from patients with particular nucleic acid detection patterns. The results showed if either type of viral nucleic acid was detectable, the liver biopsy confirmed that the patients did indeed have liver inflammation and varying degrees of fibrosis, and thus may need more active antiviral treatment. Previous studies have demonstrated that some patients who undergo spontaneous HBeAg seroconversion have HBV DNA concentrations of 2,000-20,000 IU/ml, accompanied by persistently normal ALT activity, may progress to active hepatitis [30]. A recent study also suggested untreated high viral load in chronic HBV-infected patients without significant ALT elevation had higher risks of clinical events than treated active phase patients with elevated ALT [31]. However, research into serum HBV RNA has rarely been reported. In the present study, we found that when HBV DNA was undetectable, detectable serum HBV RNA, acting as a complementary virological biomarker, can also reflect the liver inflammation status. In clinical practice, detectable serum HBV DNA is the trigger for initiating antiviral treatment and effectively monitoring a antiviral curative effect [22], but in patients with undetectable serum HBV DNA, it is difficult to decide whether to start the antiviral treatment. Our

study may therefore provide some evidence to help resolve this dilemma. Besides, considering the better pathologic results in patients with double negativity for nucleic acids, our findings suggest that both DNA and RNA negativity may serve as an end point for antiviral therapy. However, this requires confirmation in a larger cohort with appropriate follow-up. Thus, serum HBV RNA detection is necessary in HBeAg-negative patients whose serum HBV DNA was undetectable.

According to the HBV life cycle, detection of HBV DNA in the peripheral blood indicates that intrahepatic cccDNA is actively being transcribed into pgRNA. Theoretically, serum HBV RNA should be detectable. However, in our study, despite the LLoD of serum HBV RNA detection being as low as possible, there were still several HBeAg-negative patients with detectable HBV DNA but undetectable HBV RNA. The most likely explanation is that all or nearly all of the HBV RNA in the hepatocytes of some patients may be reverse-transcribed to generate relaxed circular DNA (rcDNA) [19], so there may be little or no HBV RNA remaining to enter the peripheral circulation. There is evidence for this hypothesis in the results of a study of serum HBV RNA concentrations in chronic hepatitis B patients treated with lamivudine, which were higher, rather than lower, in the early phase of the treatment (< 5 weeks), while HBV DNA concentration decreased [13]. This implies that a nucleoside analogue that blocks the conversion of RNA to rcDNA, thereby preventing RNA consumption, causes the release of the redundant RNA into the circulation. It suggests that HBeAg-negative patients with chronic HBV infection and readily detectable circulating HBV DNA may require a more robust clinical intervention, even if their HBV RNA concentration is very low or undetectable. Therefore, high-precision HBV DNA measurement is still irreplaceable for the clinical management of chronic HBV-infected patients.

There are several limitations to our study. First, the cohort was cross-sectional and small. Second, the accurate association between HBV RNA and liver inflammation, especially in HBeAg-negative phenotypes, requires more evidence, such as measuring nucleic acid levels *in situ*. Third, due to the lack of an internationally recognized method for serum HBV RNA quantitation, the comparisons of results with other studies are not conclusive.

Conclusions

Varying serum HBV RNA levels can reflect natural disease phases in untreated HBV-infected patients, indicating a similar capacity to serum HBV DNA for reflecting liver inflammation in untreated HBeAg-negative patients. Serum HBV RNA detection is necessary and can complement clinical management strategies when serum HBV DNA is undetectable.

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Disclosure of conflict of interest

None.

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