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# HBV RNA AS A PREDICTOR OF HBEAG SEROCONVERSION IN PATIENTS WITH HEPATITIS B

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**Abstract:** Objective: To study the predictive value of serum hepatitis B virus pregenomic RNA (HBV pgRNA) in the seroconversion of HBeAg-positive chronic hepatitis B (CHB) patients. Methods: 135 patients with HBeAg positive CHB patients were admitted to our hospital from June 2021 to June 2022 were selected to detect the serum HBV pgRNA and HBV DNA levels with antiviral therapy at 12 weeks, 24 weeks, 48 weeks and 96 weeks . Receiver operating characteristic analyses was used to determine baseline and and on-treatment HBV RNA levels associated with response. Result: The serum HBV pgRNA levels at 12, 24, 48, and 96 weeks after treatment were (5.11 ± 0.76) log 10 IU/ml, (4.56 ± 0.43) log 10 IU/ml, (3.22 ± 0.36) log 10 IU/ml, and (1.54 ± 0.22) log 10 IU/ml, respectively; The levels of HBV DNA in HBeAg positive CHB patients at different stages were (5.01 ± 0.84) log 10 IU/ml, (4.73±0.52) log 10 IU/ml, (3.38 ± 0.52) log 10 IU/ml, and (1.32 ± 0.35) log 10 IU/ml, respectively. The serum HBV pgRNA and HBV DNA levels were all lower than before treatment, and the differences were statistically significant($P<0.05$); significant serum conversion occurred in HBeAg positive patients at 24 weeks that HBeAg positive rate of 71.11%, HBV pgRNA positive rate of 69.63%, and HBV DNA positive rate of 36.30% after antiviral therapy. The sensitivity and specificity of serum HBV pgRNA in predicting serum HBeAg seroconversion were 89.20% and 80.95%, which were higher than those of serum HBV DNA at 67.96% and 67.92%. Conclusion: Serum HBV pgRNA and HBV DNA levels in HBeAg positive CHB patients were gradually decreased with the prolongation of antiviral treatment time. Both may serve as a certain guiding role in antiviral treatment of CHB patients, but the predictive value of HBV pgRNA is higher than HBVDNA in the seroconversion of HBeAg-positive CHB patients.
**Keywords:** Hepatitis B virus pregenomic RNA; Hepatitis B e antigens; Chronic hepatitis B; Antiviral therapy

## 1 BACKGROUND

Hepatitis B virus (HBV) infection is the cause of chronic hepatitis B and also a common cause of liver cirrhosis and hepatocellular carcinoma disease factors [1]. Chronic HBV infection is a major global public health problem which poses a particularly serious threat to the health of Chinese people. At present, the positive rate of HBeAg in the general population of China is 5%~6%, so it is estimated that there are about 70 million cases of chronic HBV infection in China, including 20 million to 30 million cases of CHB [2]. In clinic, ntiviral treatment is effective in halting progression of CHB in many patients. Antiviral therapy can effectively inhibit the replication of hepatitis B virus and control the patient's condition,There are two types of antiviral therapy drugs: nucleos(t)ide analogues (NA), such as entecavir, tenofovir, and propenovir which inhibit the viral polymerase and interfere with viral replication, and interferon, including conventional and pegylated forms, which has antiviral and immunomodula-tory effects.[3]. However, antiviral therapy has little effect on covalently closed circular DNA (cccDNA) in the nucleus of liver cells that it is difficult to completely eliminate the virus. CccDNA is a primitive template for HBV replication, and detecting cccDNA can be provided for evaluating the effectiveness of antiviral therapy and treatment plans adjustment. In recent years, serum hepatitis B virus DNA (HBV DNA) and pregenomic RNA of hepatitis B virus (HBV pgRNA) can use as serological markers of hepatitis B, they are used as the curative effect of anti-virus therapy and prediction of antiviral efficacy [4]. HBeAg(the hepatitis B E antigen) was buried inside HBcAg. When HBcAg was cleaved, HBeAg was dissolved from the nucleus of liver cells into the serum. Its existence indicates that HBV is actively replicating in the body and serious damage to liver cells that patients are highly infectious. In case HBeAg remains positive for more than 3 months, it indicates that the disease has a tendency towards chronicity. Spontaneous or drug-induced HBeAg seroconversion has important clinical significance and is considered to be a milestone in the process of CHB patients treatment. In order to further detect the value of HBV RNA in HBeAg seroconversion, 135 HBeAg positive CHB patients were admitted to our hospital from June 2021 to June 2022 that were used to analyze the application value of HBV RNA in HBeAg seroconversion on order to guide clinical standard treatment of chronic hepatitis B patients.

## 2 MATERIALS AND METHODS

### 2.1 Study Population and Parameters

135 HBeAg positive CHB patients were received antiviral treatment with nucleoside (NAs) drugs in our hospital from June 2021 to June 2022 .Diagnosed with CHB and clinically consistent with the diagnostic criteria for the disease in the "Guidelines for Primary Diagnosis and Treatment of Chronic Hepatitis B (2020)" [5]. The studied patients were excluded if they had any of the following conditions: concurrent hepatitis A virus, hepatitis C virus, cytomegalovirus

and other hepatitis B virus infections; Concurrent Human Immunodeficiency Virus (HIV) infection; Concurrent metabolic liver disease and autoimmune liver disease; Accompanied by hepatocellular carcinoma or other systemic malignancies; Merge severe heart, kidney, blood system, and nervous system diseases; Pregnant or lactating women. All patients were informed the study,This study is prospectively performed and approved by the Yongchuan People's Hospital of Chongqing ethics committee.

### 2.2 Method

Serum HBV pgRNA and HBV DNA were detected in CHB patients before and after antiviral treatment at 12, 24, 48, and 96 weeks.5ml of venous blood from CHB patients were collected, centrifuge at 4000rpm for 5 minutes, and then freeze at -20℃.Serum HBV pgRNA detection: 200μL serum was used and detected it by quantitative polymerase chain reaction (qPCR). Nucleic acid extraction were made with Shanghai Bojie automatic nucleic acid extractor and strictly followed the instrument instructions for RNA extraction. The HBV pgRNA assay kit (Beijing Re Jing Biotechnology Co.Ltd) was used to detect the serum HBV RNA levels of patients.produced by . HBV DNA quantitative detection kit (Hunan Sheng xiang Biotechnology Co.Ltd)was select to detect the serum HBV DNA levels of patients. HBV DNA or RNA nucleic acid testing was used the Shanghai Hongshi SLAN96P fluorescent quantitative instrument.

### 2.3 Observation Indicators

The expression of serum HBV pgRNA and HBV DNA were detected with realtime PCR in HBeAg positive CHB patients before and after antiviral treatment at 12, 24, 48, and 96 weeks.Observation of the predictive value (sensitivity, specificity) of serum HBV pgRNA and HBV DNA for HBeAg Seroconversion with ROC curve.

### 2.4 Statistical Analysis

SPSS 26.0 software was used for statistical analysis, and the normally distributed quantitative data in the study were represented by ( $\bar{x} \pm s$). Multiple groups of independent, normal, and homogeneous data were compared between groups using one-way analysis of variance, and pairwise comparisons were performed using Fisher's least significant difference method. ROC (Receiver Operating Characteristic Curves) were used to compare the relative sensitivity and specificity HBV DNA and HBV RNA as a predictor of HBeAg seroconversion. $p<0.05$ was considered statistically significant.

## 3 Results

### 3.1 Relationship between HBeAg Positive and Serum HBV RNA Levels, HBV DNA Levels at Different Time Points after Antiviral Therapy

After antiviral treatment with nucleoside (NAs) drugs, the number of viruses in HBeAg positive patients showed a downward trend, It was showed the expression level of HBVDNA levels was the fastest and highest decreased in HBeAg positive CHB patients.The conversion of HBeAg and HBVRNA was decreased slower and more synchronous. there was a significant difference in HBV DNA and HBV pgRNA levels After 12 weeks of antiviral treatment,$p<0.05$. The decrease was even more significant after 48 weeks of tantiviral therapy, $p<0.05$. as shown in Table 1.

**Table 1** The Results of HBV pgRNA and HBV DNA Levels at Different Time Points after Tantiviral Therapy

[log10IU/ml, （$\bar{x}$ ±s）]

| Test nucleic acid type | Before treatment | 12weeks | 24weeks | 48weeks | 96weeks |
|---|---|---|---|---|---|
| HBV pgRNA | 6.15±1.49 | 5.11±0.76 | 4.62±0.43 | 3.22±0.36 | 1.54±0.22 |
| HBV DNA | 6.03±1.27 | 5.01±0.84 | 4.57±0.52 | 3.13±0.52 | 1.32±0.35 |

### 3.2 HBV DNA and RNA Associated with HBeAg Seroconversion after Antiviral Treatment with Nucleoside (NAs) Drugs

HBeAg seroconversion in HBeAg-positive CHB patients were the desired treatment endpoints, the results of HBV DNA, and RNA were different in HBeAg-positive CHB patients. The HBeAg positive rate was 71.11%, HBV pgRNA positive rate was 69.63%, and HBV DNA positive rate was 36.30% at 24 weeks after antiviral therapy in 135 HBeAg-positive CHB patients . $p<0.05$. The HBeAg seroconversion was even more significant at 96 weeks with tantiviral therapy. $p<0.05$ as shown in Table 2.

**Table 2** HBeAg Seroconversion in 135 HBeAg-positive CHB Patients

| Testing index | Before treatment | 12weeks | 24weeks | 48weeks | 96weeks |
|---|---|---|---|---|---|
| HBeAg（+） | 135 | 123 | 96 | 71 | 29 |
| HBV pgRNA（+） | 131 | 121 | 94 | 65 | 24 |
| HBV DNA（+） | 129 | 114 | 49 | 34 | 13 |

### 3.3 Predictive Value of HBV pgRNA and HBV DNA for HBeAg Seroconversion in CHB Patients at 24weeks with Antiviral Treatment

ROC curve analysis showed the predictive value of serum HBV pgRNA and HBV DNA in HBeAg seroconversion at 24weeks . The AUC of HBV DNA was 0.725, P=0.011, and AUC of HBV pgRNA was 0.822, P=0.001, indicating that serum HBV DNA was completely suppressed by drugs to undetectable levels, changes in serum HBVRNA levels can be used to predict HBeAg seroconversion.As shown in Figure 1, Table 3.

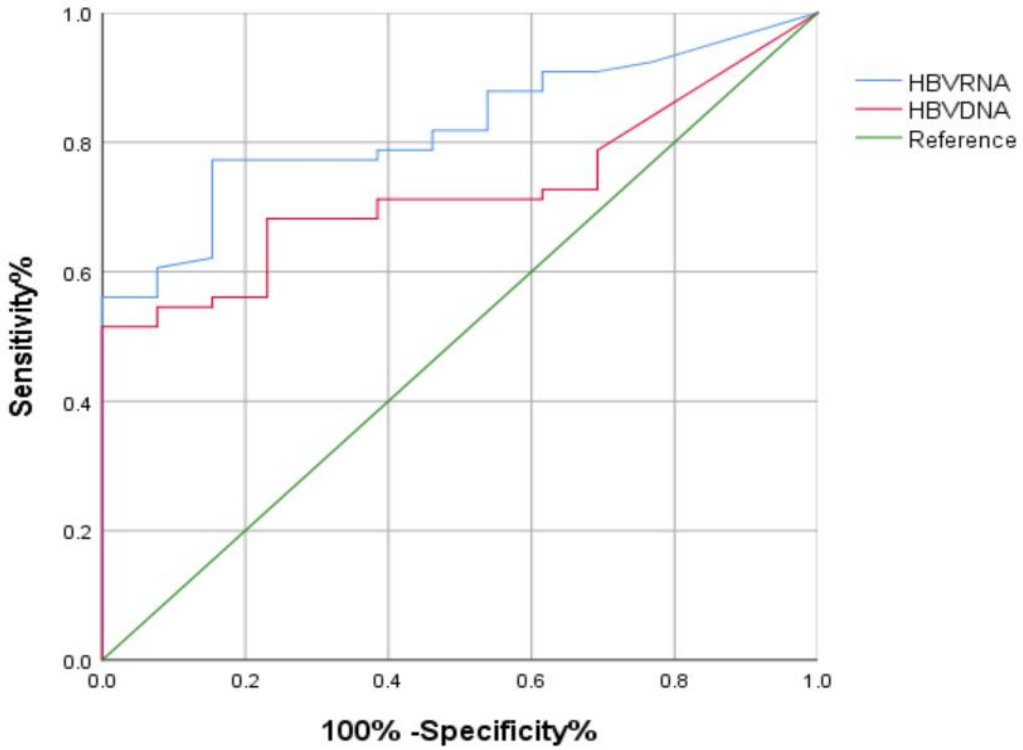


**Figure 1** The ROC Curves of Various Test Indicators Including HBV pgRNA and HBV DNA

**Table 3** The Predictive Value of Serum HBV pgRNA and HBV DNA in HBeAg Seroconversion

| Testing index | AUC | 95%CI | P value | Sensitivity（%） | specificity（%） |
|---|---|---|---|---|---|
| HBV pgRNA | 0.822 | 0.726~0.919 | 0.001 | 92.40 | 76.90 |
| HBV DNA | 0.725 | 0.612~0.838 | 0.011 | 78.80 | 69.20 |

## 4 DISCUSSION

In recent years, with the popularization of hepatitis B vaccine and maternal and infant blocking measures, more and more CHB patients are receiving standardized antiviral treatment such as nucleoside analogues (NUC) and pegylated interferon (PEG-IFN) in China.The clinical outcomes of CHB patients were significant and greatly improving with the prevention and antiviral treatment [6-7]. The HBeAg seroconversion indicates that HBV has entered a low replication stage that it own low probability of progression to cirrhosis and liver cancer, and low infectivity of HBV. It is currently an important target for antiviral treatment of CHB. However, the HBeAg seroconversion in CHB patients is influenced by multiple factors, such as the level of HBV DNA is the most intuitive indicator of virus replication and infectivity. Studies have found that low levels of HBV DNA quantification predict faster negative conversion of HBeAg [8]. Patients with HBV DNA<20 IU/mL are more likely to undergo HBeAg seroconversion at 24 and 48 weeks with antiviral treatment. The HBV DNA level at 24 weeks of antiviral treatment is a predictive factor for HBeAg seroconversion at 96 weeks in CHB patients. These patients with lower HBV DNA levels are more prone to HBeAg seroconversionat at 24 weeks [9].

This study found that serum HBV pgRNA and HBV DNA were decreased significantly in HBeAg positive CHB patients after treatment. HBV pgRNA can be used as an important indicator of HBeAg seroconversion, and the level of HBV RNA is a powerful predictor of HBeAg seroconversion at the 24 weeks. Our results are the same as existing research reports that low levels of HBV RNA can effectively predict HBeAg seroconversion at 6, 12 months with oral NUC treatment [10]. The other study demonstrated that serum HBV RNA levels can serve as a new tool for predicting HBeAg seroconversion during PEG-IFN alpha-2a treatment in CHB patients [10]. Luo found that patients who remained positive for HBV pre genomic RNA at 48 weeks with antiviral treatment were difficult to develop HBeAg seroconversion , and it need long time to develop HBeAg negative conversion [11].In addition, Wu et al investigated the predictive effect of HBV RNA levels on HBeAg seroconversion in pediatric CHB patients,they found HBV RNA

levels could effectively predict HBeAg seroconversion at 12 and 24 weeks with antiviral treatment.Compared with patients who did not undergo HBeAg seroconversion, patients who underwent HBeAg seroconversion had a decrease HBV RNA [12-13]. Therefore, whether it is baseline or dynamic changes after antiviral treatment, low levels of HBV RNA indicate that HBeAg is more likely to undergo HBeAg seroconversion.

The spontaneous or drug-induced HBeAg seroconversion in HBeAg positive CHB patients were important clinical significance and considered a milestone in the CHB treatment. Domestic and foreign studies had shown that HBeAg seroconversion was influenced by various factors, such as virological factors, host factors, immunological factors, etc. However, due to the unclear association mechanism between most factors and HBeAg seroconversion, and it had not been widely used in clinical [14-16]. The next step is to conduct depth research on the relevant mechanisms between different factors and HBeAg seroconversion, establish a predictive model for HBeAg seroconversion, clarify the application scope of different indicators, and verify them through multi center clinical studies. High sensitivity and specificity predictive indicators should be selected for clinical application in order to provide reference for the clinical diagnosis and treatment of CHB patients.

## COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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