Spontaneous relapse of hepatitis in inactive HBsAg carriers

Chia-Ming Chu · Yun-Fan Liaw

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Abstract

Background/aims The issue of spontaneous relapse of hepatitis in anti-HBe positive asymptomatic HBsAg carriers was rarely reported before and deserves further exploration.

Methods A total of 1241 anti-HBe positive asymptomatic adult HBsAg carriers were prospectively followed up. Of these, 661 (53%) were males, and the mean ($\pm$ SD) age was 35.6 ± 9.1 years. Relapse of hepatitis was defined as elevation of ALT more than twice the upper limit of normal accompanied by detectable serum HBV DNA by hybridization assays.

Results During a mean follow up of 12.3 years, hepatitis relapsed in 211 patients with an annual rate of 1.46%. The cumulative probabilities of hepatitis relapse were 10.2%, 17.4%, 19.3%, 20.2%, and 20.2%, respectively, after 5, 10, 15, 20, and 25 years of follow up. Multivariate analyses showed that the probability of hepatitis relapse correlated significantly with male sex ($P < 0.0001$) and age at entry ($P = 0.007$). The cumulative probability of hepatitis relapse after 20 years was 26.9% for males and only 12.5% for females, and was 13.1% for those of age <30 years at entry but increased to 29.4% for those of age 40–49 years at entry.

Conclusion Hepatitis relapsed in about 20% of asymptomatic HBsAg carriers during 25 years of follow up. Relapse of hepatitis occurred more frequently during

earlier years of follow up. Males were more likely to have relapse of hepatitis than females. In addition, relapse of hepatitis was significantly less frequent in patients who were younger than 30 years at study entry, possibly implicating more favorable outcome of earlier HBeAg seroconversion.

Keywords Age · Anti-HBe · HBeAg seroconversion · Gender

Abbreviations

HBV	Hepatitis B virus
HBeAg	Hepatitis B e antigen
ALT	Alanine aminotransferase
Anti-HBe	Antibody against hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
Anti-HCV	Antibodies against hepatitis C virus
Anti-HDV	Antibody against hepatitis D virus

Introduction

The epidemiological and clinical features of hepatitis B virus (HBV) infection vary between high and low HBV endemic areas [1]. In high endemic areas such as Southeast Asia and Taiwan, HBV infection occurs predominantly in the perinatal period or early childhood with high-rate of persistent infection [1]. The natural history of chronic HBV infection consists of three chronological phases: an initial immune tolerant phase (hepatitis B e antigen [HBeAg] positive and normal alanine aminotransferase [ALT] levels), followed by immune clearance phase (HBeAg positive and abnormal ALT levels), and finally the low replication phase (hepatitis B e antibody [anti-HBe] positive and normal ALT levels) [2, 3]. Patients in the third phase

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C.-M. Chu (✉) · Y.-F. Liaw
Liver Research Unit, Chang Gung Memorial Hospital and Chang Gung University, 199, Tung Hwa North Road, Taipei 10591, Taiwan
e-mail: chiamingchu@yahoo.com.tw

usually have HBV DNA undetectable by hybridization assays and are termed “inactive HBsAg carriers”. The ultimate outcome of chronic HBV infection appears to depend on the duration of the immune clearance phase as well as on the severity of liver damage during this phase [4, 5]. In addition, hepatitis may relapse after HBeAg seroconversion that also significantly contributes to the development of cirrhosis [6–8]. There were two published studies from Taiwan that prospectively followed up the natural course after spontaneous HBeAg seroconversion [6, 7]. The annual rates of relapse of hepatitis (ALT > twice the upper normal limit and detectable serum HBV DNA by hybridization assays) were 3.3% in the study involving patients with chronic hepatitis B [6] and 2.2% in the study involving asymptomatic patients [7].

HBV infection is highly endemic in Taiwan, where the HBsAg carrier rates in the general population are high up to 15–20% before the era of universal nation-wide neonatal HBV vaccination [9–11]. Most HBsAg carriers are incidentally identified during blood donation or health check up. Among them, the great majority is negative for HBeAg and has normal ALT. For example, in one large series of 10,431 asymptomatic adult HBsAg carriers from Taiwan, 19.6% were HBeAg positive and 80.4% were HBeAg negative. Of the latter, only 16.2% had abnormal ALT. It is thus estimated that 67% of incidentally recognized asymptomatic HBsAg carriers in Taiwan are HBeAg negative with normal ALT [12]. The natural course of chronic HBV infection in these inactive HBsAg carriers, however, is rarely reported.

Recently, we have noted that there was an appreciably high-rate of spontaneous HBsAg seroclearance in anti-HBe positive asymptomatic adult HBsAg carriers during a long-term follow up and that HBsAg carriers with sustained remission of hepatitis were 2.2 times more likely to have spontaneous HBsAg seroclearance than those with relapse of hepatitis [13]. In that study, the annual rate of spontaneous relapse of hepatitis was 1.6%, which correlated positively with male sex and age at entry [13]. However, the issue of spontaneous relapse of hepatitis was not particularly addressed. In this study, we tried to further analyze in more detail and discuss the rates, determinants and clinical implication of spontaneous relapse of hepatitis in 1241 anti-HBe positive asymptomatic adult HBsAg carriers from Taiwan.

Materials and methods

Patients

From 1980, asymptomatic adults who were incidentally identified as HBsAg carriers during blood donation or

health check up were recruited into the present study if they fulfilled the following criteria: (1) HBsAg positive for at least one year; (2) HBeAg negative, anti-HBe positive, normal ALT (0–36 U/l), no evidence of cirrhosis based on the clinical ground and liver ultrasonography [14] and no concomitant infection with hepatitis C virus or hepatitis D virus at baseline; (3) no anti-viral or immunomodulatory therapy before entry and during follow up; (4) regular follow up at least every year and for a minimum of 3 years; (5) available for follow up through 2005. Patients who had alcohol or drugs that might be possible etiological agents of hepatitis were excluded. No patient in this cohort admitted intravenous drug abuse or homosexuality.

Clinical data at baseline and during follow up

At entry, the patients were tested for liver biochemical tests, virological markers (HBsAg, HBeAg, anti-HBe, antibody against hepatitis C virus [anti-HCV], and antibody against hepatitis D virus [anti-HDV]), α -fetoprotein, and liver ultrasonography. Liver biochemical tests were assayed every 6 months if ALT levels were normal, and every 1–3 months or more often if ALT levels were elevated. HBsAg, HBeAg, anti-HBe, and α -fetoprotein were assayed every 6 months or more often if clinically indicated. Liver ultrasonography was assayed every 6–12 months or more often if clinically indicated. Assays for anti-HCV and anti-HDV were repeated if ALT levels elevated to more than twice the normal upper limit. Serum HBV DNA was not routinely assayed but was measured when ALT levels elevated to more than twice the normal upper limit.

Methods

HBsAg, HBeAg, anti-HBe, and anti-HDV were assayed by radioimmunoassay (Abbott Diagnostics, North Chicago, Ill.). Anti-HCV was tested by a second- or third-generation enzyme immunoassay (Abbott Diagnostics). Hepatitis B virus DNA was tested by sandwich molecular hybridization assays using a Digene Hybrid Capture System (Digene Diagnostics, Inc., Beltsville, Md.). The detection sensitivity was 0.5 pg/ml.

Relapse of hepatitis was diagnosed when ALT levels elevated to more than twice the normal upper limit, accompanied by positive serum HBV DNA by hybridization assays [6–8]. Patients that remained positive for anti-HBe with normal ALT through the course of follow up were defined as sustained remission.

Statistical analysis

To compare characteristics between groups, either chi-squared test or Fisher exact test was used for analysis of

categorical variables and Student *t* test or Wilcoxon non-parametric test was used for analysis of continuous variables. Estimates on the rate of hepatitis relapse were calculated by the actuarial analysis method, and the difference was determined by the long-rank test. Univariate and multivariate analyses were performed to identify factors associated with relapse of hepatitis. Variables found to be significant in the univariate models were tested in a multivariate setting using the Cox proportional hazards regression models. Statistical procedures were performed with SPSS statistical software (version 13.0; SPSS, Chicago, IL). *P* values <0.05 were considered significant.

Results

A consecutive series of 1241 anti-HBe positive asymptomatic adult HBsAg carriers were enrolled in this study. Their baseline and follow-up data were summarized in Table 1. The mean ($\pm$ SD) age at entry was 35.6 ± 9.1 years (median, 34.5; range, 16–76 years), and 661 (53%) were men.

During 14,484 person-years of follow up, relapse of hepatitis occurred in 211 patients (154 males and 57 females). The calculated rate of hepatitis relapse was 1.46% per year. The cumulative probabilities of hepatitis relapse were 10.2%, 17.4%, 19.3%, 20.2%, and 20.2%, respec-

tively, after 5, 10, 15, 20, and 25 years. Multivariate analyses using the Cox proportional hazards regression models showed that the probability of hepatitis relapse correlated positively with male sex (hazard ratio [95%CI] = 2.41 [1.78–3.27], *P* < 0.0001) and age at entry (hazard ratio [95%CI] = 1.22 [1.06–1.41] per decade increase, *P* = 0.007). The person-years of follow up and the events of hepatitis relapse in each age group are illustrated in Table 2. The annual rates of hepatitis relapse increased significantly with increasing age at entry. The cumulative probability of hepatitis relapse according to gender is shown in Fig. 1, and according to age at entry is shown in Fig. 2. Relapse of hepatitis was significantly more frequent in males than in females. In addition, relapse of hepatitis was least likely in carriers of age younger than 30 years and most likely in those of age 40–50 years at study entry.

Forty patients were found to progress to cirrhosis based on the clinical ground and liver ultrasonography [14] until the last visit. Of these, four subsequently developed hepatocellular carcinoma. The annual rate of progression to cirrhosis was 1.14% (33 during 2,887 person-years of follow up) for patients with relapse of hepatitis and 0.06% (7 during 11,597 person-years of follow up) for those without (*P* < 0.0001). The rates of progression to cirrhosis also increased progressively with increasing age at entry, as shown in Table 2.

Discussion

The present study showed that annual rate of spontaneous relapse of hepatitis in anti-HBe-positive asymptomatic adult HBsAg carriers was approximately 1.5%. This figure was substantially lower compared to the rates of 2.2% and 3.3%, respectively, in two previous studies reported from our unit [6, 7]. It should be pointed out that the previous two studies followed up the patients starting from the time of HBeAg seroconversion, while the onset of HBeAg seroconversion in this cohort of patients was unknown. In one of these studies, the cumulative probability of HBeAg negative hepatitis after spontaneous HBeAg seroconversion was

Table 1 Baseline and follow-up characteristics of study patients

Characteristics	<i>N</i> = 1241 Mean $\pm$ SD or number (%) (range)
Age at entry (years)	35.6 ± 9.1 (16–76)
Men:women	661:580
Follow up duration (years)	12.3 ± 5.5 (3–26)
Male	12.6 ± 5.7 (3–26)
Female	12.0 ± 5.3 (3–25)
Relapse of hepatitis	211(17.0)
Male	154(23.3)
Female	57(9.8)

Table 2 Calculated rates of relapse of hepatitis and cirrhosis in 1241 anti-HBe positive asymptomatic adult HBsAg carriers according to age at entry

Age at entry	<i>N</i>	Person-years of follow-up	No. (%/years) of patients with relapse of hepatitis	No (%/year) of patients with cirrhosis
<30 years	320	4,176	37(0.89%)*	1(0.03%)#
30–39 years	563	6,565	101(1.53%)*	17(0.25%)#
40–49 years	251	2,620	56(2.14%)*	14(0.53%)#
≥ 50 years	107	1,123	17(1.51%)*	8(0.71%)#
Total	1241	14,484	211(1.46%)	40(0.28%)

*, # *P* < 0.0001 by chi-square test for trend

Fig. 1 Actuarial analysis of the cumulative probability of spontaneous relapse of hepatitis in 1241 anti-HBe-positive asymptomatic adult HBsAg carriers according to gender ($P < 0.0001$)

Fig. 2 Actuarial analysis of the cumulative probability of spontaneous relapse of hepatitis in 1241 anti-HBe-positive asymptomatic adult HBsAg carriers according to age at entry ($P = 0.0007$)

14%, 18%, 22%, respectively, at 3, 5, and 10 years of follow up [6]. These data indicate that more than half of relapse of hepatitis occurred during the first 3 years after HBeAg seroconversion. The mean age at entry of the patients in this cohort was 35.6 years, while previous studies revealed that the mean age of HBeAg seroconversion for HBeAg-positive HBsAg carriers from endemic areas was 31–33 years [7, 15–17]. These data probably suggest that HBsAg carriers in this cohort were enrolled into this study 2–4 years after HBeAg seroconversion, which may explain the relatively lower rates of relapse of hepatitis in the present series than in the previous series. Moreover, the present results showed that the risk of spontaneous relapse of hepatitis tended to decrease with the time of follow up. The cumulative probability of spontaneous relapse of hepatitis was 10.2% after 5 years of follow up. However, the rates tended to progressively become low 5 years thereafter compared to the first 5 years: the cumulative probability of relapse of hepatitis increased to 17.4% after 10 years (7.2% increase from 6th to 10th year of follow up), 19.3% after 15 years (1.9% increase from 11th to 15th year of follow up), and 20.2% after 20 years (0.9%

increase from 16th to 20th year of follow up). Notably, the risk of relapse of hepatitis became negligible, if any, after 20 years of follow up (Fig. 1).

Two factors independently associated with spontaneous relapse of hepatitis in anti-HBe positive asymptomatic adult HBsAg carriers were identified in this study. In keeping with the previous observations that relapse of hepatitis after HBeAg seroconversion was significantly more frequent in male carriers than in female carriers [7], the present study showed that male carriers were 2.4 times more likely to have spontaneous relapse of hepatitis than female carriers. As shown in Fig. 1, the cumulative probability of spontaneous hepatitis relapse after 20 years of follow up was 26.4% for male carriers but was only 12.5% for female carriers ($P < 0.0001$). Because HBsAg carriers with relapse of hepatitis were about 20 times more likely to progress to cirrhosis compared to those with sustained remission of hepatitis, as previously observed [7] and in this cohort of carriers, the high risk of relapse of hepatitis in male gender may account for the male predominance among patients with HBV related cirrhosis [18], despite of little male sex dominance in asymptomatic HBsAg carriers in high endemic areas, as shown in this cohort.

One interesting finding of the present study is that spontaneous relapse of hepatitis correlated significantly with the age at entry. As shown in Table 2 and Fig. 2, spontaneous relapse of hepatitis occurred much less frequently in patients who were younger than 30 years at study entry. The cumulative probability of spontaneous relapse of hepatitis after 20 years of follow up was only 13.1% for carriers of age less than 30 years at entry. The cumulative probability increased much with increasing age at entry, peaked to 29.4% in those of age 40–49 years and then declined in those of age over 50 years at entry. While the onset of HBeAg seroconversion for patients of age over 30 years at entry was unknown, HBsAg carriers of age less than 30 years at entry in this cohort can be considered as those with early HBeAg seroconversion. The present results possibly implicate that HBeAg seroconversion before age 30 years may be associated with a decreased risk of spontaneous relapse of hepatitis, compared to that after age 30 years. Further studies are indicated to clarify this issue.

With regard to viral factors possibly associated with spontaneous relapse of hepatitis, the results of previous studies from Taiwan and Hong Kong have suggested that relapse of hepatitis was significantly more frequent in genotype C infection than in genotype B infection [8, 17]. Unfortunately, data on HBV genotype and HBV DNA levels at entry were not available in this study, because the majority of patients in this cohort entered into follow up long before the significance of HBV genotype and viral load [19, 20] have been explored and no adequate serum

samples were available for study retrospectively. Further studies are indicated to clarify the impact of HBV genotype as well as other viral factors such as precore stop mutant and basal core promoter mutant in spontaneous relapse of hepatitis in anti-HBe positive asymptomatic carries.

In conclusion, the annual rate of spontaneous relapse of hepatitis in anti-HBe positive asymptomatic adult HBsAg carriers is estimated to be 1.5%. Approximately 20% of these carries have spontaneous relapse of hepatitis during 25 years of follow up. Relapse of hepatitis occurs relatively more frequently during the first 5- to 10- year follow up. Male carriers are 2.4 times more likely to have relapse of hepatitis than females. In addition, relapse of hepatitis is significantly less likely to occur in patients who are younger than 30 years at study entry. The latter finding may implicate more favorable outcome of earlier HBeAg seroconversion in chronic HBV infection.

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