

## Time to seroconversion of HBsAg to anti-HBs in individuals who lost HBsAg during follow-up

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### SUMMARY

To determine the time to appearance of antibody against hepatitis B surface antigen (anti-HBs) after clearance of hepatitis B surface antigen (HBsAg) in chronically infected individuals, we followed up 3963 cases with positive antibody against hepatitis B e antigen (anti-HBe) from 1991 to 2014. Of these, 101 (67 males, 34 females) lost HBsAg. These serocleared cases were checked every 6-month interval regarding HBsAg, anti-HBs, liver function tests, and liver sonography. Hepatitis B virus DNA was assessed at the time of seroclearance or the appearance of anti-HBs. The mean age of these patients at entry to this study was  $34.4 \pm 13$  years. The mean follow-up duration until seroclearance of HBsAg was  $6.6 \pm 4.3$  years. After the mean follow-up of  $43.7 \pm 45$  months, anti-HBs appeared in 64 (63.4%) cases. The cumulative probabilities of anti-HBs appearance for 2, 5 and 10 years were 24.3%, 58% and 78.2%, respectively. The appearance of anti-HBs was associated with age  $\geq 35$  years and seroclearance of HBsAg (hazard ratio 1.96, 95% confidence interval 1.32–3.38,  $P = 0.016$ ) but not with sex. The results show that anti-HBs may develop in 78.2% of cases within 10 years of HBsAg clearance. Age  $\geq 35$  years at HBsAg loss was associated with earlier development of anti-HBs.

**Key words:** Clearance, chronic hepatitis B, follow-up, HBsAg, resolved hepatitis B.

### INTRODUCTION

More than 400 million people in the world are chronically infected with hepatitis B virus (HBV) and are at risk of developing cirrhosis and hepatocellular carcinoma. These individuals are the main source of HBV infection worldwide [1, 2]. The natural history of chronic HBV infection can be divided into five phases: immune tolerance, immune reactive hepatitis B e antigen (HBeAg)-positive, inactive HBV carrier state,

HBeAg-negative chronic hepatitis B, and hepatitis B surface antigen (HBsAg)-negative [3]. Annually, between 0.5% and 3% of inactive HBV carriers lose HBsAg [4–7]. Spontaneous HBsAg clearance usually confers a good prognosis if there is no pre-existing hepatocellular carcinoma or cirrhosis at the time of HBsAg seroclearance [8–11].

Seroconversion to antibody against hepatitis B surface antigen (anti-HBs) following HBsAg loss is more likely to prevent the progression of chronic liver diseases to cirrhosis and hepatocellular carcinoma and it represents immunity to HBV and may indicate a better prognosis [1, 2, 12]. Antibodies to HBsAg develop within 6 months in 95% cases with acute hepatitis B, but the development of anti-HBs in patients

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with chronic HBV infection who lost HBsAg during follow-up has not been clearly determined [12]. This study was conducted to determine the time to appearance of anti-HBs in inactive HBV carriers who lost HBsAg during follow-up.

## METHODS

From 1991 to 2014, 3963 cases of inactive anti-HBe-positive carriers were registered at the Infectious Diseases and Tropical Medicine Research Centre of Babol University of Medical Sciences. Patients with normal levels of aminotransferases, absence or low levels of serum HBV DNA on two occasions for 1 year plus normal liver sonographic findings were considered to be inactive HBV carriers [3, 13]. Patients co-infected with hepatitis C, D, previous history of treatment with anti-hepatitis agents, HIV, alcohol consumer and intravenous drug user were excluded from the study.

After enrolment, these cases were checked at 6-month intervals regarding viral markers (HBsAg, anti-HBs), liver function tests [serum aspartate aminotransferase (AST), alanine aminotransferase (ALT)],  $\alpha$ -fetoprotein, complete blood count and platelet count. Liver sonography was also performed for those aged >40 years and for those with a history of cirrhosis or liver cancer in their family members due to a HBV-related cause. The viral markers were tested by ELISA (HBsAg, Siemens, Germany; anti-HBs, Radim, Italy). A record was prepared for each patient and the variables were noted.

Those who lost HBsAg during follow-up, tested on two occasions at 6-month intervals, were entered into the study analysis (time of seroclearance). In these cases, serum HBV DNA levels were measured at the time of seroclearance and the appearance of anti-HBs, as well as in those who had elevated ALT or AST levels during the follow-up period. For isolation of HBV DNA, we used the QIAamp DNA mini-kit (Qiagen, Germany). All processes were performed according to the manufacturer's instructions. For quantification of HBV DNA, we used Rotor-Gene 3000 (Corbett Research, Australia) using the Artus HBV RG PCR kit (Qiagen, Germany). According to the manufacturer's instructions, the sensitivity of the test was 3.8 IU/ml (1 IU = 7 copies/ml). The study was approved by the Infectious Diseases and Tropical Medicine Research Centre of Babol University of Medical Sciences and the ethical committee approved the study.

## Statistical analysis

The data were collected and analysed using SPSS v. 22 (IBM Corp., USA). The *t* test was used to compare mean values. A multiple Cox proportional hazards regression model was used to estimate HBsAg to anti-HBs with the covariates of sex, and seroclearance age <35 or  $\geq$ 35 years. The time to appearance of anti-HBs data was plotted using a Kaplan–Meier graph. The log-rank test was used to compare the appearance of anti-HBs with the same covariates. Differences with a *P* value <0.05 were considered significant. All *P* values were two-tailed.

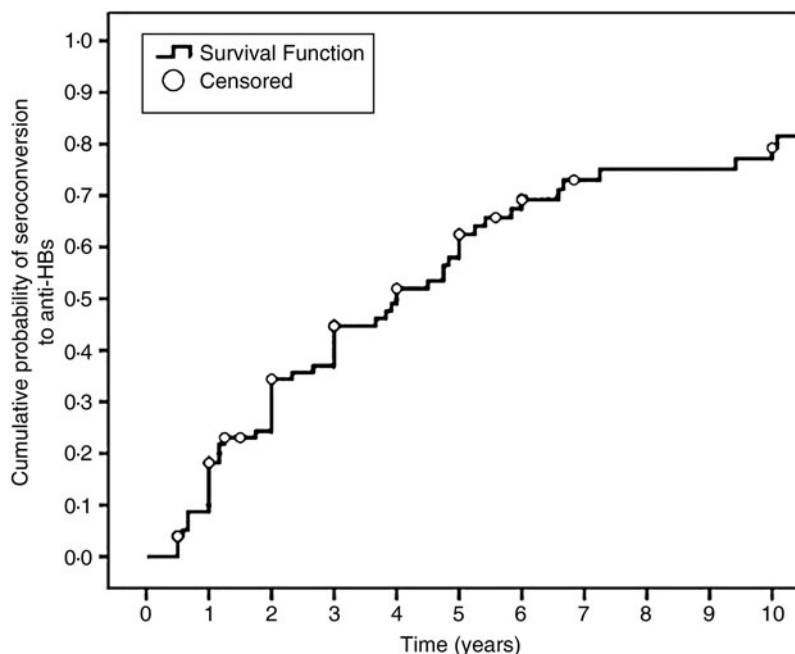
## RESULTS

During follow-up, 119 (3%) subjects became HBsAg negative and 18 cases did not attend the follow-up and were excluded from the study. Thus, data from 101 subjects (67 males, 34 females) were analysed. None had seroreverted to HBsAg positive.

The mean age of patients at entry to the study was  $34.4 \pm 13$  years (range 11–64 years). The mean duration of follow-up before seroclearance of HBsAg in these cases was  $6.6 \pm 4.3$  years (range 1–22 years). The mean age of the 67 males was  $35.7 \pm 13.8$  and of the 34 females  $31.8 \pm 10.7$  years (*P* = 0.16).

Anti-HBs developed in 40 males with a mean follow-up period of  $47.6 \pm 48$  months and in 24 females with a mean follow-up period of  $49.3 \pm 47.8$  months after seroclearance of HBsAg (*P* = 0.89). The cumulative probabilities for the appearance of anti-HBs after the seroclearance of HBsAg for 1, 2, 3, 4, and 5 years were 8.7%, 24.3%, 37%, 49.1%, and 58% cases, respectively (Fig. 1). Anti-HBs developed in 78.2% of subjects within 10 years of seroclearance. The mean duration of seroconversion in these cases after seroclearance was  $43.7 \pm 45$  months (range 6–215 months). Cox regression model showed that sex had no effect on the appearance of anti-HBs, but age  $\geq$ 35 years after seroclearance was associated with the appearance of anti-HBs (hazard ratio 1.96, 95% confidence interval 1.32–3.38, *P* = 0.016) (Table 1, Fig. 2).

HBV DNA was detected in 16 (15.8%) out of 101 cases at the time of seroclearance. After seroconversion, HBV DNA was detected in six (9.5%) out of 63 subjects who developed anti-HBs. ALT levels in all cases were in the normal range except in nine cases that had serum ALT levels <80 IU/l on occasions during follow-up, but sonography in these



**Fig. 1.** Cumulative probability of seroconversion to antibody against hepatitis B surface antigen (anti-HBs) after seroclearance of hepatitis B surface antigen (HBsAg).

**Table 1.** Estimated time to seroconversion of anti-HBs after seroclearance of HBsAg and hazard ratio regarding sex and age <35 or ≥35 years

Variable	Mean ST (95% CI)	Median ST (95% CI)	Crude HR (95% CI)	P value	Adjusted HR (95% CI)	P value
<b>Sex</b>						
Female	65.8 (40.7–90.9)	48 (31.9–64.1)	1		1	
Male	70.2 (51.7–88.6)	48 (28.1–67.9)	0.96 (0.57–1.61)	0.88	0.88 (0.52–1.48)	0.641
<b>Age (years)</b>						
<35	89.7 (62.7–116.7)	72 (43.6–100.4)	1		1	
≥35	53.7 (38.3–69.2)	36 (25.6–46.4)	1.92 (1.12–3.30)	0.018	1.96 (1.32–3.38)	0.016
Total	68.2 (53.3–83.1)	48 (28.2–67.8)	–	–	–	–

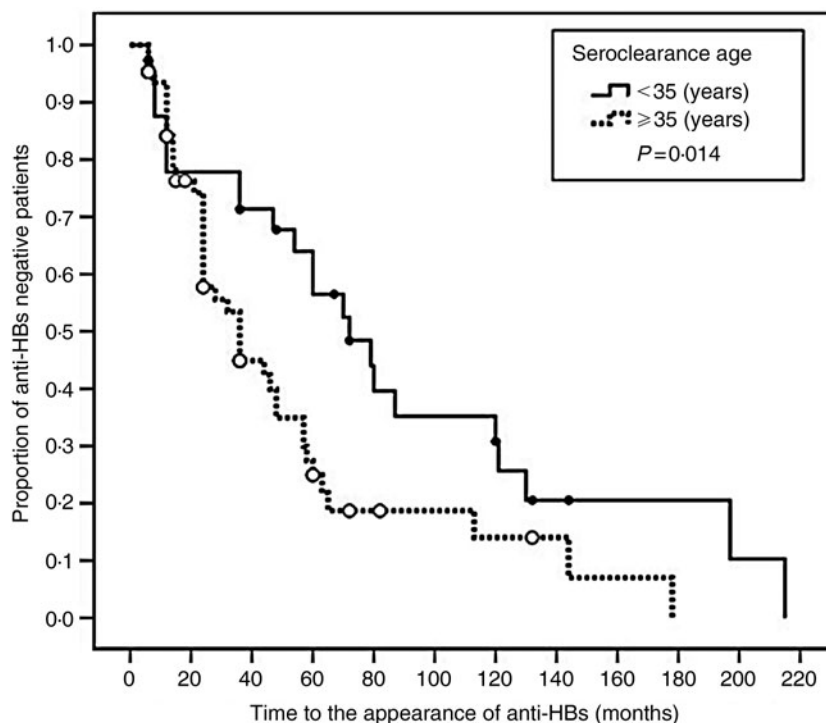
Anti-HBs; Antibody against hepatitis B surface antigen; HBsAg; hepatitis B surface antigen; ST, Survival time; CI, confidence interval; HR, hazard ratio.

cases showed mild fatty liver and their HBV DNA levels were <2000 copies/ml. No cirrhosis or hepatocellular carcinoma were found at the time of seroclearance or afterwards.

## DISCUSSION

The ideal goal for inactive HBV carriers is seroclearance of HBsAg and the appearance of anti-HBs. So far, studies with the aim of producing anti-HBs after seroclearance of HBsAg have not been published in the medical literature, and we believe that the present

study may be the first report showing the follow-up of a significant number of cases with chronic HBV infection who lost HBsAg during follow-up. Although spontaneous clearance of HBsAg usually confers a good prognosis in those without pre-existing hepatocellular carcinoma or cirrhosis at the time of HBsAg seroclearance [9, 10], other studies have shown cirrhosis and hepatocellular carcinoma in patients who were cirrhotic or non-cirrhotic at the time of HBsAg clearance [14–20]. In our study, we found that 78.2% of inactive HBV subjects who lost HBsAg during follow-up seroconverted to anti-HBs within 10 years.



**Fig. 2.** Age  $\geq 35$  years and seroclearance of hepatitis B surface antigen (HBsAg) was associated with earlier appearance of antibody against hepatitis B surface antigen (anti-HBs) than for individuals aged  $< 35$  years.

Development of anti-HBs may be a clue which shows that they have protection and are less likely to predispose to the development of chronic hepatitis, cirrhosis and hepatocellular carcinoma [1, 2]. In our series of cases, we did not find these complications at the time of seroclearance of HBsAg, or during the follow-up period. In contrast to acute hepatitis B cases, in which anti-HBs develops within 6 months of the initiation of infection, in those with chronic HBV infection, seroconversion to anti-HBs may develop at 1 year after HBsAg clearance in a minority of cases.

Arase *et al.* [8] reported that development anti-HBs was seen in 50.2% of patients with spontaneous seroclearance of HBsAg at the fifth year after HBsAg seroclearance. In our study, after the mean follow-up of  $43.7 \pm 45$  months, anti-HBs developed in 64 (63.4%) cases and no patients developed cirrhosis or hepatocellular carcinoma, which is in agreement with the results obtained by Chen *et al.* [10]. In our study, we found that 8.7% of cases seroconverted to anti-HBs within 12 months of seroclearance of HBsAg, which was lower than the results obtained in previous studies of 11.4% and 16.7% [19, 21].

An interesting finding in our study was that individuals who had HBsAg seroclearance after age  $\geq 35$  years had a lower mean time-span for the appearance

of anti-HBs compared to individuals aged  $< 35$  years (Fig. 2, Table 1). Therefore, the older the age of the patient at the time of seroclearance, the more likely they are to develop anti-HBs, a finding that has not been reported previously.

Yuen *et al.* [21] showed that if HBsAg seroclearance occurred before age 50 years, these patients would have a significantly lower risk of developing cirrhosis or HCC since we saw no cirrhosis or cancer in the follow-up of our cases. Ferreira *et al.* [22] followed-up 548 cases of chronic HBV infection for 15 years and they found that 40 cases lost HBsAg with no progression to more severe forms of the disease during follow-up and the results were similar to those obtained in our study.

It is of interest that 63.4% of carriers who became HBsAg negative developed anti-HBs, suggesting that the remaining 36.6% cases of HBV infection were not cleared, and HBsAg production decreased below the sensitivity of the detection methods. Other findings of our study were the detection of HBV DNA in 16 (15.8%) out of 101 cases at the time of seroclearance of HBsAg and that the majority of patients had persistently undetectable HBV DNA levels during follow-up. Similar to our study, taken within 1 year after HBsAg seroclearance, other studies found that

up to 13.4% of cases had detectable HBV DNA with low viral loads [18, 21].

With the appearance of anti-HBs, we observed low levels of HBV DNA (<100 copies/ml) in a small number of our cases. Low viral load, genotypes B, C and lengthy duration of HBV infection were associated with HBsAg seroclearance [6, 23]. Earlier studies [8, 16, 24] reported HBV DNA in 31–71.4% of cases at the time of HBsAg seroclearance, which decreased to 21.4% after 5 years, and 14.3% after 10 years. The weakness of the present study is that we did not determine the precise presence or absence of liver fibrosis because asymptomatic carriers are not required to be evaluated regarding liver fibrosis [3]. Long-term follow-up of these seroconverted subjects is necessary to see the clinical outcome. In conclusion, the results show that anti-HBs may develop in up to 78% of cases of anti-HBe-positive chronic carriers of hepatitis B. Age  $\geq$  35 years at HBsAg seroclearance was associated with the earlier development of anti-HBs.

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#### DECLARATION OF INTEREST

None.

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