Hepatitis B Surface Antigen Serum Levels Help to Distinguish Active From Inactive Hepatitis B Virus Genotype D Carriers

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BACKGROUND & AIMS: The accurate identification of inactive (serum HBV-DNA persistently ≤2000 IU/mL) hepatitis B virus (HBV) carriers (IC) is difficult because of wide and frequent HBV-DNA fluctuations. We studied whether hepatitis B surface antigen (HBsAg) serum levels (HBsAgsl) quantification may contribute to diagnosis of HBV phases in untreated hepatitis B e antigen-negative genotype D asymptomatic carriers. **METHODS:** HBsAgsl were measured at baseline and end of follow-up and correlated with virologic and biochemical profiles of 209 consecutive carriers followed-up prospectively (median, 29; range, 12–110 months). HBV phases were defined after 1-year monthly monitoring of HBV-DNA and transaminases. **RESULTS:** HBsAgsl were significantly lower in 56 inactive carriers (IC) than 153 active carriers (AC): median, 62.12 (range, 0.1–4068) vs median, 3029 (range, 0.5–82,480) IU/mL; $P < .001$. Among AC, HBsAgsl were lower in 31 AC whose viremia remained persistently <20,000 IU/mL (AC1) than in 122 AC with fluctuations ≥20,000 IU/mL (AC2): 883 (0.5–7838) vs 4233 (164–82,480) IU/mL, $P = .002$. HBV infection was less productive in IC and AC1 than AC2 (log_{10} HBV-DNA/HBsAgsl ratios 0.25 and 0.49 vs 2.06, respectively, $P < .001$) and in chronic hepatitis than cirrhosis (1.97 vs 2.34, respectively; $P = .023$). The combined single point quantification of HBsAg (<1000 IU/mL) and HBV-DNA (≤2000 IU/mL) identified IC with 94.3% diagnostic accuracy, 91.1% sensitivity, 95.4% specificity, 87.9% positive predictive value, 96.7% negative predictive value. During follow-up, HBsAgsl were stable in AC but declined in IC (yearly median decline, −0.0120 vs −0.0768 log_{10} IU/mL, respectively, $P < .001$), 10 of whom cleared HBsAg. **CONCLUSIONS: HBsAgsl vary during chronic hepatitis B e antigen-negative genotype D infection and are significantly lower in IC. Single-point combined HBsAg and HBV-DNA quantification provides the most accurate identification of IC, comparable with that of long-term tight monitoring.**

Keywords: HBsAg Quantification; Inactive HBV Infection; HBeAg Negative Chronic Hepatitis B.

Currently, hepatitis B e antigen (HBeAg)-negative hepatitis B virus (HBV) carriers represent the great majority of cases of HBV infection in many geographical areas, including Europe, having become more prevalent during the last decade because of the aging of the HBV-infected population.[1–4] Clinical conditions associated with chronic HBeAg-negative HBV infection are variable, ranging from inactive carrier (IC) status to active chronic HBV infection (CHB).[5] The survival of those in the IC stage is comparable with the noninfected population, at least in Western countries.[6,7] By contrast, the rate of progression to cirrhosis among those with HBeAg-negative CHB ranges from 2.8 to 9.7 × 100 per year.[8–13] Current antiviral treatments may slow disease progression in cirrhotic patients and lead to cure of CHB if identified before the development of cirrhosis.[14] Thus, an early diagnosis of CHB may allow earlier consideration of initiating antiviral therapy. However, differential diagnosis between active CHB and IC status is problematic because HBeAg-negative CHB is characterized by wide fluctuations in viral replication and biochemical activity, with intermittent reductions of HBV-DNA serum levels below the inactive carrier cut-off ≤2000 IU/mL and spontaneous normalization of alanine aminotransferase (ALT) lasting from a few weeks to several months.[10,15,16] Therefore, to warrant an accurate differential diagnosis between CHB and IC, it is mandatory to monitor serum HBV-DNA by sensitive and quantitative polymerase chain reaction assays and ALT over a period of at least 1 year.[1,16,17] In addition, ALT levels are not a specific marker of viral-induced liver damage, and their fluctuation in an inactive carrier may be caused by nonviral liver disease cofactors.[18]

Abbreviations used in this paper: AC, active carriers; AC1, active carriers with HBV-DNA persistently <20,000 IU/mL; AC2, active carriers with HBV-DNA ≥20,000 IU/mL; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; AUROC, area under the receiver operating characteristic; cccDNA, covalently closed circular DNA; DA, diagnostic accuracy; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBsAgsl, HBsAg serum levels; HBV, hepatitis B virus; IC, inactive carriers; IgM, immunoglobulin M; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

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Thus, the identification of IC status is not always straightforward. Recently, hepatitis B surface antigen (HBsAg) serum levels were shown to correlate with intrahepatic covalently closed circular DNA (cccDNA) levels (the hallmark of infected hepatocytes),[19,20] to decline during therapy, and to be associated with long-term sustained virologic response.[21,22] These findings suggest that quantification of HBsAg might help to characterize the phase of HBV infection in HBeAg-negative carriers. We studied HBsAg serum levels and assessed their potential diagnostic value in a large cohort of untreated, HBeAg-negative/antibody to hepatitis B e antigen (anti-HBe)-positive, asymptomatic HBV genotype D carriers who had been followed up prospectively.

Patients and Methods

Patients

We studied 209 consecutive HBeAg-negative/anti-HBe-positive chronic HBsAg carriers infected with HBV genotype D (median age, 48 years; range, 18–77 years; 104 males and 105 females), admitted to the Liver Unit of the University Hospital of Pisa between 2000 to 2006. All the carriers were untreated and asymptomatic. The study was approved by the Ethical Committee of our hospital, and participants gave their written consent. Inclusion criteria were as follows: HBsAg/anti-HBe positive for at least 2 years; negative for HBeAg and antibodies against hepatitis C virus (HCV), hepatitis D virus, human immunodeficiency virus; absence of autoimmune disease and high alcohol intake (>40 g/day). They were part of a cohort of 314 HBeAg-negative/anti-HBe-positive HBsAg carriers followed up at our unit: 24 of them were excluded because they were symptomatic, 52 were infected with HBV genotypes other than D, 20 were already in treatment at the time of their first observation, 9 had missing clinical data.

To define accurately the infection profile of each carrier, serum tests were performed monthly during the first 12 months of the prospective follow-up and every 3 months thereafter. At the end of the 1-year monthly monitoring, HBV carriers were classified according to their biochemical and virologic profiles: (1) inactive carriers (IC) had persistently serum HBV-DNA levels ≤2000 IU/mL and normal ALT (<40 U/L); (2) active carriers (AC) had HBV-DNA serum levels >2000 IU/mL, with or without elevated ALT. Transient elastography became available in our unit on April 2004 and was performed in all newly enrolled carriers during the first year of follow-up from April 2004 on and at the first visit for carriers already in follow-up at that time. Liver biopsy specimens were obtained, within the first year of monitoring, from 109 of the 153 individuals in the AC phase; biopsy was not performed in the remaining 44 carriers because of persistently normal ALT and transient elastography values <6 kPa in 21; histologic diagnosis of cirrhosis already present in 11; signs of cirrhosis at ultrasound in 7; 5 refused to undergo biopsy.

Quantitative HBsAg was performed in all cases at baseline and end of the follow-up or at the time of initiation of antiviral therapy. Additional samples (median, n = 5; range, 3–16) were obtained in 67 HBV carriers to analyze the kinetics of HBsAg during the natural course of the infection.

Serology and Histology

Serum biochemistry included aspartate transferase (AST) and ALT, γ-glutamyl transpeptidase, alkaline phosphatase, albumin, globulins, total bilirubin, prothrombin time, and α-1 fetoprotein. HBsAg, antibody to hepatitis B surface antigen, antibody to hepatitis B core antigen (anti-HBc), HBeAg and anti-HBe, antibody to HCV, antibody to hepatitis D virus, and antibody to human immunodeficiency virus were detected by commercially available immunoassays (Abbott Laboratories, N Chicago, IL). Immunoglobulin M (IgM) anti-HBc level were determined by CORE-M-IMx (Abbott Laboratories), using 0.200 and 0.100–0.200 Index as cut-off and grey zone of chronic hepatitis respectively.[23]

HBsAg was quantified using the Architect HBsAg assay (Abbott Laboratories; dynamic range, 0.05–250.0 IU/mL) after 1:100 dilution. Samples with HBsAg levels >250.0 IU/mL at 1:100 dilution were retested at 1:1000 final dilution. Samples with HBsAg levels <0.05 IU/mL at 1:100 dilution were retested undiluted. Serum HBV-DNA levels were quantified by COBAS Amplicor Monitor 2.0 HBV assay (Roche Diagnostic Systems Inc, Mannheim, Germany) with a lower limit of detection of 200 copies/mL and linearity range from 200 to 20,000 copies/mL (conversion factor, 5.6 copies = 1 IU) until 2005 and thereafter by COBAS TaqMan assay, sensitivity 12 IU/mL, dynamic range 6–1.10×10^8 IU/mL. HBV genotyping was performed by direct sequencing of the region encoding for the small hepatitis B surface protein. Liver specimens were processed using standard criteria; grading of inflammation and staging of fibrosis were assessed by Ishak score.

Statistical Analysis

Data were expressed as median and range values. The logarithmic transformation was used for quantitative data without normal distribution. The Spearman correlation test was used to analyze the correlations between HBsAg serum levels and other continuous variables. Differences between subgroups were analyzed using Mann–Whitney rank sum test or Kruskal-Wallis test where appropriate. To identify factors independently correlated with HBsAg serum levels and HBsAg δ variations, variables with statistical associations ($P < .05$) or trends ($P < .10$) at univariate analysis were included in multiple regression analysis. Factors independently associated with HBsAg clearance were identified by using logistic regression analysis. The diagnostic performance of HBsAg serum levels was evaluated by receiver operating characteristic (ROC) curve. The cut-off value to discriminate chronic hepatitis patients