Diagnostic value of serum pgRNA detection in HBV-infected patients with different clinical outcomes

Ni Lin, a,b,c* Aizhu Ye, a,b,d* Jinpiao Lin, a,b Can Liu, a,b Jinlan Huang, a,b Ya Fu, a,b Songhang Wu, a,b Siyi Xu, a,b Long Wang, a,b Qishui Ou a,b

*First Clinical College, Fujian Medical University, Fuzhou, China
bDepartment of Laboratory Medicine, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China
cSchool of Medical Technology and Engineering, Fujian Medical University, Fuzhou, China
dDepartment of Blood Transfusion, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China

Correspondence: Qishui Ou, E-mail addresses: ouqishui@fjmu.edu.cn.

*These authors contributed equally to this research.

Abstract

Pregenomic RNA (pgRNA) is a direct transcription product of HBV covalently closed circular DNA (cccDNA) and plays important roles in viral genome amplification and replication. This study was designed to investigate whether serum pgRNA is a strong alternative marker for reflecting HBV cccDNA levels and to analyze the correlation between serum pgRNA, serum HBV DNA and hepatitis B surface antigen (HBsAg). A total of 400 HBV-infected patients who received nucleos(t)ide analogs (NAs) therapy with different clinical outcomes were involved in this research. Case group included asymptomatic hepatitis B virus carrier (ASC), chronic hepatitis B (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC), 100 patients for each group. The results showed that the level of HBV pgRNA had significant difference in these 4 groups. Serum pgRNA levels correlated well with serum HBV DNA levels and HBsAg levels (HBV pgRNA levels vs. HBV DNA levels, r=0.58, P<0.001; HBV pgRNA levels vs. HBsAg levels, r=0.47, P<0.001). In addition, we focused on the 108 HBV-infected patients with HBV DNA <500 IU/ml, it was surprising to find that 17.57% (13/74) of HBV pgRNA can be detected even when HBV DNA level was below 20 IU/ml. In conclusion, HBV pgRNA levels in serum can be a surrogate marker for intrahepatic HBV cccDNA compared with serum HBV DNA and HBsAg. The detection of serum HBV pgRNA levels may provide a reference for clinical monitoring of cccDNA levels and the selection of appropriate timing for discontinuing antiviral therapy, especially when HBV DNA levels are below the detection limit.
Key words: real-time fluorescence quantitative PCR, hepatitis B virus, pregenome RNA, covalently closed circular DNA, clinical outcomes

Introduction

Hepatitis B is a potentially life-threatening liver infection caused by Hepatitis B virus (HBV) (1). Hepatitis B creates a serious global public health problem. About 2 billion people worldwide are infected with HBV, including about 350 million patients with chronic hepatitis B (CHB) (2). CHB is closely related to the occurrence of liver cirrhosis and liver cancer, therefore, the control and treatment of HBV infection has important medical and social significance. While the covalently closed circular DNA (cccDNA) in liver tissue is a key factor reflecting the replication of HBV and the formation of infectious state, the complete elimination of HBV cccDNA has become the “gold standard” for evaluating the cure of CHB patients (3-6). In theory, as long as there is one replication-capable HBV cccDNA remaining in the infected liver cells, there may be a risk of recurrence once antiviral therapy is stopped (7). Studies (8-10) have proposed a “para-functional cure” as the new endpoint of HBV treatment. It is based on the disappearance or silence of HBV cccDNA and uses the sustained negative level HBV RNA and low level of HBsAg as the criteria. It is of great significance in guiding new antiviral drug research and safe drug withdrawal. Therefore, it is important to comprehensively monitor the transcriptional activity of HBV cccDNA in infected cells. However, the detection of intrahepatic HBV cccDNA relies on liver biopsy. Unfortunately, many factors including the damage caused by invasive examination, the low specimen yield, the subjectivity between different observers, and the potential complications of hepatic puncture hinder the widespread application of liver biopsy (11-13).

HBV pregenomic RNA (pgRNA) is an intermediate of HBV replication (14). In recent years, many researchers have confirmed that the HBV pgRNA in serum is derived from the active transcription of HBV cccDNA in the infected hepatocytes (15-17). In particular, the serum HBV RNA level in patients receiving nucleoside(t)ide analogs (NAs) treatment can reflect the presence of the cccDNA and its transcriptional activity in hepatocytes, when the reverse transcription and DNA synthesis of the virus are inhibited (8, 18). These pgRNAs are present in the nucleocapsid of mature viral particles in the form of HBV RNA virus-like particles and are associated with persistent viral infection and virological rebound risk (19). To further verify whether HBV pgRNA in serum is a good alternative marker for reflecting HBV cccDNA levels in hepatocytes, we collected serum samples from 400 patients with different clinical outcomes after HBV infection and who received NAs treatment. We examined the serum levels of HBV pgRNA, HBV DNA, HBsAg and HBeAg, and performed statistical analysis of these serological indicators. The
results will facilitate a better understanding of the infection status of HBV and a better evaluation of the antiviral treatment efficacy. The promising findings here will provide new and potential predictive index for the disease progression of HBV-infected patients with different clinical outcomes and for the judgement of the treatment endpoint.

Materials and methods

1. Study subjects

The subjects of this study were 400 HBV-infected patients with different clinical outcomes from 2015 to 2018 in the department of the Liver Disease Center of the First Affiliated Hospital of Fujian Medical University, who received entecavir (ETV) or tenofovir (TDF) for more than 6 months. Case group included ASC, CHB, LC, and HCC, 100 cases in each of the four groups.

The clinical specimens were collected according to diagnostic criteria of the Chinese Medical Association’s 2013 Expert Consensus on Standardized Diagnosis and Treatment of Primary Liver Cancer, and the 2015 Guidelines for Prevention and Treatment of Chronic Hepatitis B. Serum samples were rapidly stored at -80 ℃ until further use. It should be noted that in all patients in ASC group, HBV-DNA are positive and Histological activity index (HAI) or Fibrosis index (F) is greater than or equal to 2 according to Metavir scoring system. The control group included healthy people and people with other viral infections, such as patients infected with hepatitis C, herpes simplex virus, and Epstein–Barr virus. The above studies are in line with the ethical principles of the Helsinki Declaration and approved by the Ethics Committee of Fujian Medical University. Each patient enrolled has signed informed consent.

2. Cell lines

The stable HBV-expressing human liver cancer cell line HepG2.2.15 was maintained in Dulbecco’s Modified Eagle Medium (Thermo Fisher scientific, Shanghai, China) supplemented with 10% fetal bovine serum (Gibco, Mexico) at 37 ℃ in 5% CO₂.

3. Real-time qPCR to detect serum pgRNA level

The EasyPure Viral RNA Kit (TransGen Biotech, Beijing, China) was used for extraction of total HBV RNA from HepG 2.2.15 cells supernatant. After treated with DNase I (Thermo Fisher Scientific, Waltham, MA, USA), the sample was then reverse transcribed into cDNA using RevertAid First Strand DNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) with a HBV RT primer. The specific upstream and downstream primers of HBV pgRNA were
used in the PCR amplification. After regular PCR amplification, the PCR product was purified with Universal purification and Recovery Kit (Tiangen, Beijing, China), then ligated with pEASY®-Blunt plasmid (TransGen, Beijing, China). The construct was transformed into Trans1-T1 competent cells. Positive colonies were picked and further screened by PCR. The positive plasmids were isolated using Tiangen mini plasmid isolation kit (Tiangen, Beijing, China) and were sequenced. Standard was prepared in 10-fold dilutions by serial dilution of the plasmid with Easy Dilution Buffer (Tiangen, Beijing, China) at $1 \times 10^4$ to $1 \times 10^{10}$ copies/ml. StepOne Plus Fluorescence quantitative PCR machine (Life Technologies, USA) was used to detect the serum pgRNA level of the HBV-infected patients. The specific primers and TaqMan probe used are the same as those mentioned in the previous article (15).

4. Detection of serum HBV DNA, HBsAg and HBeAg levels

HBV DNA was extracted using a Viral Genomic DNA Extraction kit (Beijing Xinmou Company, China) according to the manufacturer’s instruction and then stored at -80°C until used. The level of HBV DNA and high-sensitivity HBV DNA were quantified by the commercially available real-time fluorescence quantitative kit (Shengxiang Biotech Company, Hunan Province, China), then measured on LightCycler 480 fluorescence quantitative PCR machine (F. Hoffmann-La Roche AG, Basel, Switzerland) and ABI 7500 Real-Time PCR machine (Life Technologies, USA). The levels of HBV serum markers HBsAg and HBeAg were quantitatively examined by the commercially available kit (Abbott Laboratories, USA) with the Architect i4000 microparticle chemiluminescence immunoassay analyzer (Abbott Laboratories, USA).

5. Statistical analysis

Serum HBV DNA levels and HBsAg and HBeAg concentrations were log-transformed; data statistics and analysis were performed using SPSS 22.0 software (SPSS Inc., Chicago, USA). Figures was prepared using GraphPad Prism 5.0 software (GraphPad Software, Inc., LaJolla, USA). The data were subjected to the One-Sample Kolmogorov-Smirnov normality test. Data with normal distribution were presented as mean ± standard deviation, and the mean between the two groups was compared using the t test. The One-Way ANOVA test was used to compare normal distribution variables. Data with non-normal distribution were presented as median (P25, P75), and the Kruskal–Wallis test was used to compare abnormal variables. Correlation degree was analyzed using Pearson correlation analysis. The difference was statistically significant at $P < 0.05$.

Results
1. Successfully established Real-Time qPCR method to detect serum pgRNA levels

The established Real-Time qPCR method was evaluated in linear range, lower detection limit, repeatability, specificity and sensitivity. The linear range of the plasmid standard detected by Real-Time qPCR was $1 \times 10^{10} \sim 1 \times 10^3$ copies/ml; the detection limit was $1 \times 10^7$ copies/ml; the intra-assay coefficient of variation was between 0.13% and 0.73%; the inter-assay coefficient of variation was between 0.16% and 0.63%. The specificity was tested with fifteen different serum specimens of control group, including healthy people and people with other viral infections, such as HCV, HSV and EBV. The results showed that the serum levels of HBV pgRNA of those samples were negative and were very specific. In consideration of the complex background of serum samples, we also did recovery experiments to verify the accuracy of the method. According to the calculation formula ($\text{recovery rate} = \frac{\text{recovery concentration}}{\text{addition concentration} \times 100\%}$), the average recovery rate was 93.785%, in line with the requirements of the “PRC Pharmaceutical Industry Standards”(20).

2. Analysis and comparison of serum HBV pgRNA, HBV DNA and HBsAg levels in patients with different clinical outcomes of HBV infection

This study involved 400 HBV-infected patients with different clinical outcomes, including 100 cases in ASC, CHB, LC and HCC groups. The essential information including gender, age, HBV DNA level, HBV pgRNA level, HBsAg level and HBeAg level in 4 groups were presented at Table 2.

In the analysis of the changes in different serological markers of HBV-infected patients with different clinical outcomes, we found that there was a significant difference in HBV pgRNA levels between ASC and LC groups ($4.32 \pm 2.36 \log_{10}$ copies/ml vs. $3.22 \pm 2.13 \log_{10}$ copies/ml, $t = 3.389, P = 0.0009$, FIG 1A), between ASC and HCC groups ($4.32 \pm 2.36 \log_{10}$ copies/ml vs. $3.14 \pm 2.12 \log_{10}$ copies/ml, $t = 3.766, P = 0.0002$, FIG 1A), between CHB and LC groups ($4.41 \pm 2.51 \log_{10}$ copies/ml vs. $3.22 \pm 2.13 \log_{10}$ copies/ml, $t = 4.911, P < 0.001$, FIG 1A), and between CHB and HCC groups ($4.41 \pm 2.51 \log_{10}$ copies/ml vs. $3.22 \pm 2.13 \log_{10}$ copies/ml, $t = 5.275, P < 0.001$, FIG 1A).

In the analysis of the HBV DNA levels in HBV-infected patients with different clinical outcomes, a significant difference in HBV DNA levels was found between ASC and LC groups ($4.42 \pm 1.72 \log_{10}$ copies/ml vs. $3.68 \pm 0.89 \log_{10}$ copies/ml, $t = 3.700, P = 0.0003$, FIG 1B), between ASC and the HCC groups ($4.42 \pm 1.72 \log_{10}$ copies/ml vs. $3.64 \pm 0.65 \log_{10}$ copies/ml, $t = 4.238, P < 0.001$, FIG 1B), between CHB and LC groups ($4.7 \pm 1.77 \log_{10}$ copies/ml vs. $3.68 \pm 0.89 \log_{10}$ copies/ml, $t = 5.275, P < 0.001$, FIG 1B).
In the analysis of the HBsAg levels of HBV-infected patients with different clinical outcomes, the results showed that there was a significant difference in HBsAg levels between ASC and LC groups (7393.32 ± 16302.77 IU/ml vs. 1831.56 ± 6005.84 IU/ml, t = 3.552, P = 0.0014, FIG 1C), between ASC and the HCC groups (7393.32 ± 16302.77 IU/ml vs. 1276.72 ± 1697.07 IU/ml, t = 2.784, P = 0.0081, FIG 1C), between CHB and LC groups (6157.9 ± 11583.95 IU/ml vs. 1831.56 ± 6005.84 IU/ml, t = 5.076, P < 0.001, FIG 1C), and between HCC and CHB groups (6157.9 ± 11583.95 IU/ml vs. 1276.72 ± 1697.07 IU/ml, t = 4.405, P < 0.001, FIG 1C).

3. Correlation analysis of different serological indicators in HBV-infected patients

By analyzing the correlation between different serological indicators of HBV-infected patients, a positive correlation was found between HBV DNA, HBV pgRNA and HBsAg levels (HBV pgRNA group vs. HBV DNA group: r = 0.58, P < 0.001, FIG 2A; HBV pgRNA group vs. HBsAg group: r = 0.47, P < 0.001, FIG 2B; HBsAg group vs. HBV DNA group: r = 0.45, P < 0.001, FIG 2C).

4. Correlation analysis of different serological indicators of HBV-infected patients with different clinical outcomes

In the correlation analysis of different serological markers of HBV-infected patients with different clinical outcomes, it showed that there was a positive correlation between HBV pgRNA and HBV DNA levels in ASC and CHB groups (ASC group: r = 0.71, P < 0.001, FIG 3A; CHB group: r = 0.62, P < 0.001, FIG 3B), between HBV pgRNA and HBsAg levels in ASC and CHB groups (ASC group: r = 0.44, P < 0.001, FIG 4A; CHB group: r = 0.54, P < 0.001, FIG 4B), between HBV DNA and HBsAg levels in ASC and CHB groups (ASC group: r = 0.51, P < 0.001, FIG 5A; CHB group: r = 0.45, P < 0.001, FIG 5B).

5. Comparing the levels of HBV pgRNA and HBV DNA and their correlation in HBeAg positive and negative groups

The specimens of 400 HBV patients were divided into HBeAg positive group and HBeAg negative group. Analysis showed that HBV pgRNA and HBV DNA levels were significantly different in HBeAg positive group (5.04 ± 2.49 log10 copies/ml and 4.85 ± 1.97 log10 copies/ml) and HBeAg negative group (3.11 ± 1.99 log10 copies/ml and 3.72 ± 0.91 log10 copies/ml) (HBV pgRNA group: t = 9.987, P < 0.001; HBV DNA group: t = 7.545, P < 0.001, FIG 6A).
pgRNA and HBV DNA levels were positively correlated in both HBeAg positive and HBeAg negative groups, but it was better in the former than in the latter (HBeAg positive group: \( r = 0.57, P < 0.001 \), FIG 6B; HBeAg negative group: \( r = 0.31, P < 0.001 \), FIG 6C).

6. Analysis of detection rate of HBV pgRNA in specimens with HBV DNA < 500 IU/ml

Detection and statistical analysis of serological markers in 400 HBV-infected patients indicated that, the detection rate of HBV pgRNA in serum samples with HBV DNA < 500 IU/ml was 78.9%, and the undetected rate was 21.1%. Based on this result, we selected 108 HBV-infected and HBeAg negative patients for further analysis, including 74 specimens with HBV DNA less than 20 IU/ml and 34 specimens with HBV DNA more than 20 IU/ml but less than 500 IU/ml.

The statistical analysis of the detection rate of HBV pgRNA in both groups indicated 17.57% (13/74) and 41.18% (14/34), respectively. The undetected rate of HBV pgRNA in both groups was 82.43% (61/74) and 58.82% (20/34), respectively. The essential information of 108 patients were presented at Table 3.

Discussion

Chronic hepatitis B affects approximately 2 billion people worldwide, with an estimated 15%-40% progressing to cirrhosis or hepatocellular carcinoma(21). Early diagnosis and treatment of CHB can reduce the incidence of cirrhosis and HCC. It always has been a challenge to find biological markers in peripheral blood that can effectively reflect the transcriptional activity of hepatitis virus and observe the therapeutic effect of drugs. Currently, we mainly rely on the levels of HBsAg, HBeAg, HBV DNA and ALT in serum, combined with liver fibrosis examination, in order to divide the process of HBV infection, determine the timing of treatment and judge the timing of drug withdrawal. In this study, we successfully established Real-Time qPCR method to quantitatively detect HBV pgRNA levels in serum of HBV-infected patients. Analysis of the correlation between pgRNA and traditional serological and molecular markers to prove whether it can be used as an alternative marker.

Studies have reported that the level of serum HBsAg in CHB patients is closely related to the transcriptional status of HBV cccDNA in the nucleus of hepatocytes (22). The decrease of serum HBsAg level was correlated with the decrease of serum HBV DNA level or HBV cccDNA level in hepatocyte nucleus, suggesting that to a certain extent the level of serum HBsAg could reflect the clearance of HBV cccDNA in hepatocyte nucleus. Our results showed that HBV DNA and HBsAg levels were significantly different among the four groups. It is worth noting that both HBV DNA and HBsAg levels were higher in the CHB group than in the LC group (\( t = 5.375, P <\))
0.001; \( t = 5.076, P < 0.001 \), which is consistent with the literature mentioned above. In addition, the expression of serum HBV pgRNA in CHB and LC groups \( (t = 4.911, P < 0.001) \) was also consistent with that of HBV DNA and HBsAg. The disappearance of serum HBsAg is considered to be the standard for CHB “functional cure” \( (13, 23) \). However, due to the frequent integration of HBV DNA into HBV-infected hepatocytes \( (18, 24) \), it may be the reason for the sustained low expression of serum HBsAg. Under these circumstances, serum HBsAg may not reflect the activity of cccDNA in the liver. Unlike serum HBsAg, 3.5 kb serum pgRNA is produced only from cccDNA. Therefore, serum HBV RNA can accurately reflect the status of cccDNA in the liver.

Studies have shown that there is a positive correlation between full-length HBV RNA and HBV DNA in serum of CHB patients \( (25) \). Our results also confirmed that serum HBV pgRNA level and HBV DNA expression level were well correlated in HBV-infected patients \( (r = 0.58, P < 0.001) \). Serum HBV pgRNA level was also positively correlated with HBsAg level \( (r = 0.47, P < 0.001) \). There were some correlations between serum HBV pgRNA, HBV DNA and HBsAg levels in HBV-infected patients with different clinical outcomes. Particular in ASC group and CHB group, the levels of HBV pgRNA and HBV DNA \( (\text{ASC group: } r = 0.71, P < 0.001; \text{CHB group: } r = 0.62, P < 0.001) \), HBV pgRNA and HBsAg \( (\text{ASC group: } r = 0.44, P < 0.001; \text{CHB group: } r = 0.54, P < 0.001) \) and HBV DNA and HBsAg \( (\text{ASC group: } r = 0.51, P < 0.001; \text{CHB group: } r = 0.45, P < 0.001) \) were positively correlated. These results suggest that the detection of HBV pgRNA levels in serum of HBV-infected patients is comparable with the detection of other traditional diagnostic indicators. It is worth noting that despite positive correlations among the various HBV serologic and molecular markers, the degree of correlation was not good. The weaker correlation coefficient value might be attributed to the accumulation of viral variation, as well as the diversity of host background in clinical research.

At present, the evaluation of the efficacy of antiviral therapy relies on the detection of HBV DNA levels. However, HBV DNA in HBV-infected patients below the detection limit only indicates that the reverse transcription of the virus is inhibited, and cannot be used as a clinical index for drug withdrawal \( (7, 26) \). To validate this theory, we analyzed the serological markers of 400 HBV-infected patients with HBV DNA < 500 IU/ml, from which the detection rate of HBV pgRNA was 78.9%. Intrigued by this finding, we selected 108 HBeAg negative patients with HBV DNA < 500 IU/ml and further divided these clinical specimens into two groups according to HBV DNA levels. In one group, 74 patients had HBV DNA levels below 20 IU/ml. In another group, 34 patients had HBV DNA levels above 20 IU/ml but below < 500 IU/ml. We were
surprised to find that 17.57% (13/74) of HBV pgRNA can be detected even when HBV DNA level was below 20 IU/ml. On the other hand, this suggested that the HBV DNA level below the detection limit cannot be used as a clinical index for drug withdrawal and that it provides a favorable proof for the previously proposed concept of para-functional cure (27, 28). Therefore, the detection of HBV DNA copy only to assess the efficacy of antiviral therapy has certain limitations. Compared with HBV DNA, serum HBV pgRNA has an advantage in monitoring changes in sustained viral response and cccDNA levels during treatment.

In summary, the detection of serum HBV pgRNA levels in HBV-infected patients can be a potential alternative marker and provides a reference for clinical monitoring of cccDNA levels and the selection of appropriate timing for discontinuing antiviral therapy. Especially when HBV DNA levels are below the detection limit, the detection of HBV pgRNA levels can help to determine whether the criteria for para-functional cure are met and to evaluate the efficacy of antiviral therapy. It can provide meaningful guidance for rational clinical medication.

Acknowledgments

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References


FIG 1. Analysis and comparison of serum HBV pgRNA, HBV DNA and HBsAg levels in patients with different clinical outcomes of HBV infection. The baseline serum levels of HBV pgRNA (1A), HBV DNA(1B), HBV HBsAg(1C) were compared between the ASC group (n = 100), CHB group (n = 100), LC group (n = 100) and HCC group (n = 100).

FIG 2. Correlation analysis of different serological indicators in HBV-infected patients. The correlation between HBV pgRNA and HBV DNA was analyzed in patients with HBV infection patients. (2A); The correlation between HBV pgRNA and HBsAg was analyzed in patients with HBV infection patients. (2B); The correlation between HBsAg group and HBV DNA was analyzed in patients with HBV infection patients. (2C).

FIG 3. Analysis of the correlation between serum HBV pgRNA and HBV DNA in patients with ASC (3A), CHB (3B), LC (3C) and HCC (3D).

FIG 4. Analysis of the correlation between serum HBV pgRNA and HBsAg in patients with ASC (4A), CHB (4B), LC (4C) and HCC (4D).

FIG 5. Analysis of the correlation between serum HBV DNA and HBsAg in patients with ASC (5A), CHB (5B), LC (5C) and HCC (5D).

FIG 6. Comparison of serum levels of HBV pgRNA and HBV DNA according to HBeAg classification and the correlation of serum levels of HBV pgRNA and HBV DNA classified by HBeAg. The baseline serum levels of HBV pgRNA and HBV DNA were compared between the HBeAg(+) and HBeAg(-) patients(6A); The correlation of serum levels of HBV pgRNA and HBV DNA was analyzed in HBeAg(+) patients(6B); The correlation of serum levels of HBV pgRNA and HBV DNA was analyzed in HBeAg(-) patients(6C).
The figure illustrates the relationship between HBV DNA (log10 copies/ml) and HBSAg (log10 IU/ml) in different groups: LC group, ASC group, HCC group, and CHB group.

- **A**: LC group
- **B**: ASC group
- **C**: HCC group
- **D**: CHB group

The y-axis represents HBSAg (log10 IU/ml), and the x-axis represents HBV DNA (log10 copies/ml). The trend lines and p-values are as follows:

- **LC group**: 
  - HBV DNA: $10^7$ copies/ml, $r = 0.3$, $p = 0.026$
  - HBSAg: $r = 0.2$, $p = 0.045$

- **ASC group**: 
  - HBV DNA: $10^7$ copies/ml, $r = 0.51$, $p < 0.001$
  - HBSAg: $r = 0.51$, $p < 0.001$

- **HCC group**: 
  - HBV DNA: $10^7$ copies/ml, $r = 0.045$, $p = 0.001$
  - HBSAg: $r = 0.045$, $p = 0.001$

- **CHB group**: 
  - HBV DNA: $10^7$ copies/ml, $r = 0.02$, $p = 0.045$
  - HBSAg: $r = 0.02$, $p = 0.045$

The data suggests a significant correlation between HBV DNA and HBSAg levels in all groups, with stronger correlations observed in the ASC and CHB groups.
Table 1. Primers and probe for real-time qPCR

<table>
<thead>
<tr>
<th>Primer names</th>
<th>Sequences (5'-3')</th>
<th>Length (bp)</th>
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<tbody>
<tr>
<td>Forward primer</td>
<td>AYAGACCATCAA ATGCC</td>
<td>167 bp</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>ATTTCAGACGTCAGACACGACAC</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>CTTATCAACACTTCGGARACTACTGTTGTTAGAC</td>
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Table 2. Clinical characteristics of patients with different clinical outcomes of HBV infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>ASC</th>
<th>CHB</th>
<th>LC</th>
<th>HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>67 / 33</td>
<td>56 / 44</td>
<td>72 / 28</td>
<td>87 / 13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.87±12.5</td>
<td>36.75±12.02</td>
<td>54.9±12.69</td>
<td>55.79±12.39</td>
</tr>
<tr>
<td>HBV viral load (Log10 copies/ml)</td>
<td>4.42±1.72</td>
<td>4.7±1.77</td>
<td>3.68±0.89</td>
<td>3.64±0.65</td>
</tr>
<tr>
<td>HBV pgRNA (Log10 copies/ml)</td>
<td>4.32±2.36</td>
<td>4.41±2.51</td>
<td>3.22±2.13</td>
<td>3.14±2.12</td>
</tr>
<tr>
<td>HBeAg (IU/ml)</td>
<td>7393.32±16302.77</td>
<td>6157.9±11583.95</td>
<td>1831.56±6005.84</td>
<td>1276.72±1697.07</td>
</tr>
<tr>
<td>HBeAg (S/CO)</td>
<td>251.15±537.47</td>
<td>207.48±485.05</td>
<td>52.39±215.95</td>
<td>9.15±77.28</td>
</tr>
</tbody>
</table>

Abbreviations: ASC, Asymptomatic hepatitis B virus carrier; CHB, Chronic hepatitis B; LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; pgRNA, pregenomic RNA; HBsAg, hepatitis B virus surface antigen; HBeAg, hepatitis B virus e antigen.
<table>
<thead>
<tr>
<th>Variables</th>
<th>20IU/ml &lt; HBV DNA &lt;500IU/ml</th>
<th>HBV DNA&lt; 20IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>25/9</td>
<td>54/20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.5±12.20</td>
<td>45±14.58</td>
</tr>
<tr>
<td>HBV viral load (Log&lt;sub&gt;10&lt;/sub&gt; copies/ml)</td>
<td>2.70±0.37</td>
<td>2.04±0.39</td>
</tr>
<tr>
<td>HBV pgRNA (Log&lt;sub&gt;10&lt;/sub&gt; copies/ml)</td>
<td>0 (0, 0.63)*</td>
<td>2.04 (1.68, 2.04)*</td>
</tr>
<tr>
<td>HBsAg (IU/ml)</td>
<td>2241.47±717.09</td>
<td>1394.34±370.55</td>
</tr>
<tr>
<td>HBeAg(S/CO)</td>
<td>0.47±0.25</td>
<td>0.39±0.19</td>
</tr>
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*: Non-normal distribution data, results expressed in median(P25, P75)