

## A persistently low HBV DNA level is a predictor of spontaneous HBsAg clearance in patients with chronic hepatitis B

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Received: 28.11.2014 • Accepted/Published Online: 16.02.2015 • Final Version: 05.01.2016

**Background/aim:** The incidence and predictors of spontaneous hepatitis B surface-antigen (HBsAg) seroclearance in patients with chronic hepatitis B virus (HBV) were evaluated.

**Materials and methods:** A total of 1427 patients with chronic HBV infection, who were followed between 1994 and 2013, were investigated in this retrospective study. All data were extracted from patient files.

**Results:** Spontaneous HBsAg seroclearance occurred in 84 patients during 8798 person-years of follow-up. The patients were categorized into 3 groups at follow-up based on HBV DNA features as continuously <100 copies/mL (Group A), 0–10,000 copies/mL (Group B), and 0 to >10,000 copies/mL (Group C). Alanine aminotransferase features in the 2 groups were categorized as continuously normal (<40 U/L) and 0 to >40 U/L. Spontaneous HBsAg seroclearance was seen primarily in patients with Group A HBV DNA features, and continuously low HBV DNA values were the main predictor of HBsAg seroclearance ( $P < 0.001$ ).

**Conclusion:** These results suggest that a continuously low viral load is the most important factor affecting spontaneous HBsAg seroclearance.

**Key words:** Spontaneous HBsAg seroclearance, incidence, predictor, HBV DNA

### 1. Introduction

Chronic hepatitis B virus (HBV) infection is a serious public health problem due to its worldwide prevalence and potential to cause other adverse consequences, such as cirrhosis and hepatocellular carcinoma (HCC) (1). More than 240 million patients have chronic (long-term) liver infections. Approximately 600,000 patients die every year due to the acute or chronic consequences of HBV. Several epidemiological studies have shown that hepatitis B surface-antigen (HBsAg) seropositivity is an important risk factor for further disease, including cirrhosis and HCC (1). Current antiviral therapies result in relatively low HBsAg clearance rates of 0.5%–2.3% (2). Spontaneous HBsAg seroclearance in untreated individuals is a relatively rare event, particularly in highly endemic areas. Previous studies have found annual seroclearance rates of 0.5%–1.4% (2). Predictors of HBsAg seroclearance include old age, HBeAg seronegativity, male sex, HBV genotype B, and low serum HBV-DNA level (<10<sup>5</sup> copies/mL) (3). We retrospectively evaluated the incidence and determinants of spontaneous HBsAg seroclearance in patients with

chronic HBV, who were followed up at the Infectious Diseases and Clinical Microbiology Department of a tertiary-care hospital.

### 2. Materials and methods

#### 2.1. Patient data source

A total of 2625 patients were evaluated from 1994 to 2013, and 1427 patients (aged 13–86 years) were included. All data were extracted from patient files. Tests on serum HBsAg, HBeAg, anti-HBe, anti-HBs serum HBV-DNA, and alanine aminotransferase (ALT) levels were measured using commercial kits. HBsAg, anti-HBsAb, HBeAg, anti-HBeAb, anti-HCV, and anti-Delta were measured with an autoanalyzer using commercial reagents. HBV DNA was assayed by real-time polymerase chain reaction (RT-PCR) assay using the *Artus* HBV RG PCR Kit with the QIAGEN Rotor-Gene Q 6000 device (Valencia, CA, USA). The analytical lower detection limit of the assay was 3.8 IU/mL (constant: 8.2 to conversion copy: 31.4,  $P = 0.05$ ). Ultrasounds were performed regularly for HCC surveillance.

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## 2.2. Inclusion and exclusion criteria

Patient inclusion criteria for the study were: 1) HBsAg, HBeAg, anti-HBe, anti-HBs, HBV DNA, and ALT monitoring at least once annually, 2) 3 HBV DNA tests with PCR, and 3) follow-up duration of >1 year.

Exclusion criteria were: 1) antiviral therapy, 2) immunosuppressive therapy and/or immunosuppressive status, and 3) coinfection with a Delta virus or HCV.

## 2.3. Patient classification

We observed various HBV DNA and ALT values at follow-up in the patients with HBV. Because of these features, the patients were classified not as unique values but rather as spectral values. The first group was defined as continuously <100 copies HBV DNA/mL band (Group A), the second group as 0–10,000 copies/mL band (Group B), and the third group as 0 to >10,000 copies/mL (Group C). Similarly, the patients were classified according to ALT values as <40 U/L and >40 U/L. We considered that the 0–100 copies/mL category represented continuously low HBV DNA, the 0–10,000 category represented a heterogeneous group, and the 0 to >10,000 copies/mL category represented patients who had histologic active disease potential.

## 2.4. Statistical analysis

All data were extracted from the patients' files retrospectively. Statistical analysis was conducted using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Comparison of continuous variables was performed with Student's t-test. The log-rank test with adjusted Kaplan–Meier time curve analyses was performed to determine the significance of the predictive factors (age, sex, HBV DNA, and ALT) for loss of HBsAg. Significant variables were analyzed with the Cox regression model. The estimated probability curves for loss of HBsAg were calculated according to the Cox regression model, adjusted for the influence of the significant variables (Figure 1). Data are expressed as means  $\pm$  standard deviation (SD), ranges, and percentages (%) where appropriate. All statistical tests were two-sided, and  $P < 0.05$  was considered to indicate significance.

## 3. Results

The patients (660 [46.3%] females and 767 [53.7%] males) were aged 13–86 years, with a total of 8798 person-years of follow up. The mean follow-up period was  $6.17 \pm 4.13$  years (range: 2–18 years). The mean age at the beginning of the follow-up was  $37.13 \pm 12.21$  years. According to the questionnaire and blood test results, 647 family members (45.3%) and 167 mothers (11.7%) of patients had chronic HBV or had developed immunity. The DNA and ALT levels in all patients are shown in Table 1. An ALT level of <40 IU/mL was detected in 1145 patients (80.2%). The prevalence of the HBV DNA level categories of 0–100, 0–10,000, and 0 to >10,000 IU/mL were 22.6%, 47.7%, and 29.6%, respectively.

Eighty-four (33 [39.3%] females and 51 (60.7%) males) patients (5.7%) showed HBsAg seroclearance during the follow-up period. The DNA and ALT levels in all patients are shown in Table 2. HBsAg seroclearance rates are shown in Figure 1.

Among the 84 patients who had spontaneous HBsAg seroclearance, 73% had inactive disease with persistently <100 copies HBV DNA/mL and 0–40 IU/L ALT. One patient was HBeAg-positive (1.2%) at first admission. The relationships between HBsAg seroclearance, age, male sex, HBV DNA, and ALT levels were investigated. Of these parameters, only 0–100 copies HBV DNA/mL ( $P = 0.000$ ) and age at beginning of follow-up ( $P = 0.009$ ) were significantly correlated with spontaneous HBsAg seroclearance. The HBV DNA categories were a unique predictor of loss of HBsAg in the Cox regression analysis. The cumulative probability of HBsAg loss during the 14-year follow-up period is shown in Figure 2. A total of 43% of patients were in the 0–100 copies HBV DNA/mL category. No patients with spontaneous HBsAg seroclearance developed cirrhosis or HCC during the follow-up period. Fifty patients (59.2%) with spontaneous HBsAg seroclearance were anti-HBs-positive.

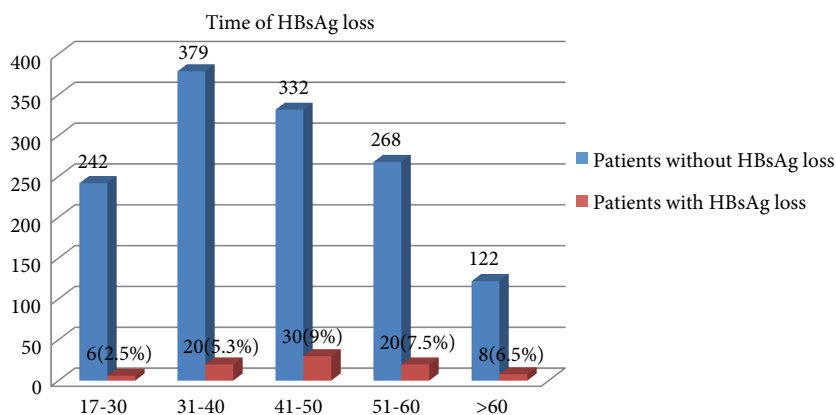


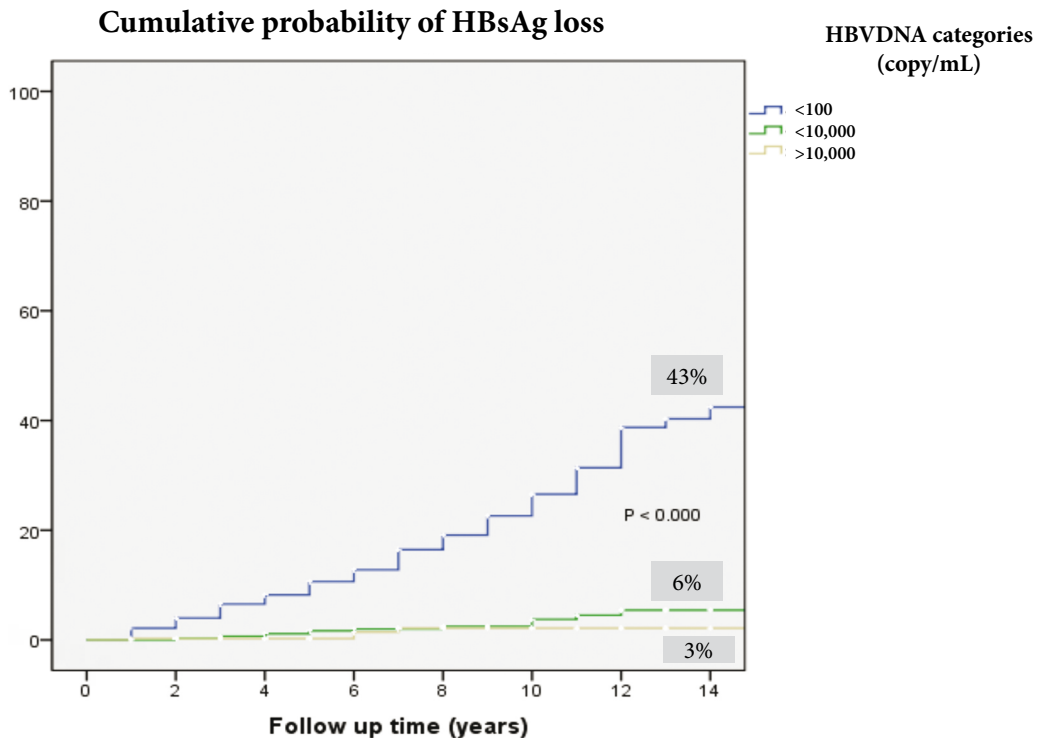
Figure 1. Hepatitis B virus surface antigen (HBsAg) seroclearance rate according to age.

**Table 1.** Hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) classifications and number of patients.

	ALT 0–40, n (%)	ALT 0 to >40, n (%)	Total, n (%)
HBV DNA <100, n (%)	286 (20%)	37 (2.6%)	323 (22.6%)
HBV DNA 0–10,000, n (%)	588 (41.2%)	93 (6.5%)	681 (47.7%)
HBV DNA 0 to >10,000, n (%)	271 (19%)	152 (10.7%)	423 (29.6%)
Total, n (%)	1145 (80.2%)	282 (19.8%)	1427 (100%)

**Table 2.** Hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) classifications of all patients who achieved spontaneous hepatitis B virus surface antigen (HBsAg) seroclearance.

DNA (copies/mL)	ALT 0–40, n (%)	ALT 0 to >40, n (%)	Total, n (%)
HBV DNA <100, n (%)	62 (73.8%)	4 (4.8%)	66 (78.6%)
HBV DNA 0–10,000, n (%)	9 (10.8%)	5 (6%)	14 (16.7%)
HBV DNA 0 to >10,000, n (%)	0 (0%)	4 (4.8%)	4 (4.8%)
Total, n (%)	71 (84.6%)	13 (15.5%)	84 (100%)



**Figure 2.** Cumulative probability of hepatitis B virus surface antigen (HBsAg) loss according to HBV DNA level during the follow-up period.

Forty-eight (3.4%) patients were HBeAg-positive at first admission, 31 (64.5%) were persistently HBeAg-positive, and 3 (6.2%) were anti-HBe-positive simultaneously.

Seventeen patients (35.47%) lost the HBeAg antigen. Of these, 13 (27%) developed anti-HBe. One (1.1%) patient who was HBeAg-positive achieved HbsAg seroclearance.

#### 4. Discussion

HBV infection is an important cause of acute and chronic liver disease worldwide. Patients with chronic HBV infection are at increased risk of developing cirrhosis, hepatic decompensation, and HCC. It is estimated that the lifetime risk of developing complications is 25%–40% in HBV carriers who acquire the virus early in life. Providing clear information about disease progression is the most important criterion in terms of patient safety (4).

HBsAg seroclearance is the ultimate goal of immune clearance. Spontaneous HBsAg seroclearance is defined as loss of serum HBsAg on two occasions at least 6 months apart. Maintaining the absence of serum HBsAg (annual incidence: 1%–2%) at the last visit is rare during the natural history of a chronic HBV infection (3,5). In this study, the cumulative probability of losing HBsAg during a 14-year follow-up was 43% for Group A. The cumulative probability of spontaneous HBsAg seroclearance increased from 8.1% to 24.9% after 10 years, and to 44.7% after 20 years in a study by Chu and Liaw (6).

In the present study, HBsAg seroclearance developed in 5.8% of all patients who met the inclusion criteria. The relationships between HBsAg seroclearance and age, male sex, HBV DNA, and ALT levels were investigated; however, only HBV DNA was statistically significant.

HBsAg seroclearance rates according to age are 8.3% for 41–50 years, 6.9% for 51–60 years, and 6.2% for >60 years. The epidemiological features of HBV infection in Turkey are similar to those in Mediterranean and Middle Eastern countries. The most common transmission route for infection in this region is horizontal transmission during early childhood and adolescence (7). This finding may be attributed to the long-term follow-up of these patients.

Loss of HBeAg in HBV carriers is a sign of remission of hepatitis and suppression of HBV replication (3). However, applying a sensitive method to detect serum HBV showed that HBV can continuously or intermittently replicate, even in HBeAg-negative patients (8).

During the follow up, clinicians observed more than one HBV DNA and ALT value for the patients with chronic HBV and defined the disease categories as inactive carrier state, active disease, and other serious complications that should be treated, such as cirrhosis or HCC (9,10). As a result of this spectral character, the patients were categorized as spectral. Initial HBV DNA level was not associated with loss of HBsAg in some previous studies; nevertheless, if patients are evaluated according to spectral character, HBV DNA is the most important feature (5).

Although major guidelines define the inactive chronic HBV carrier state as anti-HBe-positive, these patients have normal ALT and HBV DNA of <2000 IU/mL (~10,000

copies/mL) levels. Although most inactive carriers remain stable throughout their life and some eventually achieve HBsAg seroclearance, up to 30% of these inactive carriers may encounter reactivation of HBV and develop HBeAg-negative chronic hepatitis (9,10). According to European Association for the Study of the Liver guidelines, some inactive carriers may have HBV DNA levels of >10,000 copies/mL (usually <100,000 copies/mL) accompanied by a persistently normal ALT level, but this definition can hide cirrhotic disease (9). Some patients classified as inactive carriers have differing fibrotic activity (8). In this study, most patients had heterogeneous features. A total of 681 patients (47.7%) had a HBV DNA level of 0–10,000 copies/mL, and 271 (19%) had 0 to >10,000 copies/mL DNA and a 0–40 IU/mL ALT level. These groups included 66.7% of all patients. Our results show that continuously low HBV DNA level not only predicted HBsAg loss but also identified patients with inactive disease.

Most studies have not associated sex with the incidence of HBsAg seroclearance, as we found, with the exception of two studies conducted in Alaska and Taiwan. In the study conducted in Alaska, female carriers were more likely to clear HBsAg than males. In contrast, the study in Taiwan showed that HBsAg seroclearance was significantly more common in male carriers in univariate analysis, although the difference became marginally significant in multivariate analysis (3).

The antigenic determinants of HBsAg are classified into 4 subtypes or are based on serotype. The subtypes are divided into subdivisions within 9 serotypes. Eight HBV genotypes (A–H) have been identified, and the roles of the genotypes have been investigated in several studies. HBsAg seroclearance is more likely in patients with genotype B than in those with genotype C, and more likely in genotype A than in genotype D (3). Several genotype studies on patients with chronic HBV have been conducted in Turkey. Genotype D is the most prevalent genotype in patients with HBV in Turkey (11).

The disappearance of HBsAg and the appearance of anti-HBs in sera indicate HBV clearance (12). Several studies have reported that serum HBV DNA may still be detectable by PCR and more often in the liver (8,9). The prognosis improves in carriers that clear HBsAg, although HCC has been reported years after HBsAg clearance, particularly in older patients or in those who progressed to cirrhosis before clearing HBsAg (3). None of the patients who achieved spontaneous HBsAg seroclearance developed cirrhosis or HCC during the follow-up in our study. However, we found that serum HBV DNA was not detectable in patients who lost HBsAg, even in those who did not develop detectable anti-HBs. The detectable anti-HB rate was 59.2% in this study.

In conclusion, we showed that a continuously low HBV DNA level was a significant predictor of spontaneous HBsAg seroclearance. Patients with a continuously low HBV DNA level may have inactive disease.

### Acknowledgment

The authors thank Hatice Uluer, Department of Biostatistics, Faculty of Medicine, Ege University, for the statistical evaluation.

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