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Spontaneous loss of hepatitis B surface antigen in chronic carriers, based on a long-term follow-up study in Goto Islands, Japan

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Abstract: Annual mass examination was performed between 1972 and 1997 in Tomie-town, Goto Islands, Japan, where hepatitis B virus (HBV) infection is very prevalent. In the present study, the incidence of spontaneous loss of hepatitis B surface antigen (HBsAg) in HBsAg carriers was determined in this area. Three thousand and nineteen inhabitants were tested for HBsAg two or more times in our annual surveys. Among them, 131 (4.3%) were defined as chronic HBsAg carriers based on the persistence of HBsAg for 1 or more years. These 131 subjects were followed for 12.2 ± 7.6 years. During the follow-up period, spontaneous loss of HBsAg occurred in 38 (29%) of the 131 carriers, with a yearly incidence of 2.5%. This loss was seen more frequently in carriers aged 40 years or more on enrollment than in those aged less than 40 years during the same observation periods ($P = 0.0141$), irrespective of sex or the results of liver function tests. The values for liver function test results were similar before and after loss of HBsAg in these carriers. Stored serum samples were available for later analysis of HBV-DNA by polymerase chain reaction in 32 carriers with loss of HBsAg. The HBV-DNA sequence was detected in 26 (81%) and 2 of the 32 carriers (6%) before and after loss of HBsAg, respectively. These results indicate that spontaneous loss of HBsAg, largely attributable to clearance of viremia, occurs age-dependently in chronic carriers.

Key words: hepatitis B surface antigen, epidemiology, polymerase chain reaction

Introduction

Hepatitis B virus (HBV) is the most common cause of acute and chronic liver disease worldwide, although its prevalence shows striking geographic and ethnic variations.[1–3] Persistent HBV infection evolves into chronic hepatitis, and a proportion of patients with chronic disease progress to liver cirrhosis, hepatocellular carcinoma (HCC), or both.[4–8] In Japan, current studies indicate that hepatitis C virus has had an important role as a causative agent in chronic liver disease and HCC.[7–10] HBV-associated liver cirrhosis and HCC are still common in the southern and western parts of Japan, where the mortality rate due to liver disorders is especially high in Goto Islands, Nagasaki Prefecture.[11,12]

Annual mass examinations have been done since 1968 in Tomie-town, Goto Islands, and hepatitis B surface antigen (HBsAg) in the inhabitants has been determined annually between 1972 and 1997. We previously demonstrated that the positivity rate for HBsAg in the inhabitants was approximately 5%, and that the rate for inhabitants born during 1946–1950 was more than 10%, followed by a decreasing trend, with the rate being only 0.6% in those born during 1971–1975, mainly due to a reduction in the occurrence of horizontal transmission of HBV in infancy.[13] Further, recent annual surveys revealed a marked reduction in the prevalence of HBV carriage, which was partly responsible for the decrease in the HBsAg carrier rate in relatively younger inhabitants.[13]

Although many studies of HBV carriers have been done, the course of chronic HBV infection is not well understood. The aim of the present study was to clarify the natural course of HBsAg carriers in this area on the basis of a long-term follow-up study.

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Subjects and methods

In annual mass examinations between 1972 and 1997 in Tomie-town, Goto Islands, Japan, 4015 inhabitants (including more than 80% of the adult inhabitants of this area) were tested for HBsAg, using the reverse passive hemoagglutination method. In addition to the test for HBsAg, levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in the inhabitants at each annual survey. Among them, 3019 inhabitants (1210 men and 1809 women) were tested for HBsAg two or more times in our annual surveys, and 157 (5.2%) were positive for HBsAg. However, in this study, to rule out false-positive and false-negative results and to exclude those inhabitants who were transiently exposed to HBV, those who were persistently positive for HBsAg for 1 or more years were defined as chronic carriers. According to this definition, 131 (4.3%) of these 3019 inhabitants were defined as chronic HBsAg carriers (Table 1). This sample population included 60 men and 71 women, aged 44 ± 12 years on enrollment (range, 8–81 years). On enrollment in this study, 11 HBsAg carriers were tested for hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe), using radioimmunoassay. Levels of serum AST and ALT on enrollment were elevated (is AST >35 IU/l and ALT >30 IU/l) in 12 (9.2%) and 5 (3.8%) of the 131 chronic HBsAg carriers, respectively, and 14 (10.7%) had elevated values on one or more liver function tests on enrollment. The subjects were followed for 12.2 ± 7.6 years (range, 2–24 years).

During the follow-up period, 38 (29%) of the 131 HBsAg carriers showed loss of HBsAg. Serum samples obtained before and after loss of HBsAg and stored at −40°C were available for later analysis by polymerase chain reaction (PCR) for the presence of HBV-DNA in 32 of the 38 carriers with loss of HBsAg. In brief, DNA was isolated from each serum sample as described previously.[14–16] PCR was performed using a set of oligonucleotide primers; sense primer, 5′-TACAGGCGGGGGTTTTTCTTG-3′, and anti-sense primer, 5′-AAGCCCTACGAACCACTGAA-3′, by which the surface sequence of HBV-DNA was amplified. The reaction involved 40 cycles with denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. The PCR product was extracted with phenol-chloroform, precipitated with ethanol and later separated by electrophoresis on a 2% agarose gel. The separated band corresponding to the HBV-DNA fragment was visualized using ethidium bromide staining. With respect to the analytical sensitivity of the qualitative PCR assay for HBV-DNA, the value is 100 genome equivalents/ml.

Table 1. Characteristics of 131 chronic HBsAg carriers on enrollment

Sex (M/F)	60/71
Age in years[a] (range)	44.2 ± 12.2 (8–81)
Liver function tests	
AST, ≤35 IU/l	n = 119
>35 IU/l	n = 12 (range, 37–72 IU/l)
ALT ≤30 IU/l	n = 126
>30 IU/l	n = 5 (range, 32–62 IU/l)
Follow-up period in years[a] (range)	12.2 ± 7.6 (3–24)

HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase
[a] Data values are expressed as means ± SD

The cumulative rate of loss of HBsAg in chronic carriers was evaluated using the Kaplan-Meier method.[17] The observation period started at the time of enrollment and ended at the end of August, 1997. The analysis of individual records showed that none of the 131 chronic HBsAg carries received anti-viral medications such as interferons or nucleotide analogues or vaccination against HBV during the observation period. Fourteen subjects (11%) died (of whom 7 died of advanced liver cirrhosis, HCC, or both) and 10 (8%) were lost to follow-up during the study.

Statistical analyses were performed using the χ^2 test, Student's t-test (two-tailed), or multivariate analysis by Cox's regression model. P values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were carried out using Stat-View-J 4.5 software (Abacus Concepts, Berkeley, CA, USA) for the Macintosh Computer.

Results

Spontaneous loss of HBsAg in chronic carriers

Approximately 1000 to 1500 individuals, including 50 to 60 HBsAg carriers and those who cleared HBsAg, received our survey every year; consequently, 4015 inhabitants were tested for HBsAg between 1972 and 1997. One carrier was positive for HBeAg and 10 carriers were positive for anti-HBe. During the follow-up period, 38 (29%) of the 131 chronic HBsAg carriers showed loss of HBsAg. The cumulative rate of loss of HBsAg in chronic carriers was analyzed using the Kaplan-Meier method, and this showed that loss of HBsAg occurred time-dependently, with a yearly incidence of approximately 2.5% (Fig. 1). To elucidate the factors involved in loss of HBsAg in chronic carriers, we investigated their backgrounds (Table 2). Loss of HBsAg was seen more frequently in carriers aged 40 years or more on enrollment than in those aged less than 40 years during the same observation periods (P = 0.0141), but this was not dependent on sex or on the values for liver function test results on enrollment. Mul-

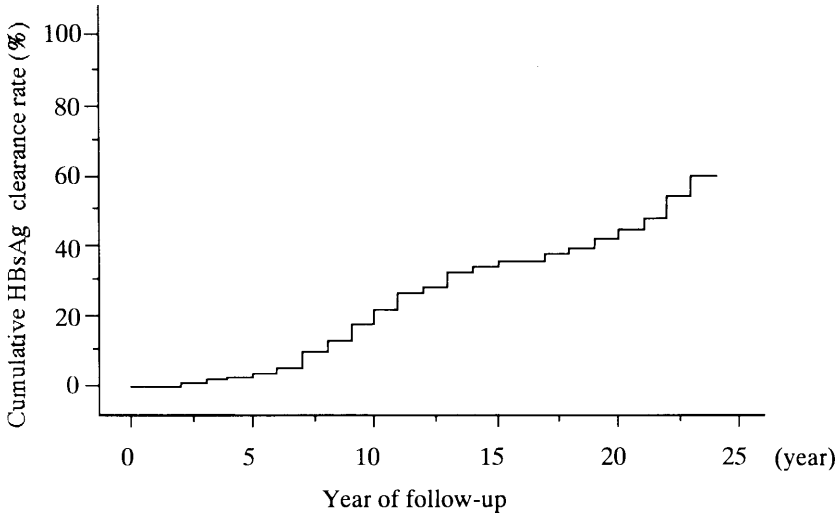

Fig. 1. Analysis of cumulative hepatitis B surface antigen (*HBsAg*) clearance rate. One hundred and thirty-one HBsAg carriers were followed in our annual surveys. During the mean follow-up period of 12.2 years, loss of HBsAg was found in 38 (29%) of the 131 carriers

Table 2. Analysis of factors involved in loss of HBsAg in chronic carriers

Factors	Loss of HBsAg (%)	Follow-up period (years)[a]
Sex		
Male (n = 60)	18 (30%)	11.5 ± 7.4
Female (n = 71)	20 (28%)	12.9 ± 7.7
Age on enrollment (years)		
<40 (n = 45)	7 (16%)	12.0 ± 7.5
≥40 (n = 86)	31 (36%)*	12.6 ± 7.7
Liver function test results on enrollment		
AST		
Normal[b] (n = 119)	35 (29%)	12.7 ± 7.9
Abnormal[c] (n = 12)	3 (25%)	9.7 ± 5.2
ALT		
Normal[d] (n = 126)	38 (30%)	12.6 ± 7.8
Abnormal[e] (n = 5)	0 (0%)	8.2 ± 5.8

* P = 0.0141 AST, aspartate aminotransferase; ALT, alanine aminotransferase
[a] Data values are expressed as means ± SD
[b] Normal, ≤35 IU/l
[c] Abnormal >35 IU/l
[d] Normal ≤30 IU/l
[e] Abnormal >30 IU/l

tivariate analysis also revealed that age at entry was significantly associated with loss of HBsAg (P = 0.0126) (Table 3). In addition, serial changes in the values for liver function test results were evaluated in the 38 carriers with loss of HBsAg (data not shown); the values were similar before and after loss of HBsAg.

PCR analysis of HBV-DNA in carriers with loss of HBsAg

PCR analysis for the presence of HBV-DNA was performed in 32 of the 38 carriers with loss of HBsAg;

Table 3. Multivariate analysis of variables correlated with loss of HBsAg in chronic carriers

Factors	Standard coefficient	P value
Sex	−1.362	0.1731
Age on enrollment	2.494	0.0126
<40 years		
≥40 years		
AST	0.033	0.9733
Normal		
Abnormal		

AST, aspartate aminotransferase
Normal and Abnormal, as defined in Table 2 footnote

Table 4. Polymerase chain reaction analysis of HBV-DNA in 32 carriers with loss of HBsAg

HBV-DNA	Male (n = 14)	Female (n = 18)	Total (n = 32)
No. positive before loss of HBsAg	12	14	26 (81%)
No. positive after loss of HBsAg	1	1	2[a] (6%)

[a] Both carriers were also positive for hepatitis B virus (HBV)-DNA before loss of HBsAg

26 of these 32 subjects (81%; 12 men and 14 women) were positive for HBV-DNA before loss of HBsAg (Table 4). In contrast, only 2 (6%) were persistently positive for HBV-DNA after loss of HBsAg ($P < 0.001$). PCR failed to detect the HBV-DNA sequence in 6 carriers during the study.

Discussion

Perinatal transmission of HBV, particularly mother-to-child transmission, is the most common mode of infection, especially in East Asia, and frequently leads to a chronic carrier state.[18,19] During persistent HBV infection, some patients progress to chronic liver disease, HCC or both, and others remain asymptomatic.[6] However, the natural course of persistent HBV infection is not fully understood. In the present study, loss of HBsAg in chronic carriers was analyzed on the basis of a long-term follow-up study in an endemic area.

Our annual surveys indicated that 38 (29%) of 131 chronic HBsAg carriers showed loss of HBsAg during the follow-up period. Loss of HBsAg in chronic carriers occurred time-dependently, with a yearly incidence of 2.5%. This was not associated with the values for liver function test results on enrollment or with a flare-up of hepatitis manifested by changes in the values before and after loss of HBsAg. However, the short period of flare-up of hepatitis may have been missed in some subjects because this study had loose annual surveillance. These results suggest that delayed clearance of serum HBsAg occurs frequently in chronic carriers. In several long-term follow-up studies, clearance of HBsAg in chronic carriers was believed to be an unusual event.[20–23] Hsu et al.[20] showed that spontaneous loss of HBsAg in carrier children occurred with a yearly incidence of 0.6% during a mean follow-up period of 4.3 years. In a long-term follow-up study of volunteer blood donors and HBsAg carriers in Montreal, Villeneuve et al.[21] found that the yearly incidence of loss of HBsAg in carries with a mean age of 30 years on enrollment was approximately 0.7%. Similar results were reported by some other investigators.[22,23] In Taiwan, where the HBsAg carrier rate was as high as approximately 15%,[24] recent studies estimated that the incidence of delayed HBsAg clearance was 1% to 2% among chronic carries with a mean age of 33 years.[23] Ethnic variations may account for differences in HBsAg clearance rates in chronic carriers. However, the discrepancy between our results and the previous results is, in part, attributable to the age distribution and the follow-up period; in fact, HBsAg carrier inhabitants with a mean age of 44 ± 12 years were followed for 12.2 ± 7.6 years in our study, and the HBsAg clearance rate in carriers aged 40 or more years on enrollment was significantly higher than that in carriers aged less than 40 years. In addition, the majority of our sample population were asymptomatic carriers and they were thought to be seronegative for HBeAg. A previous report showed that spontaneous loss of HBsAg occurred more frequently in asymptomatic carriers, comparted with patients with chronic hepatitis B.[22] Another report showed that HBsAg clearance also occurred more frequently in those who were seronegative for HBeAg, compared with those who were seropositive for HBeAg.[23] These reports support the relatively high incidence of HBsAg clearance in our studies.

PCR is a sensitive method for detecting virus genomes and is considered to be reliable marker of virus replication and infectivity. In patients with HBV, PCR assay can detect HBV-DNA at amounts as low as 10^{-5} pg.[25,26] Accordingly, to evaluate the state of viremia, we performed PCR analysis in 32 chronic carriers with loss of HBsAg. Before loss of HBsAg, 26 (81%) were positive for HBV-DNA, but only 2 (6%) were persistently positive for HBV-DNA after loss of HBsAg. Previous studies demonstrated that HBV-DNA was detected by PCR in almost all HBeAg-positive carriers examined and in 70%–80% of anti-HBe-positive carriers.[27] Mutations in viral genes allow viral persistence after serological recovery from HBV infection in some patients.[28] Yotsuyanagi et al.[29] reported that, in 10 of 11 patients with self-limited acute hepatitis B, HBV-DNA was detected by PCR for a long period after recovery. The incidence of HBV-DNA detection was significantly higher than our result. However, consistent with our results, Baker et al.[27] reported that 5 of 21 patients with chronic hepatitis B who had cleared HBeAg and HBsAg had HBV-DNA detectable by PCR on initial testing, but all eventually became negative for HBV-DNA on subsequent testing. The reason for this discrepancy between these results is not clear. Some differences in immune response may be involved.

In conclusion, spontaneous loss of HBsAg, largely attributable to clearance of viremia, occurs age-dependently in chronic carriers. Further investigation of factors associated with the virus, such as HBeAg status and serum HBV-DNA levels, involved in HBsAg clearance or the development of HCC after loss of HBsAg

could clarify the long-term prognosis of chronic HBsAg carriers.

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