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Prediction value of serum HBV large surface protein in different phases of HBV infection and virological response of chronic hepatitis B patients

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ABSTRACT

Background: Serum HBV large surface protein (HBV-LP) is an envelope protein that has a close relationship with HBV DNA level. This study is to explore the prediction value of HBV-LP in different phase of HBV infection and during antiviral therapy in chronic hepatitis B (CHB) patients.

Methods: A retrospective study was conducted in 2033 individuals, which included 1677 HBV infected patients in different phases and 356 healthy controls. HBV-LP, HBV serum markers and HBV DNA were detected by ELISA, CMIA and qRT-PCR, respectively. 85 CHB patients receiving PegIFNα or ETV were divided into virological response (VR) and partial virological response (PVR). The dynamic changes of HBV DNA and HBV-LP were observed.

Results: The level of HBV-LP in 2033 individuals was shown as: HBeAg-positive hepatitis > HBeAg-positive infection > HBeAg-negative hepatitis > HBeAg-negative infection > healthy controls. HBV-LP was positive in all patients whose HBV DNA > 1.0E + 06 IU/ml. When HBsAg was < 0.05 IU/ml or > 1000 IU/ml, HBV DNAs were all negative if HBV-LP < 1.0 S/CO. When HBsAg was between 0.05 IU/ml and 1000 IU/ml, the consistency of HBV-LP with HBV DNA was 100% in case of HBV-LP > 4.0 S/CO in HBeAg-positive patients and HBV-LP > 2.0 S/CO in HBeAg-negative ones. During antiviral therapy, baseline HBV-LP was lower in VR patients than that in PVR patients. The optimal cut-off points to predict VR by baseline HBV-LP were 32.4 and 28.6 S/CO for HBeAg-positive and HBeAg-negative hepatitis patients, respectively.

Conclusions: HBV-LP may be a useful marker for distinguishing the different phases of HBV infection. Moreover, baseline HBV-LP level can be used for predicting VR of CHB patients.

1. Introduction

Infection with hepatitis B virus (HBV) remains a serious public health problem worldwide [1]. In China, 2.64% of individuals between 1 and 29 years old are HBV carriers [2,3]. According to 2017 Clinical Practice Guidelines of EASL, the natural history of HBV infection can be classified into four phases: HBeAg-positive chronic infection, HBeAg-positive chronic hepatitis, HBeAg-negative chronic infection and HBeAg-negative chronic hepatitis. However, despite this nomenclature, for many patients in clinic, a single determination of HBV replication markers and disease activity markers does not allow an immediate classification to one of the phases [1]. Serial monitoring of serum HBeAg, HBV DNA and ALT levels are required in most cases but even after a complete assessment, some subjects fall into an indeterminate grey area and result in the dilemma in antiviral therapy [4].

Moreover, in chronic hepatitis B (CHB) patients with PegIFNα therapy for 24 weeks and entecavir (ETV) therapy for 48 or 52 weeks, the virological response rates are 20%–30% and 60%–90%, respectively [1]. Current numerous studies suggest that quantitative HBV DNA and HBsAg have a predictive value in the antiviral therapeutic efficacy which happens in 24 or 48 weeks later [1,5]. Therefore, during the early phase of antiviral therapy, measures should be taken to optimize the application of available therapies and predictive factors determining the therapeutic outcomes are of great importance.

The HBV pre-S/S domains encode three different envelope proteins: large (preS1, preS2 and S), middle (preS2 and S) and small (S) [6]. All three proteins contain the S domain. HBV was secreted into serum in the forms of Dane and subviral particles (filamentous or spherical particles). Most serum hepatitis B virus large surface protein (HBV-LP) exists on Dane particles but less on subviral particles (SVPs) [7]. The

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HBsAg detected small protein was the common component of these three particles while HBV DNA exists mainly on the Dane particles. HBV-LP, HBsAg and HBV DNA showed much better consistency with each other which secreted in the form of Dane particles but not in SVPs. However, in the different stages of CHB, what are the secreted forms of HBV in the serum? Whether HBV-LP can remedy the deficiency of HBsAg and HBV DNA in clinical diagnosis and antiviral therapy? What is the relationship among HBV-LP, HBV DNA and HBsAg in large populations? All above questions need further analysis.

In this study, serum HBV-M, HBV-LP and HBV DNA of 2033 individuals including healthy controls and CHB patients in different phases and the dynamic changes of HBV-LP and HBV DNA in 85 CHB patients receiving antiviral therapy were tested. Statistical analyses were performed to discuss the clinical value of HBV-LP.

2. Materials and methods

2.1. Patients

A total of 2033 specimens were collected from June 1, 2014 to June 1, 2017 at the First Hospital Affiliated to Fujian Medical University, including 1677 HBV infection patients and 356 healthy controls. According to 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection of EASL, HBV infection were further divided into HBeAg-positive chronic infection, HBeAg-positive chronic hepatitis, HBeAg-negative chronic infection and HBeAg-negative chronic hepatitis [1]. Furthermore, the superinfection or coinfection of hepatitis A, C, D or E and HIV had been excluded by texting serum markers of those pathogens above. The clinical characteristics of the study population were summarized in Table 1.

44 CHB patients (included 24 HBeAg-positive hepatitis and 20 HBeAg-negative hepatitis) receiving PegIFNα (180 μg weekly) and 41 CHB patients (included 21 HBeAg-positive hepatitis and 20 HBeAg-negative hepatitis) receiving ETV (0.5 mg daily) were consecutively recruited and followed up for 48 weeks from the Liver Research Center of the First Affiliated Hospital of Fujian Medical University, between January 2015 and June 2017. Sera were collected at baseline, week 4, week 8 and every 12 weeks (weeks 12, 24, 36 and 48). Based on 2017 Clinical Practice Guidelines of EASL, virological response (VR) during PegIFNα is defined as serum HBV DNA level < 2000 IU/ml at 6 months or at the end of therapy and during ETV is defined as HBV DNA < 20 IU/ml at 12 months of therapy in compliant patients. Other cases were defined as partial virological responses (PVR) [1]. Study protocols were approved by the Institutional Medical Ethics Review Board of the First Affiliated Hospital of Fujian Medical University.

2.2. Detection and imprecision evaluation of HBV-LP

ELISA method (Hotgen Biotech Co., Ltd., Beijing, China) was used for the determination of serum HBV-LP and the positive results were interpreted as Optical Density $\geq 2.1 \times$ NC (average of negative control, calculated as 0.05 if < 0.05). The concentrations of samples were calculated by S/CO.

Assay imprecision has been estimated according to Clinical and Laboratory Standards Institute (CLSI) EP15-A3 guidelines [8]. Manufacturers' quality control materials as well as patient serum samples have been analyzed over 5 days with five measurements a day. From the obtained 25 measurements, within-run, between-run, and total imprecision have been estimated.

2.3. Measurement of ALT and AST

The kit (Roche Diagnostics, Switzerland) was used in an automatic biochemical analyzer cobas8000 (Roche Diagnostics, Switzerland) on the principle of Enzyme Kinetics with standard substance and quality control substance from American Bio-Rad Laboratories (Hercules). The upper limit of normal (ULN) for ALT and AST was 40 U/l and 35 U/l respectively.

2.4. Quantization of HBsAg and HBeAg

Quantitative HBsAg and HBeAg were detected by an automated chemiluminescent microparticle immunology analyzer (Abbott I2000, Abbott Laboratories, Chicago) with original reagents supplied by Abbott. Specimens with HBsAg concentration values ≥ 0.05 IU/ml or HBeAg ≥ 1.0 S/CO are considered reactive. The samples with concentration of HBsAg exceeding the upper detection (250 IU/ml) were automated further diluted by 1:500.

2.5. Hypersensitivity HBV DNA detection

HBV DNA was detected by quantitative real-time PCR (qRT-PCR) method (Sansure Biotech Inc., Hunan, China) and Roche Lightcycler 480 (Roche Corporation, Basel, Switzerland). The quality control substance was from Controls & Standards Biotechnology (Beijing, China). The lower limit of detection (LLoD) for HBV DNA was 20 IU/ml. The linear range was 20 IU/ml–1.0E + 09 IU/ml of serum HBV DNA.

2.6. Statistical analysis

Data was analyzed by the SPSS version 20.0 software. Pearson's chi-squared test was used to assess the differences of categorical variables. Student's *t*-test and Mann-Whitney *U* test were used to evaluate the differences of normally distributed and abnormally distributed continuous variables, respectively. Spearman's correlation test was used to assess the relationship between continuous variables. What's more, the HBV-LP and HBV DNA content was categorized into several groups based on a cut-off point and the difference of each variable was evaluated by One-Way ANOVA test or Kruskal-Wallis test. The receiver operating characteristic (ROC) curve was obtained and area under the curve (AUC) was calculated to identify the best HBV-LP to predict virological response in CHB patients with antiviral therapy. All statistical tests were two-sided and the level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Imprecision of HBV-LP

To ensure the accuracy of the HBV-LP results, the imprecision of HBV-LP was analyzed first. Two quality control materials and three pooled serum specimens with different HBV-LP concentrations were

Table 1
Baseline characteristics of patient population.

Characteristics	HBeAg positive		HBeAg negative		healthy controls
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis	
No.	372	263	825	217	356
Male/female	259/113	190/73	583/242	158/59	248/108
Age (mean ± SD, yr)	37.0 ± 10.6	36.4 ± 12.1	37.6 ± 9.4	37.8 ± 10.6	36.9 ± 10.5