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Correlation between HBeAg and Hepatitis B Virus DNA and RNA Levels in Diverse Liver Disease Cohorts

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Abstract: *Objective:* To investigate the disparities and associations between HBV DNA and HBV RNA in various liver disease groups with respect to HBeAg status. *Methods:* Between September 2020 and September 2023, 90 patients diagnosed with chronic hepatitis B (CHB), 74 patients diagnosed with liver cirrhosis (LC), and 102 patients diagnosed with hepatocellular carcinoma (HCC) from the Department of Gastroenterology or Infection at the First Affiliated Hospital of Xi'an Jiaotong University were selected. HBV DNA, HBV RNA, and HBeAg quantitative tests were conducted using serum samples from the same patients. *Results:* In the three groups of cases, the HBV RNA load was higher when HBeAg was positive than when HBeAg was negative, and this difference was statistically significant. Only in the HCC group was the HBV DNA load significantly higher when HBeAg was positive than when HBeAg was negative. Additionally, there was a positive correlation between HBV DNA and HBV RNA regardless of HBeAg status. *Conclusion:* During HBeAg conversion, HBV RNA demonstrates a more sensitive response than HBV DNA. As CHB progresses to LC or HCC, HBV RNA exhibits better diagnostic value than HBV DNA.

Keywords: Hepatitis B virus DNA; Hepatitis B virus RNA; HBeAg

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1. Introduction

Human hepatitis B virus (HBV) is a small group of hepatophilic DNA viruses that infect more than 258 million people worldwide [1]. Chronic HBV infection is the primary cause of viral hepatitis, with approximately one-third of HBV carriers developing liver cirrhosis (LC), and 11% progressing to hepatocellular carcinoma (HCC) [2]. While prophylactic vaccines are available to prevent HBV infection, there is currently no cure for patients with chronic hepatitis B (CHB) [3].

Hepatitis B e antigen (HBeAg) is produced from the core pre-mRNA of HBV. It is an unstructured protein that can be released from infected hepatocytes into the bloodstream, where it functions as a soluble protein [4]. HBeAg can act as a tolerogen, modulating the host immune response and inhibiting the cytotoxic activity of host T-cells, leading to immune tolerance against HBV infection. Additionally, HBeAg acts as an immunogen, protecting hepatocytes from apoptosis by interfering with hepatocyte signaling pathways as a target of the

immune response. While HBeAg is not essential for viral replication or infection, it plays a crucial role in virus-hepatocyte interactions and the establishment of chronic HBV infection. It also serves as a marker of HBV replication and infectivity [5].

HBV DNA reflects the extent of active replication of the hepatitis B virus. A higher viral load indicates more active replication and increased infectivity. Serum HBV RNA is directly transcribed from covalently closed circular DNA (cccDNA) in infected hepatocytes, and most of it is reverse-transcribed into HBV DNA and released into the serum. Serum HBV RNA levels can reflect the presence of cccDNA and its transcriptional activity in hepatocytes [6]. However, the biological significance and clinical relevance of serum HBV RNA in the course of HBV infection remain unclear. An early study suggested that serum HBV RNA might be a valuable marker for distinguishing the stages of chronic HBV infection [7]. In this study, the levels of HBV DNA and HBV RNA before and after HBeAg conversion were compared separately in different disease groups, aiming to assess the clinical utility of these two assays.

2. Materials and methods

2.1. General information

A total of ninety patients diagnosed with CHB, 74 patients diagnosed with LC, and 102 patients diagnosed with HCC who had sought treatment at the Department of Gastroenterology or the Department of Infection at the First Affiliated Hospital of Xi'an Jiaotong University from September 2020 to September 2023 were selected.

The inclusion criteria included patients with HBV infection for more than half a year, patients aged between 18–80 years, and patients willing to provide informed consent.

The exclusion criteria included patients with severe liver-related complications (including decompensated cirrhosis, hepatocellular carcinoma, and liver transplantation) and patients co-infected with human immunodeficiency virus (HIV) or hepatitis C virus (HCV).

This study adhered to the ethical principles of the Declaration of Helsinki, and the protocol received approval from the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All patients provided informed consent.

2.2. Laboratory testing

HBV DNA and HBV RNA were quantified using real-time fluorescence quantitative polymerase chain reaction (PCR), following the reagent instructions (Zhongshan Daan, Guangzhou, China). An ABI7500 fluorescence quantitative PCR instrument from the USA was used. The lower limit of detection for HBV DNA was 20 IU/mL, and for HBV RNA, it was 100 copies/mL.

HBeAg was detected using the chemiluminescence method with the fully automatic chemiluminescence immunoassay analyzer (ALINITYI, Abbott, USA), and the associated reagents for HBeAg detection. The test unit was S/CO, with results considered negative if < 1 and positive if ≥ 1.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (IBM, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD), and differences in means were analyzed using Student's *t*-test. According to the reagent manufacturer's instructions, HBV DNA was compared to HBV RNA using a conversion factor of 1 IU = 5 copies. Correlation tests were analyzed using Pearson's correlation analysis. Significance levels were denoted as follows: * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$, **** for $P < 0.0001$, and ns for $P > 0.05$. A significance level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. HBV DNA and HBV RNA in CHB in relation to HBeAg in the CHB group

In the hepatitis group, the HBV DNA load in the HBeAg-positive group was higher than that in the HBeAg-negative group, but this difference was not statistically significant ($P > 0.05$). However, the HBV RNA load in the HBeAg-positive group was significantly higher than that in the HBeAg-negative group ($P < 0.0001$), as shown in **Figure 1**.

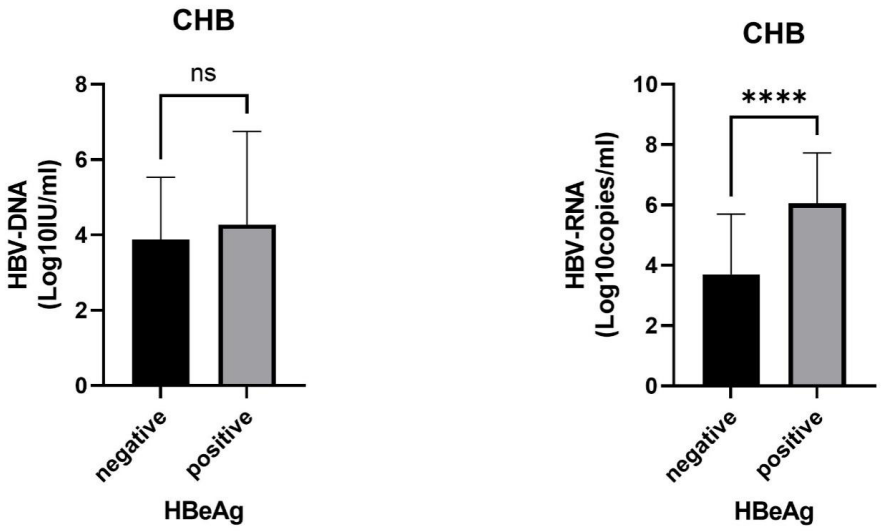

Figure 1. HBV DNA and HBV RNA load changes before and after HBeAg conversion in the CHB group

3.2. HBV DNA and HBV RNA in relation to HBeAg in the LC group

In the cirrhosis group, the HBV DNA load in the HBeAg-positive group was higher than that in the HBeAg-negative group, but this difference was not statistically significant ($P > 0.05$). However, the HBV RNA load in the HBeAg-positive group was significantly higher than that in the HBeAg-negative group ($P < 0.001$), as shown in **Figure 2**.

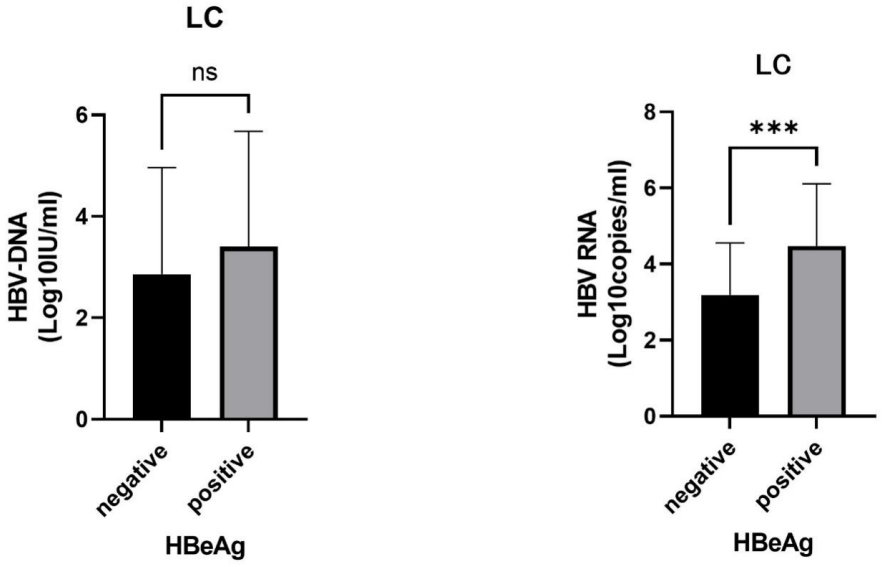

Figure 2. HBV DNA and HBV RNA load changes before and after HBeAg conversion in the LC group

3.3. HBV DNA and HBV RNA in relation to HBeAg in the HCC group

In the hepatocellular carcinoma group (**Figure 3**), the HBV DNA load in the HBeAg-positive group was substantially higher than that in the HBeAg-negative group ($P < 0.01$), while the HBV RNA load in the HBeAg-positive group was significantly higher than that in the HBeAg-negative group ($P < 0.0001$).

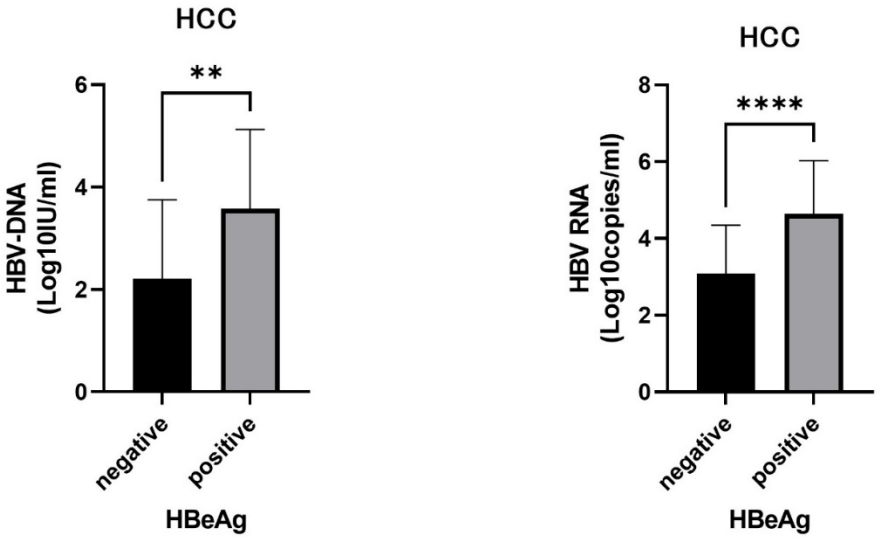

Figure 3. HBV DNA and HBV RNA load changes before and after HBeAg conversion in the HCC group

3.4. Correlation of HBV DNA with HBV RNA

The correlation between HBV DNA and HBV RNA was further examined. The results indicated a positive correlation between HBV DNA and HBV RNA, both in the HBeAg-negative group ($r = 0.6085$) and the HBeAg-positive group ($r = 0.5741$).

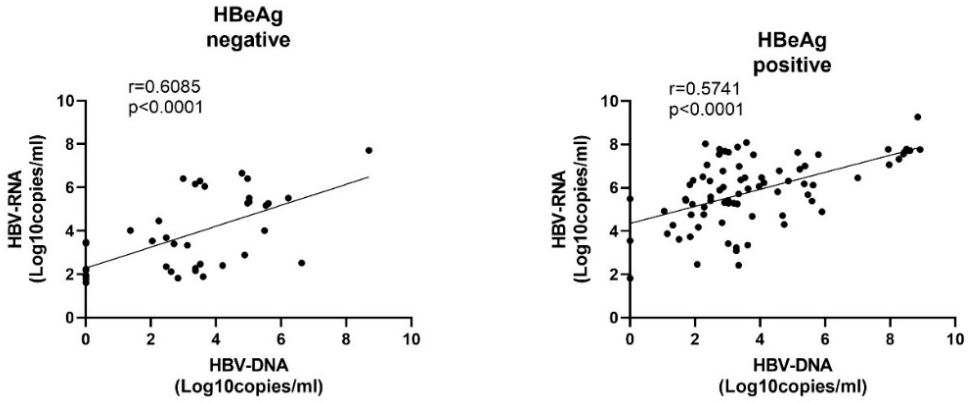

Figure 4. Correlation analysis of HBV DNA and HBV RNA before and after HBeAg conversion

4. Discussion

The core HBV gene encodes HBeAg, which is secreted from infected hepatocytes and serves as a marker of active viral replication. Both HBeAg and its antibody, HBeAb, can be quantitatively measured through immunoassays. HBeAg typically becomes detectable 6–12 weeks after exposure to HBV, indicating a high level of HBV DNA and infectivity. Seroconversion from HBeAg to anti-HBe is a crucial milestone in disease

progression, often signaling the transition to an inactive infection state [8]. The seroconversion of HBeAg is a key prognostic factor. Persistent HBeAg positivity in patients with CHB is indicative of ongoing HBV infection, which is associated with active hepatitis and an increased risk of LC. High serum HBeAg levels are correlated with the progression to LC and HCC [9].

In this study, higher HBV DNA and HBV RNA loads were observed in the HBeAg-positive group compared to the HBeAg-negative group within the CHB cohort. Notably, the difference in HBV DNA load was not statistically significant, but the difference in HBV RNA load was statistically significant (**Figure 1**). The HBeAg-positive phase in the context of long-term persistent HBV infection is characterized by immune-mediated liver injury and fluctuating HBV titers. High HBV titers and active viral replication are observed in this phase [10]. The transition to an anti-HBeAg-positive status signifies the entry into the inactive HBV-carrying phase, known as HBeAg-negative chronic hepatitis, characterized by lower HBV titers and slower progression of liver injury compared to the previous immune response phase [11].

A small subset of CHB patients may ultimately clear HBV, entering the HBsAg-negative phase in which serum HBV DNA becomes undetectable [12], marking a "functional cure." However, since viral cccDNA persists in the liver, HBV can reactivate with certain immunosuppressive treatments. Typically, end-stage liver diseases related to HBV (including LC and HCC) may develop within a few decades after this stage [13]. When HBV infection tends to resolve, clinical symptoms may become less apparent and easily overlooked. Symptoms that arise before treatment often indicate advanced liver cancer, which is challenging to treat at this stage. HBV DNA positivity in HBeAg-negative patients suggests viral mutations that escape immune surveillance, increasing the risk of cancerous cell formation and the likelihood of developing cirrhosis and liver cancer.

In this study, the HBV DNA load in HBeAg-positive patients in the LC group was higher than in HBeAg-negative patients, although this difference was not statistically significant. However, the HBV RNA load in the LC group was significantly higher in the HBeAg-positive group compared to the HBeAg-negative group (**Figure 2**). In contrast, in the HCC group, both HBV DNA and HBV RNA levels were higher in the HBeAg-positive group than in the HBeAg-negative group, and these differences were statistically significant, with HBV RNA demonstrating a slightly stronger difference compared to HBV DNA (**Figure 3**). These findings suggest that HBV RNA is more responsive to HBeAg conversion in earlier stages of the disease, such as CHB and LC. However, as the disease progresses to HCC, both HBV DNA and HBV RNA become equally valuable in assessing changes before and after HBeAg conversion.

HBV RNA levels were correlated with HBV DNA levels, varying with the stage of infection. The highest levels were observed during the immune tolerance period, whereas patients with inactive HBV infection had the lowest levels [14]. This study found a positive correlation between HBV DNA and HBV RNA, regardless of HBeAg status (**Figure 4**). This indicates that HBeAg conversion does not alter the relationship between HBV DNA and HBV RNA. Some studies have investigated whether measuring HBeAg levels can serve as a substitute for quantifying HBV DNA. In a study of 82 patients in Malaysia, HBeAg levels were correlated with HBV DNA levels above 300 IU/mL ($r = 0.893$) but not with HBsAg levels [15].

While seroconversion of HBeAg is widely regarded as a treatment endpoint [16], it is important to note that HBeAg presence primarily indicates active viral replication and active liver disease. In a subset of HBeAg-negative patients, detectable HBV-DNA and active hepatitis may still be present when using low-sensitivity molecular hybridization techniques for serum HBV DNA detection [17]. Furthermore, first-line antiviral agents (e.g., nucleotide analogs) can reduce serum HBV DNA to undetectable levels by interfering with the viral transcription process, but HBV RNA quantification remains unaffected [18]. Thus, serum HBV RNA is considered a novel marker for monitoring antiviral efficacy, especially in virologically suppressed patients who

cannot achieve HBV DNA suppression under nucleotide analogs therapy [19]. This study illustrates that while HBV DNA and HBV RNA exhibit a certain degree of correlation, HBV RNA demonstrates greater sensitivity and superior predictive value in disease assessment compared to HBV DNA.

Disclosure statement

The authors declare no conflict of interest.

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