

Research Article

Relationship between Serum Hepatitis B Virus-RNA Levels and Histopathological Assessment in Chronic Hepatitis B Patients Treated with Entecavir

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Abstract

Hepatitis B Virus RNA (HBV RNA) is a new serum marker and a direct product of the covalently closed circular DNA (cccDNA). Entecavir (ETV) has been widely used for the treatment of Chronic Hepatitis B Virus (CHB) infection. The following study investigates the correlation between HBV RNA and histopathological changes in the liver, in response to ETV therapy. A cross-sectional set of serum and liver biopsy samples was obtained from patients treated with ETV. The correlations between serum HBV-RNA concentration and levels of peripheral viral replicative forms, as well as histological scores were analyzed before and after treatment. At baseline, serum HBV-RNA ($5.807 \pm 2.503 \log_{10}$ copies/ml) was detected in 29 patients. These levels were correlated with the serum HBV-DNA level ($5.828 \pm 1.586 \log_{10}$ IU/ml) and HBeAg levels (median, 11.733; 25%~75% range, 0.311~385.75 S/CO). Nevertheless, low correlation was found between the serum HBV-RNA, ALT levels and the histological scores for grading and staging of the liver. Moreover, after 53 ± 5 weeks of treatment, the concentrations of serological indicators have significantly decreased, and the characteristics of histology have also improved. When grouped according to the pathological changes, no statistical differences in HBV RNA levels were detected between groups, before or after treatment. To conclude, the serum HBV-RNA levels reflect the intrahepatic viral transcriptional activity and when combined with liver histopathological analysis they can more precisely reflect the effect of ETV therapy.

Keywords: HBV RNA; Entecavir; Histopathology; cccDNA; ALT

Abbreviations

HBV: Hepatitis B Virus; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TBIL: Total Bilirubin; DBIL: Direct Bilirubin; AFP: Alpha Fetoprotein; HBVEA: Hepatitis B Virus E Antigen; LLOD: Lower Limit of Detection

Introduction

Hepatitis B Virus (HBV) infection cause a wide spectrum of clinical manifestations, ranging from fulminant acute hepatitis to chronic infection with varying degrees of liver disease [1]. The goal of the treatment for Chronic Hepatitis B (CHB) infection is to prevent disease progression to (decompensated) cirrhosis, Hepatocellular Carcinoma (HCC) and death [2]. In clinical practice, there are two types of therapies for patients with CHB: Pegylated Interferon Alpha (Peg-IFN) or Nucleoside/Nucleotide Analogues (NAs) [3-6]. Compared to Peg-IFN, NAs exhibit a direct antiviral effect potentially reducing the HBV DNA level and its associated complications [7-9]. Nevertheless, blocking reverse transcription by NAs has no impact on

the formation of HBV pregenomic RNA (pgRNA) or on production of viral proteins, as the HBV covalently closed circular DNA (cccDNA) is unaffected and remains transcriptionally active. Consequently, treatment discontinuation of NAs usually results in recurrence of disease activity and most patients usually require a lifelong therapy [10]. In addition to HBV DNA, Wang et al. [10,11] have found the presence of HBV RNA in the serum of chronically HBV-infected patients. Moreover, Jansen et al. [12] have discovered that RNAs do not affect plasma HBV RNA levels and that the large majority of measured HBV RNA are pgRNA. The amount of circulating pgRNA should mirror the amount of pgRNA present in the liver and hence the presence of transcriptionally active cccDNA in patients [13].

HBV RNA levels vary significantly from those of established viral markers during antiviral treatment, which highlights its potential as an independent marker in the evaluation of patients with CHB. Traditionally liver biopsy has been the mainstay of HBV disease assessment, but with the emergence of non-invasive markers for liver fibrosis, this approach has become less popular. Whether HBV RNA can reflect the changes in histology still needs to be explored given that HBV-related inflammation and fibrosis can be reversed after ETV antiviral therapy. However, treatment of patients with CHB with NAs suppresses HBV DNA production but does not affect the synthesis of the RNA pregenome or HBV messenger RNA. Though HBV RNA-containing particles continue to be secreted into the bloodstream, detailed changes of liver characteristics before and after therapy and the correlation with HBV RNA remain unclear. In this study, Polymerase Chain Reaction (PCR)-based assay was used to specifically quantify the HBV RNA in plasma during therapy with ETV, and paired biopsy samples obtained from patients were used to assess disease inflammation and fibrosis levels. Herein we discuss the relationship between histological and serological changes in liver after treatment of ETV. The results showed that liver biopsy remains

Citation: Zhou L, Zhang Y, Zhang M, Ren Y, Ma A. Relationship between Serum Hepatitis B Virus-RNA Levels and Histopathological Assessment in Chronic Hepatitis B Patients Treated with Entecavir. *J Med Public Health*. 2022;3(1):1022.

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Publisher Name: Medtext Publications LLC

Manuscript compiled: Jan 07th, 2022

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an important tool, not only for the clinical assessment of HBV but also for evaluation of research progress.

Materials and Methods

Patients and samples

In this study, we selected patients with CHB (Hepatitis B Surface Antigen (HBsAg) positive for at least 6 months) who were admitted to China-Japan Friendship Hospital from 2015 to 2017. The exclusion criteria were the following: the diagnosis or history of other virus infection; alcohol consumption above moderate levels (more than 30 g/day), hepatic injury caused by metabolism dysfunction or other causes; history of treatment for hepatic diseases; liver biopsy not suitable for analysis; or patients with missing key data concerning clinical information and laboratory data. After baseline enrollment, the following criteria were used to additionally exclude patients during antiviral treatment: (i) lost to follow up, (ii) stopped NUC, (iii) HCC occurrence during antiviral treatment, (iv) Hepatic decompensation during antiviral treatment, or (v) refused liver biopsy measurement after treatment.

A total of 29 patients were eligible for the current analysis, including 27 male and 2 female patients. The mean age was 38 years (SD, ± 8 years). For chronic HBV-infected individuals, 20 were HBeAg-positive and 9 were HBeAg-negative at baseline. Those patients received a NA-ETV (Bristol-Myers Squibb) monotherapy with doses of 0.5 mg daily, according to the guidelines for the prevention and treatment of CHB in China [14]. A summary of patient characteristics is presented in Table 1.

Plasma samples from enrolled patients were collected before the initiation of ETV therapy (mean time of collection (\pm SD), 2.8 ± 2.1 weeks) and after therapy (53 ± 5 weeks). All samples were stored at -80°C until further use. Needle liver biopsies were performed in all patients before and after therapy. Each specimen (>15 mm in length) was formalin-fixed, paraffin-embedded and used for diagnostic histological examination.

The study was carried out according to the guidelines of the Declaration of Helsinki and the principles of good clinical practice, and was approved by the ethics committee of China-Japan Friendship Hospital. All patients gave a written informed consent.

Histological assessment

Liver biopsies were prepared for subsequent histological analysis. Tissue were cut into $4\mu\text{m}$ thick sections, and stained with hematoxylin and eosin, Masson's Trichrome and Reticulin were used for standard histological assessment. The degree of inflammation was graded using Ishak modified Histology Activity Index (HAI) grading system; the fibrosis was staged by Ishak fibrosis scoring system [15,16] by two senior experts. Histological evaluations were performed using a blind approach [17].

Laboratory tests and data collection

A standardized data collection form was designed to retrieve all the relevant information on demographic data (age, gender, history of alcohol consumption, family history of HBV infection); laboratory data (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Bilirubin (TBil), Serum Albumin (ALB), Gama-Glutamyl Transpeptidase (GGT), Prothrombin Activity (PTA) and so on). HBV surface antigen (HBsAg), HBV e antigen (HBeAg), HBV e antibody (HBeAb), HBV c antibody (HBcAb), hepatitis C virus antibody (anti-HCV), and human immunodeficiency virus antibody (HIV Ab) were

measured by commercially available enzyme-linked immunosorbent assay kits (Abbott Japan, Tokyo, Japan). In addition, all the relevant assays were analyzed in the clinical laboratory and the data were collected from the patient medical records.

Extraction and reverse transcription of HBV RNA

According to the manufacturer's instructions, HBV RNA was isolated using the Easy Pure Viral RNA Kit (TransGen Biotech, Beijing, China) and then treated with DNase I (Thermo Fisher Scientific, Waltham, MA, USA). The ratio of all RNA samples 260/280 is in the range of 1.8 to 2.1 tested by Nanodrop. Isolated HBV RNA was reverse-transcribed using Revert Aid™ First Strand DNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) with either random primers or a HBV specific RT primer. Primer sequences were listed as previous Wang J's article [13].

Quantification of HBV RNA and HBV DNA

The levels of HBV RNA were detected by quantitative real-time Polymerase Chain Reaction (qPCR) using 1206352 SYBR Green qPCR Master Mix (Applied Biosystems, Warrington, UK) in Light Cyclor 480 II Real-time PCR Detection System (Roche, Mannheim, Germany). This experiment was done in the Peking University Health Science Center. HBV DNA was detected in our laboratory. The detection range of DNA was 1×10^3 to 1×10^8 IU/ml. If the levels were lower than $1 \text{ IU/ml} \times 10^3 \text{ IU/ml}$, we recorded it as $3 \log_{10} \text{ IU/ml}$; if the levels were higher than $1 \times 10^8 \text{ IU/ml}$, we recorded it as $8 \log_{10} \text{ IU/ml}$.

Statistical analyses

The repeated measures analysis of variance and two groups of related nonparametric Wilcoxon symbols test were performed using the statistical software package SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA). Continuous variables were presented as mean \pm standard deviation or medians with interquartile range, while categorical variables as the frequencies or percentages of events. The Students t-test or Mann-Whitney U test was used only for continuous data. All tests of significance were two-tailed and $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients

A total of 29 patients with paired liver biopsies were enrolled in this study. Overall, most patients were male (93%). After 53 ± 5 -week therapy, significant decreases were observed in ALT (from median, 66; 25%~75% range, 39~101 IU/L to median, 34; 25%~75% range, 20~41 IU/L, $p < 0.01$) and AST (from median, 38; 25%~75% range, 31~58 IU/L to median, 23; 25%~75% range, 19~28 IU/L, $p < 0.05$). Serum HBV DNA was detectable in nearly all patients before the initiation of ETV therapy, while low levels (lower limit of detection i.e., LLoD = $3.0 \log_{10} \text{ IU/ml}$) were detected after therapy in 27 patients (93%). Nevertheless, serum HBV RNA was persistently detectable, which decreased from 5.807 ± 2.503 to $4.325 \pm 2.153 \log_{10} \text{ copies/ml}$ after therapy.

In addition, liver biopsy samples were analyzed to determine changes in Ishak stage and Fibrosis score. The response to 53 ± 5 -week antiviral therapy was categorized as: reverse (decrease in Ishak stage ≥ 1 ; $n=13$, 44.83%), stable (increase or decrease in Ishak stage 1; $n=10$, 34.48%), or in progression (increase in Ishak stage ≥ 1 ; $n=6$, 20.69%). No statistical differences among demographic and clinical features were observed between the three groups at baseline.

Association between HBV-RNA levels and liver injury

To determine whether HBV-RNA levels are linked to injury or morphological changes in patients who were experiencing CHB, all liver biopsy specimens were stained with hematoxylin-eosin and histologic grading of necroinflammation (G), and fibrosis (S) were performed by the Ishak scoring system. Furthermore, using paired serum-liver biopsy samples, we were able to examine the correlations between serum HBV-RNA, HBV DNA concentration, liver necroinflammation, and fibrosis. Despite the fact that ALT and AST levels did not reflect necroinflammation score and fibrosis levels; neither HBV-RNA nor HBV-DNA levels in the serum were correlated with liver injury (Figure 1A and 1B). Since HBV DNA was undetectable after therapy in most patients, we only compared the relationship between HBV RNA levels and tissue lesions (Figure 1C). Briefly, no statistical differences between HBV RNA/ALT and G/S score were found 1 year after ETV therapy.

When stratifying the patients according to HBeAg status, no correlations between severity of histopathology and HBV-RNA levels were observed; this data were consistent with previous reports. However, there was a correlation between serum HBV DNA levels and necroinflammation severity in both HBeAg(+) and HBeAg(-) patients (Figure 2A). In addition, no statistical significance between HBV DNA levels and fibrosis level were observed in all groups (Figure 2B).

Serum ALT and AST levels were not correlated with histopathological score for necroinflammation and fibrosis during ETV therapy (Figure 2C and 2D). Nevertheless, due to the limited sample size in each category, further studies with more samples are required to confirm these findings. The above data suggested that HBV-RNA is not linked to morphological changes of the liver. In addition, compared with serum HBV-DNA, it is not useful for evaluating liver histopathology, especially fibrosis. These data support the conclusion that accumulated immune damage, results in the liver injury and the progression of liver disease.

Baseline HBV RNA levels and response to therapy

As shown in Figure 3, in the cohort of 29 patients with CHB treated with ETV, the serum level of HBV RNA was significantly correlated with HBV DNA measured before starting the therapy ($r=0.634$,

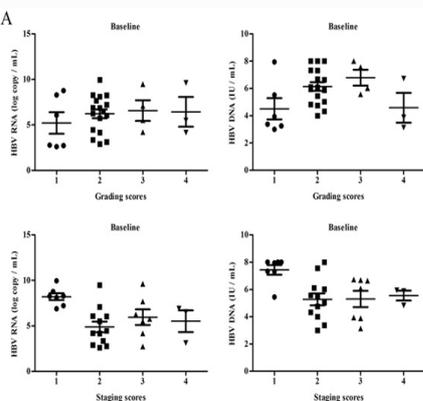


Figure 1A: Association between HBV-RNA levels and liver injury. (A) Correlations of grading scores and staging scores with serum HBV-RNA levels and serum HBV-DNA levels in treatment-naïve patients experiencing CHB (Pearson's test).

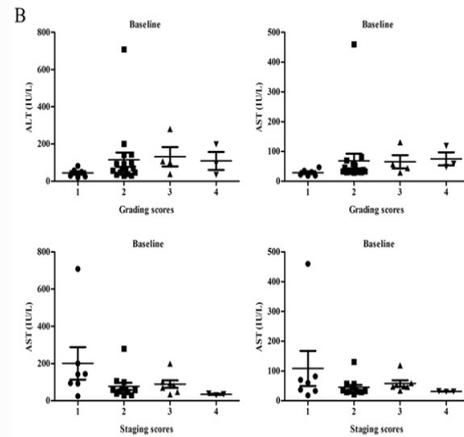


Figure 1B: Correlations of grading scores and staging scores with ALT and AST levels at baseline (Pearson's test).

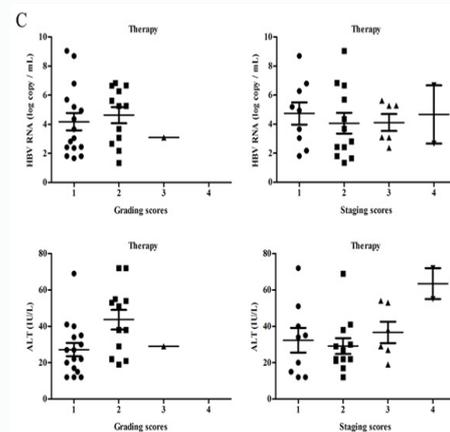


Figure 1C: Correlations of grading scores, staging scores with serum HBV-RNA and ALT levels after ETV therapy for 53 ± 5 weeks (Pearson's test). $p < 0.05$, there were statistical differences.

$p < 0.0001$). Serum HBV RNA was also significantly correlated with that of HBeAg ($r=0.679$, $p < 0.0001$). Moreover, the serum HBV-RNA and DNA were slightly correlated with both ALT ($r=0.352$, $r=0.386$) and AST ($r=0.264$, $r=0.240$) (Figure 3A and 3B). In addition, the HBV RNA levels were strongly correlated with HBV DNA levels in both HBeAg-positive ($n=20$) and HBeAg-negative ($n=9$) patients before the treatment ($r=0.869$, $r=0.563$) (Figure 3C). In contrast, HBV RNA levels showed a strong correlation with HBeAg seroconversion group ($r=0.983$) 53 ± 5-weeks after treatment, whereas the remaining seven showed neither HBeAg loss nor seroconversion. In HBeAg-negative patients, HBV RNA levels were lower than HBeAg-positive patients before ($p < 0.01$) and 53 ± 5-weeks after the therapy ($p < 0.05$) (Figure 3D). During treatment, there were no significant differences of HBV DNA levels in those HBeAg-positive patients and HBeAg-negative ones.

The serological and histological changes during antiviral treatment

After treatment, serum levels of HBV DNA ($p < 0.001$), HBV RNA ($p < 0.001$), and ALT ($p < 0.01$), AST ($p < 0.05$) all decreased significantly throughout the course of ETV therapy (Figure 4A). Mean declines in

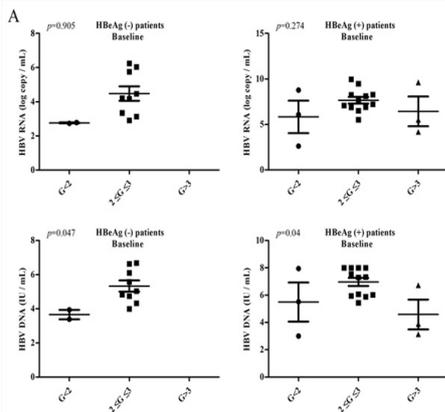


Figure 2A: Correlation between HBV-RNA or HBV DNA level and Histopathology severity in HBeAg(+) and HBeAg(-) CHB patients. Correlations of grading scores and staging scores.

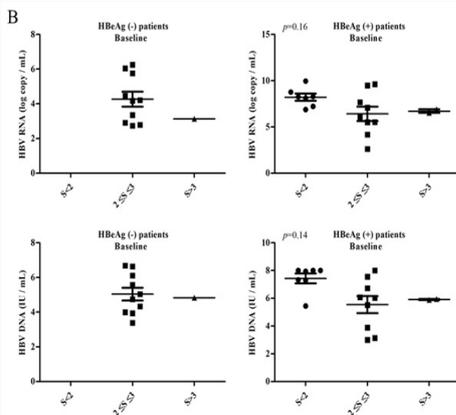


Figure 2B: Correlation between HBV-RNA or HBV DNA level and Histopathology severity in HBeAg(+) and HBeAg(-) CHB patients. Correlations of grading scores with serum HBV-RNA levels and serum HBV-DNA levels at baseline (Pearson's test).

HBV DNA levels were considerably larger than HBV RNA in patients with CHB. Treatment of CHB patients with ETV suppresses HBV DNA synthesis but does not affect synthesis of HBV pregenomic RNA (pgRNA). During long-term ETV treatment of patients, HBV RNA levels remained higher than HBV DNA levels. Serum HBV RNA has been proposed as a marker of HBV cccDNA activity. Serum HBV-RNA levels reflect the amount of intrahepatic HBV-RNA and levels of cccDNA-directed viral transcriptional activity [18]. HBV-RNA accumulation and low levels of viral replication may lead to liver disease progression. According to the response to antiviral therapy, liver biopsy samples were categorized as: reverse, stable, and in progression. During treatment, though the decrease in HBV RNA, DNA and ALT levels was significant within groups, there was no difference between each group (Figure 4B). These data suggested that ETV was effective in antiviral therapy, while there was no weighty correlation with serum and liver histological changes.

Discussion

Entecavir (ETV) a guanosine nucleoside analogue is a highly powerful inhibitor of HBV, with low toxicity, which has been approved by the US FDA for the treatment of CHB, though it was originally developed for the treatment of herpes simplex virus infections [8].

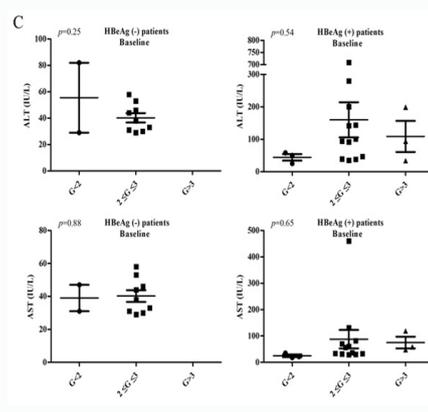


Figure 2C: Correlations of grading scores and staging scores.

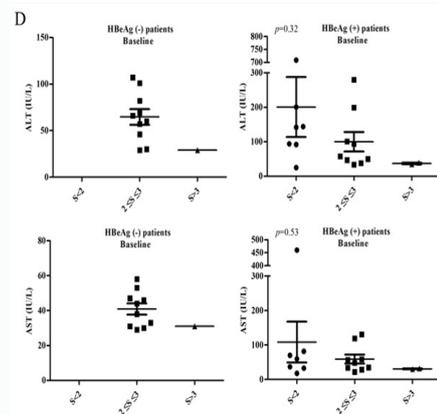


Figure 2D: Correlations of grading scores with serum ALT levels and AST levels at baseline (Pearson's test).

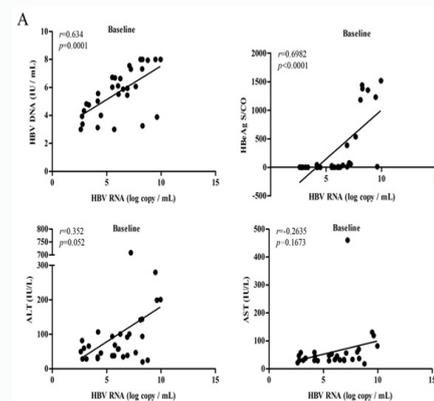


Figure 3A: Baseline HBV RNA Levels and Response to ETV Therapy. Correlation was evaluated within the whole CHB study population at the baseline. There was very strong correlation between HBV RNA and HBV DNA in patients ($r = 0.634, p < 0.0001$) but no correlation between HBV RNA and ALT ($r = 0.352, p = 0.052$).

Previous studies have shown that due to its compelling effect in adults with evidence of active viral replication, elevated serum ALT or AST or evidence of histologically active disease, ETV is superior to lamivudine on the co-primary endpoint of histological improvement [19]. Chang et al. [20], have found that ETV not only inhibits HBV replication but is also able to improve fibrosis score after continuous

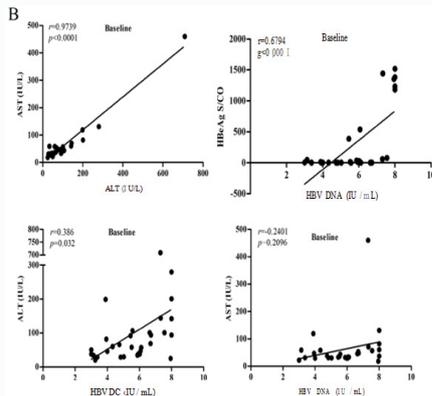


Figure 3B: Baseline HBV RNA Levels and Response to ETV Therapy. Correlation was evaluated within the whole CHB study population at the baseline. There was very strong correlation between HBV RNA and HBV DNA in patients ($r = 0.634, p < 0.0001$) but no correlation between HBV RNA and ALT ($r = 0.352, p = 0.052$).

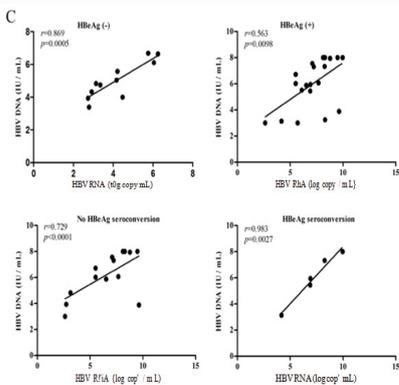


Figure 3C: Correlation between serum HBV RNA and HBV DNA in the HBeAg (+) / (-) patient group, and the HBeAg(+) patient group with/ without seroconversion. (D) HBeAg-negative patients have significantly lower viral productivity before and after the therapy.

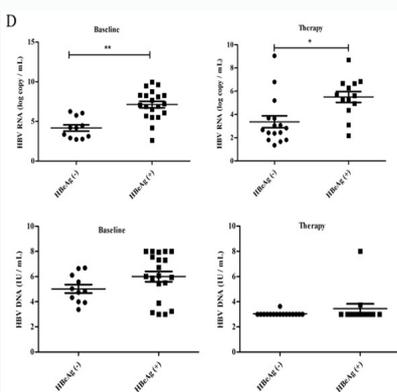


Figure 3D: HBeAg-negative patients have significantly lower viral productivity before and after the therapy.

therapy in 88% of CHB patients. Furthermore, ETV therapy may reduce the risk of HCC and liver-related events, particularly in patients with cirrhosis [21-24].

Accumulating evidence has already demonstrated that serum

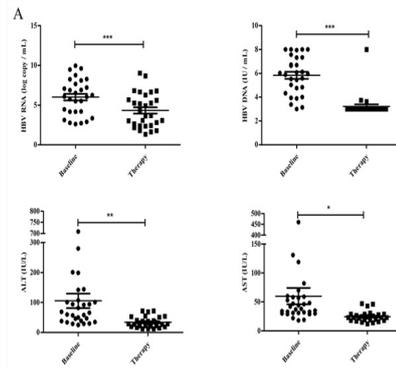


Figure 4A: The Serological and Histological Changes during Antiviral Treatment Changes in serum levels of HBV RNA, HBV DNA, ALT and AST during therapy in 29 patients with CHB.

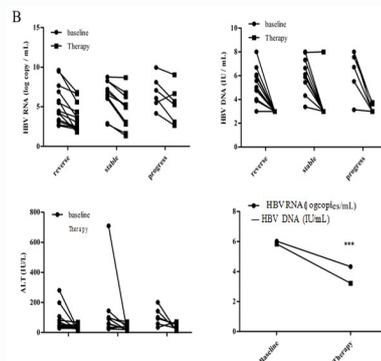


Figure 4B: Patients were divided into reverse, stable, progress three groups according to histopathological results. Changes of serum HBV RNA, HBV DNA and ALT before and after treatment in each group. (* $p \leq 0.005$, ** $p \leq 0.001$, *** $p \leq 0.0001$).

HBV RNA could still reflect the activity of intrahepatic cccDNA [25,26], thus highlighting its potential as an independent marker in the evaluation of patients with CHB [27,28]. Whether ETV can regulate RNA, and whether HBV RNA level in serum can reflect the improvement of liver histological changes after ETV treatment, are the issues we addressed in this study.

Firstly, we used qPCR assay to detect HBV RNA in serum at the start and after ETV treatment. HBV RNA in serum was correlated with activity of the main HBV replication template, such as the level of HBV DNA and HBeAg. But ALT, AST level had very low correlation with HBV markers. At the baseline and after treatment, HBV RNA levels were consistently lower in HBeAg-negative group compared to HBeAg-positive patients. However, one patient in both HBeAg-negative and HBeAg-positive groups didn't have HBV DNA levels below the limit at the end of ETV treatment. At the same time, patients receiving ETV therapy showed a strong decline in HBV RNA level, HBV DNA level, ALT and AST level. Also, the decrease in HBV RNA level was associated with response to therapy, which highlighted a possible role for HBV RNA levels in predicting response to ETV therapy.

Interestingly, patients treated with ETV had a strong decrease in the HBV RNA level, even those who were HBeAg-positive without HBeAg loss. This discovery is consistent with data from previous

Table 1: Patient characteristics and Laboratory results at baseline and after ETV therapy.

	Baseline	Therapy
Total	n=29	n=29
Age (years)	38 ± 8	39 ± 8
Male, n (%)	27 (93%)	27 (93%)
HBV RNA (log ₁₀ copies/ml)	5.807 ± 2.503	4.325 ± 2.153
HBV RNA below LLD, n (%)	0 (0%)	0 (0%)
HBV DNA (log ₁₀ IU/ml)	5.828 ± 1.586	3.2120 ± 0.936
HBV DNA below LLD, n (%)	1 (3.44%)	27 (93%)
HBeAg positive, n (%)	20 (68.97%)	13 (44.83%)
ALT (IU/L)	66 (39, 101)	34 (20, 41)
AST (IU/L)	38 (31, 58)	23 (19, 28)
TBIL (µmol/L)	16 (13.42, 20.3)	17.92 ± 7.36
DBIL (µmol/L)	4.28 (3.22, 5.33)	4.32 ± 1.82
ALB (g/L)	45.18 ± 4.72	47 (46, 49)
ALP (IU/L)	82.2 ± 33.33	77.62 ± 18.72
GGT (IU/L)	46 (33, 73)	26 (18, 43)
WBC (10 ⁹ /L)	5.42 (4.58, 6.72)	5.7 ± 1.58
NC (10 ⁹ /L)	3.09 (2.59, 4.01)	3.07 (2.56, 3.64)
LC (10 ⁹ /L)	1.879 ± 0.635	1.72 (1.57, 1.99)
PLT (10 ⁹ /L)	186.48 ± 63.51	183.975.7 ± 59.78
PTA (%)	102.1 ± 15.52	106.245.7 ± 9.99
INR	0.98 (0.94, 1.04)	0.975.7 ± 0.053
AFP (ng/ml)	4.6 (3.39, 7.64)	2.68 (2.28, 3.55)
Grading scores <2, n (%)	5 (17.24%)	16 (55.17%)
2 ≤ Grading scores ≤ 3, n (%)	21 (72.42%)	13 (44.83%)
Grading scores >3, n (%)	3 (10.34%)	0 (0%)
staging scores <2, n (%)	7 (24.14%)	9 (31.03%)
2 ≤ staging scores ≤ 3, n (%)	19 (65.52%)	18 (62.07%)
staging scores >3, n (%)	1 (3.45%)	2 (6.90%)

Data are presented as mean value ± SD, median (interquartile range) or no. (%) of patients.

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TBIL: Total Bilirubin; DBIL: Direct Bilirubin; ALB: Serum Albumin; ALP: Alkaline Phosphatase; GGT: γ -glutamyltranspeptidase; WBC: White Blood Cell Count; NC: Absolute Neutrophil Count; LC: Absolute Lymphocyte Count; PLT: Platelet Count; PTA: Prothrombin Activity; INR: International Normalized Ratio; AFP: Alpha Fetoprotein; HBeAg: Hepatitis B virus e Antigen; HBV: Hepatitis B Virus; LLoD: Lower Limit of Detection

studies showing that ETV exerts antiviral effects, such as prevention of the formation of pgRNA-containing capsids and epigenetic cccDNA modifications [29]. In conclusion, in this study, we confirmed that HBV RNA is present in plasma specimens from patients with CHB and that it remains present in higher levels compared to HBV DNA during ETV therapy. ETV therapy effectively suppresses viral replication, and in the current study nearly all patients achieved an undetectable HBV DNA. While the influence of ETV on HBV RNA levels is limited, despite there is a strong reduction, too. Additional studies on HBV RNA in HBV pathogenesis and its virological and therapeutic characteristic are needed [12].

Substantial elevation of ALT is usually required to initiate antiviral therapy for CHB, no matter how high the viral load is. HBV RNA has shown to be an effective marker for monitoring anti-HBV therapy [30], and it has also been associated with the persistence of chronic HBV infection and rebound [13]. However, HBV RNA is slightly correlated with ALT. Needle core biopsy is the most accurate way used to assess the degree of liver inflammation and fibrosis for initial HBV disease staging and for governing out other causes of liver disease. In this article, we argue that liver biopsy remains an important tool for the majority of patients with HBV infection, for clinical disease assessment and for research progress. Histology provides a more detailed assessment of the liver, reflecting cumulative damage over

time in addition to ongoing inflammation. Ishak has been routinely used to assess disease activity [31]. A liver biopsy can additionally be used to identify the coexistence of other diseases; in particular coincident steatosis and non-alcoholic steatohepatitis, increasingly common comorbidities, would be indistinguishable from HBV-related liver inflammation without a biopsy [17,32,33]. Liver biopsy remains an indispensable tool in the pursuit of HBV cure. When the viral infection is suppressed or eliminated, features of regression often start to dominate. Though Wang's study have revealed significant differences in baseline serum HBV-RNA levels across the different phases of CHB infection, we claim that HBV RNA cannot replace liver penetrating examination.

Our study has several limitations. Firstly, because we did not have paired liver biopsy and HBV RNA data at different time points, we just collected the data before and after ETV treatment. Secondly, we did not have follow-up liver biopsy results for patients with long time necroinflammation and fibrosis improvement. Thirdly, the sample size was small, and thus the statistical efficiency effect may be low. A larger cohort with comparable clinicopathological features and complete follow-up data are necessary in order to confirm the findings. Therefore, further research is needed to substantiate our result that the serum HBV RNA cannot be used to precisely monitor the characteristics of the liver. However, to the best of our knowledge, this is the first study with paired data of HBV RNA and liver biopsy both before and after ETV treatment performed to demonstrate the effect of antiviral.

In conclusion, in this study, we detected the correlation between HBV RNA levels and histological changes before and after the ETV treatment. This study has paved the way for a better understanding of the nature of serum HBV RNA and its clinical implications in the future. The use of serological markers in combination with liver biopsy for the continuous assessment of liver histology is more favorable diagnostic approach, especially for difficult cases.

Acknowledgement

We thank Professor Anlin Ma who designed the research study. We thank Yujiao Zhang, Yuanyuan Ren and Ming Zhang for their excellent language editing excellent technical assistance. Li Zhou analysed the data and wrote the study.

Funding

This study was supported by the National key project Foundation of China (Grant Number: 2013ZX10002005), and the Key Project of Beijing Natural Science Foundation (Grant Number: ZC-301).

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