Correlation between hepatitis B virus DNA levels and diagnostic tests for HBsAg, HBeAg, and PreS1-Ag in chronic hepatitis B

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ABSTRACT. The aim of this study was to investigate the diagnostic value of the serum markers HBsAg and HBeAg and PreS1 protein (PreS1-Ag) in quantifying the levels of hepatitis B virus (HBV) DNA in patients with chronic hepatitis B (CHB). One thousand CHB patients were recruited from Beijing You'an Hospital between June and December 2012. Serum HBsAg and HBeAg levels were detected by electrochemiluminescence immunoassay. Enzyme-linked immunosorbent assay and fluorescence quantitative PCR were used to determine the level of PreS1-Ag and HBV DNA, respectively. We observed a low correlation between HBsAg and HBV DNA ($r = 0.172$, $P < 0.001$) expression; however, the correlation coefficient increased gradually with the increase in HBV DNA levels, and was more significant when HBV DNA $\log_{10} > 7$ ($r = 0.597$, $P < 0.001$). Additionally, HBsAg and HBV DNA showed a significant positive correlation in the HBeAg+ group ($r = 0.321$, $P < 0.001$), whereas no correlation was observed in the HBeAg- group ($r = -0.016$, $P = 0.825$).

HBV DNA expression was correlated with HBeAg (χ^2 = 83.07, P < 0.001) and PreS1-Ag (χ^2 = 36.01, P < 0.001). HBV DNA-positive rate was higher in HBeAg/PreS1-Ag++ patients (72.26%) than that in the single-positive groups (P < 0.001). Therefore, serum HBsAg is not a good marker for the prediction of HBV replication, and co-detection of HBeAg and PreS1-Ag, which can better predict HBV DNA replication, can be used as a reliable method for the clinical diagnosis and treatment of CHB.

Key words: Clinical diagnosis; Hepatitis B e antigen; Hepatitis B surface antigen; Hepatitis B virus DNA; PreS1 protein

INTRODUCTION

Chronic hepatitis B (CHB) infections are a major global health concern. According to a 2012 report by the World Health Organization, approximately 2 billion people have been infected with the hepatitis B virus (HBV) worldwide so far. Moreover, an estimated 350-400 million people have been identified as carriers of the chronic HBV surface antigen (HBsAg) by serological examination (EASL, 2012). HBV infections lead to CHB, liver cirrhosis, and hepatocellular carcinoma.

Several clinical diagnostic tests have been developed for the detection of HBV infections. The serum HBV DNA level is a key factor affecting the initiation of antiviral therapy and evaluation of its efficacy (Mommeja-Marin et al., 2003; Degertekin and Lok, 2009; Lim et al., 2009). Evaluation of the relationship between the serum HBV DNA levels and hepatic pathology is a current hotspot in the diagnosis and treatment of CHB (Nguyen and Keeffe, 2009; Chien, 2010; Hansen et al., 2010). Quantification of the HBsAg levels has received renewed attention because of its diagnostic potential in predicting the response to antiviral treatment and identifying the infection status of an individual (Brunetto et al., 2010; Chan et al., 2011; Liaw, 2011; Moucari and Marcellin, 2011). Determination of the circulating levels of HBsAg could provide crucial information that could complement the measurement of HBV DNA. Studies of HBV infections conducted under various clinical settings have suggested that serum HBsAg could be used as a combinative or substitutive marker of HBV DNA levels (Chan et al., 2011; Moucari and Marcellin, 2011). The hepatitis B e antigen (HBeAg) is secreted by the HBV envelope and is believed to directly predict the replication and infectivity of HBV DNA. The PreS1 protein (PreS1-Ag) has multiple biological functions, such as mediating the attachment of virions to putative cellular receptors on the membrane of host hepatocytes, playing a vital role in viral replication, and regulating a wide range of promoter elements (Chen et al., 2011). The PreS1 region is located on the surface of HBV, and is reported to significantly influence viral infectivity (Petersen et al., 2008). Experimental evidence indicates that the PreS1-Ag directly participates in hepatocyte receptor binding (Xiao et al., 2003), thereby serving as a reasonable biomarker for the detection of HBV viral infection.

A number of related markers are involved in the prediction of HBV DNA levels in clinical practice. In this study, the levels of serum markers HBsAg and HBeAg and the PreS1 antigen were determined and their correlation with serum HBV DNA levels was analyzed to investigate their diagnostic value in the determination of HBV DNA levels in patients with CHB.

MATERIAL AND METHODS

Patients

Serum samples were collected from 1000 CHB patients of the Beijing You'an Hospital between June and December 2012. The diagnostic criteria for CHB was set according to the 2010 revised edition of the guidelines for the prevention and treatment of CHB (Sun and Hou, 2010). Patients with CHB who were co-infected with hepatitis C, hepatitis D, or human immunodeficiency virus were excluded from this study. The selected patients [age range: 37 (4-80) years; 688 (68.8%) males] were at different levels of disease progression, and presented different HBsAg levels (>1 IU/mL).

Quantification of serum HBsAg and HBeAg levels

Serum HBsAg and HBeAg levels were detected by an automated electrochemiluminescence immunoassay using a Roche Cobas e601 (Roche Diagnostics, Basel, Switzerland). The upper limit of the HBsAg concentration is 5200 IU/mL. The HBV DNA load was determined using the completely automated COBAS AmpliPrep-COBAS TaqMan 48 system (v.2.0; Roche Molecular Systems, Branchburg, NJ, USA) with a lower detection limit of 20 IU/mL. The linear determination range was 2.0×10-1.7×10^8 IU/mL. The PreS1-Ag levels were determined by a standard enzyme-linked immunosorbent assay (Alpha, Shanghai, China) according to the manufacturer instructions.

Statistical analysis

The data were statistically analyzed using SPSS v.17.0 (SPSS Inc., Chicago, IL, USA). All continuous variables are reported as the median and range. The correlation between serum HBsAg levels and HBV DNA levels was analyzed by the Spearman rank correlation test. The variables were compared between groups using the Pearson χ^2 test, the Mann-Whitney U-test, and the Kruskal-Wallis test. The association between categorical variables was analyzed using the log-likelihood ratio test. Matching analysis was performed using the McNemar chi-square test. P values <0.05 were considered to indicate statistically significant results.

RESULTS

Overall correlation between HBsAg and HBV DNA levels

The association between HBsAg and HBV DNA was analyzed in 473 samples, obtained after excluding those samples with HBsAg or HBV DNA levels beyond the linear range. This analysis revealed a positive correlation between HBsAg and HBV DNA (relative coefficient (r) = 0.172, P < 0.001; Figure 1).

Association between HBsAg and HBV DNA based on the HBV DNA levels

The 473 cases were divided into four groups based on the HBV DNA levels, as shown in Figure 2. We observed a significant correlation (r = 0.597, P < 0.001) between HBsAg and

HBV DNA in the HBV DNA $\log_{10} > 7$ group. Moreover, we observed an increase in the r value with the gradual increase in HBV DNA level, except in the $\log_{10} = 3\text{-}5$ group.
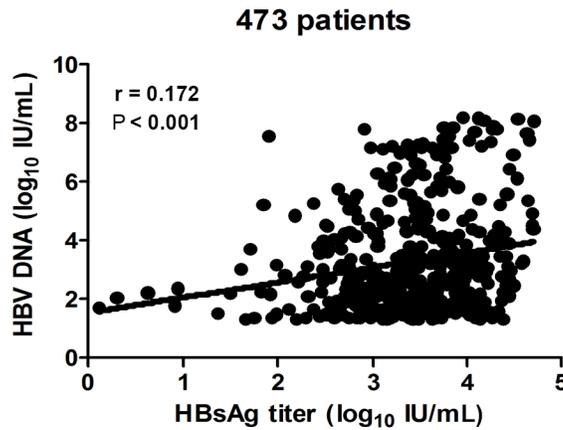
473 patients

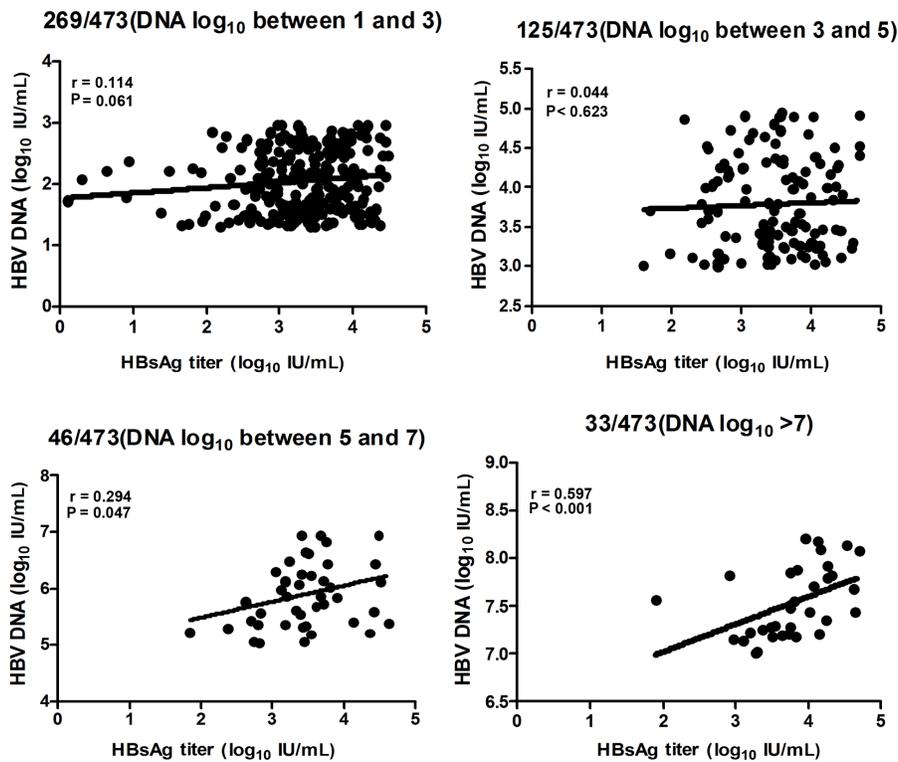**Figure 1.** Overall correlation between HBsAg and HBV DNA.

Figure 2. Correlations between HBsAg and HBV DNA, based on HBV DNA levels.

Association between HBsAg and HBV DNA based on HBeAg status

The 473 chronic hepatitis B patients were divided into two groups; the HBeAg+ group was comprised of 273 patients and the remaining 200 patients were in the HBeAg- group. The correlation between HBsAg and HBV DNA levels in these two groups was analyzed by the Spearman test. As shown in Figure 3, we observed a significant positive correlation between HBsAg and HBV DNA in the HBeAg+ group ($r = 0.321$, $P < 0.001$), while no such correlation was observed in the HBeAg- group ($r = -0.016$, $P = 0.825$).
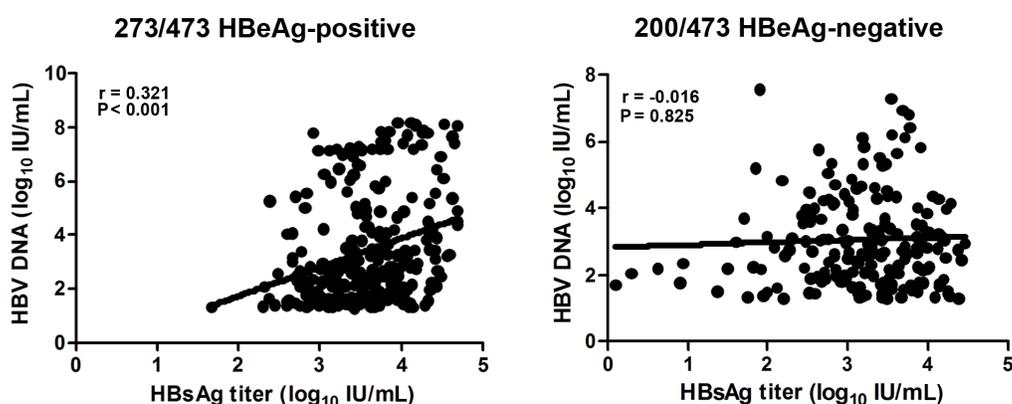

Figure 3. Correlation between HBsAg and HBV DNA, based on HBeAg status.

Association between HBeAg, PreS1-Ag, and HBV DNA

The rate of positive HBeAg, PreS1-Ag, and HBV DNA expression in the 1000 CHB serum samples was 49.3, 74.5, and 54.2%, respectively. The relationship between HBeAg, PreS1-Ag, and HBV DNA is summarized in Table 1. The rate of positive expression of HBeAg and PreS1-Ag was higher in the HBV DNA+ group than that in the HBV DNA- group ($P < 0.001$). HBV DNA was observed in 31.79% of the cases that were double-negative for HBeAg and PreS1-Ag (Table 2), which was significantly lower than that seen in the single-negative group ($P < 0.001$, respectively). The McNemar chi-square test comparing the HBeAg and PreS1-Ag with the HBV DNA showed a κ-value of 0.29 and 0.17 ($P < 0.001$), respectively. However, only 97 of all HBV DNA+ samples (97/542, 17.90%) were PreS1-Ag-.

Table 1. Relationship between HBeAg, PreS1-Ag, and HBV DNA [N (%)].

Group	N	HBeAg		PreS1-Ag	
		+	-	+	-
HBV DNA+	542	339 (62.55%)	203 (37.45%)	445 (82.10%)	97 (17.90%)
HBV DNA-	458	154 (33.62%)[a]	304 (66.38%)	300 (65.50%)[b]	158 (34.50%)
Total	1000	493 (49.30%)	507 (50.70%)	745 (74.50%)	155 (15.50%)

[a]Compared to the number of HBeAg+ patients in HBV DNA+ group, $\chi^2 = 83.07$, $P < 0.001$. [b]Compared to the rate of positive expression of PreS1-Ag+ in HBV DNA+ group, $\chi^2 = 36.01$, $P < 0.001$. The samples were matched using the McNemar chi-square test. κ value between HBeAg and HBV DNA was 0.29 ($P < 0.001$), and the κ value between PreS1-Ag and HBV DNA was 0.17 ($P < 0.001$).

Table 2. Prediction of HBV DNA level by co-detection of HBeAg and PreS1-Ag in CHB patients [N (%)].

Pattern	N	HBV DNA+	HBV DNA-
HBeAg+ PreS1-Ag+	411	297 (72.26%)	114 (27.74%)
HBeAg+ PreS1-Ag-	82	42 (51.22%)[a]	40 (48.78%)
HBeAg- PreS1-Ag+	334	148 (41.31%)[b]	186 (58.69%)
HBeAg- PreS1-Ag-	173	55 (31.79%)[c]	118 (68.21%)
Total	1000	542 (54.2%)	458 (45.8%)

[a]Compared to the rate of HBV DNA expression in the double-positive group, χ^2_{HBeAg+} = 14.09, P < 0.001. [b]Compared to the positive rate of HBV DNA expression in the double-positive group, $\chi^2_{PreS1-Ag+}$ = 54.85, P < 0.001. [c]Compared to the rate of HBV DNA expression in HBeAg/PreS1-Ag++ group, χ^2 = 83.28, P < 0.001.

Prediction of HBV DNA levels in CHB patients by co-detection of HBeAg and PreS1-Ag

The samples were divided into four groups (Table 2) based on the status of HBeAg and PreS1-Ag. HBV DNA was observed in 72.26% (297/411) of the samples that were double positive for HBeAg and PreS1-Ag; this percentage was higher than that seen in the HBeAg+ and PreS1-AG+ single-positive groups (P < 0.001). As expected, 31.69% of the HBeAg and PreS1-Ag double-negative population (55/173) showed HBV DNA expression, which was lower than that seen in the other three groups (P < 0.001). These results showed that the HBeAg/PreS1-Ag++ group contained the largest proportion of individuals expressing the HBV DNA (297/542). These results indicated that co-detection of HBeAg and PreS1-Ag could better reflect the HBV DNA levels.

DISCUSSION

In this study, CHB patients were selected from different stages of disease progression to ensure the universality of the correlation between HBsAg and HBV DNA. We discovered that the correlation between HBsAg and HBV DNA was not significant (Figure 1), with the correlation coefficient increasing with the increase in HBV DNA levels (Figure 2), which was similar to the results reported by several previous studies (Martinot-Peignoux et al., 2002; Wiegand et al., 2008). In addition, the correlation between HBsAg and HBV DNA was more significant in HBeAg+ samples than in HBeAg- samples (Figure 3), as reported in a previous study (Wiegand et al., 2008).

Recent studies have yielded conflicting results regarding the correlation between HBsAg and HBV DNA. Deguchi et al. (2004) reported a correlation between HBsAg and the levels of serum HBV DNA and HBV DNA polymerase. Ozaras et al. (2008) suggested that the HBsAg level was correlated with HBV DNA, and that it could replace HBV DNA to monitor the efficacy of pegylated interferon in the presence or absence of lamivudine marker. On the other hand, Ozdil et al. (2009) reported a negative correlation between HBV DNA and HBsAg in non-cirrhotic chronic HBV-infected patients, whereas Kuhns et al. (2004) found no correlation between HBsAg and HBV DNA in 200 blood samples. This could be attributed to various factors: 1) differences in the sensitivity of the HBV DNA detection protocol; most previous studies set a lower limit of detection as 103 copies/mL (not 1.16 x 10^2 copies/mL); 2) antiviral treatment (that some of the patients were subjected to) can induce mutations in the HBsAg sequence; viral variants appear as a result of endogenous (host immunity) and/or

exogenous (immune-prophylaxis and antiviral therapy) factors (Locarnini and Zoulim, 2010); and 3) the high spontaneous error rate in the reverse transcriptase induces mutations in the virus, such as the preS/S variants (Pollicino et al., 2012).

The fact that HBeAg and HBV DNA are not associated ($\kappa = 0.29$) is not very surprising. In this study, 40.04% (203/507) of the HBeAg- patients showed HBV DNA expression. We concluded that this could be related to a G1896A mutation (PC mutation) in the PreC region, where the 28th codon undergoes a mutation from TGG to TAG. TAG is a termination codon that signals the end of protein translation in the PreC region; therefore, HBVs expressing the G1896A mutation fail to produce HBeAg (Carman et al., 1989). Thus, HBsAg is a useful clinical serum marker of HBV infection for the diagnosis and monitoring of treatment; however, this cannot replace HBV DNA, especially in HBeAg- CHB patients. Consequently, HBeAg detection helps predict HBV DNA replication only in some types of CHB, and can only be used as a complementary index.

As shown in Table 1, PreS1-Ag and HBeAg were expressed in 82.10% and 62.55% of the population in the HBV DNA+ group, respectively. Therefore, PreS1-Ag had better sensitivity than HBeAg as a marker of HBA DNA replication, resulting from the higher mutation frequency of the PreC region (Carman et al., 1989). However, 65.5% of the HBV DNA population also displayed PreS1-Ag expression, which indicated its low specificity in predicting HBV DNA expression, compared to HBeAg. This may be attributed to the low expression of PreS1 proteins on the surface of Dane particles, which show predominant expression of HBA DNA and HBeAg. Moreover, most PreS1 proteins located in other particles are expressed in the absence of HBV DNA (Theilmann et al., 1987). This indicated that PreS1-Ag reflects the HBV DNA replication to a certain extent, but cannot displace HBeAg and HBV DNA as detection markers.

Additionally, we observed that 72.26% of the HBeAg/PreS1-Ag++ CHB patients expressed the HBV DNA (Table 2); this was higher than the number of HBV DNA+ patients in the HBeAg+, PreS1-Ag+, and HBeAg/PreS1-Ag-- groups. The number of CHB patients expressing the HBV DNA was lowest in the HBeAg/PreS1-Ag-- group (31.69%) as expected. These results indicated that co-detection of HBeAg and PreS1-Ag could better reflect the HBV DNA levels. Co-detection of serum HBeAg and PreS1-Ag levels is a more convenient and efficient method for the clinical diagnosis and treatment of CHB, compared to the detection of HBV DNA levels by fluorescence quantitative PCR.

In conclusion, the serum HBsAg level can significantly predict the HBV DNA expression only in the HBeAg+ group, or at high levels of HBV DNA. Co-detection of HBeAg and PreS1-Ag can better predict the HBV DNA levels; therefore, this can be used as a reliable method for the clinical diagnosis and treatment of CHB.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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