Level of Hepatitis B Virus DNA in Inactive Carriers With Persistently Normal Levels of Alanine Aminotransferase

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BACKGROUND & AIMS: Little is known about the level of hepatitis B virus (HBV) DNA in individuals with chronic, inactive HBV infections. Patients who test positive for the antibody to hepatitis B e antigen (anti-HBe) and have normal levels of alanine aminotransferase for more than 10 years have a low risk of HBV reactivation and are considered to be inactive carriers. We investigated HBV DNA levels in inactive carriers and identified factors that correlated with this state among anti-HBe–positive carriers with HBV DNA levels of 10^4 copies/mL or greater (5.26 copies/mL = 1 IU/mL). **METHODS:** HBV DNA levels were assayed in 250 inactive carriers with persistently normal alanine aminotransferase levels for more than 10 years. Clinical and virologic features were compared between inactive carriers (with HBV DNA levels $\geq 10^4$ copies/mL) and age-matched patients with HBe antigen–negative chronic hepatitis (controls, n = 90). **RESULTS:** The median level of HBV DNA among inactive carriers was 3.70 $\log_{10}$ copies/mL (range, undetectable to 5.98 $\log_{10}$ copies/mL). Ninety (36%) had levels of 10^4 copies/mL or greater. Compared with control patients, significant differences of inactive carriers included sex (more female patients), lower HBV DNA levels, and lower prevalence of genotype C virus and the basal core promoter mutation T1762/A1764. The prevalence of the precore mutation A1896 was similar between groups. Multiple logistic regression analyses identified male sex, HBV DNA levels greater than 10^5 copies/mL, and the basal core promoter mutation as independent factors that correlated with active disease. **CONCLUSIONS: Nearly 40% of inactive carriers had HBV DNA levels of 10^4 copies/mL or greater. Female sex, HBV DNA levels of 10^4 to 10^5 copies/mL, and wild-type basal core promoter correlated with inactive carrier state.**

Keywords: Chronic Hepatitis B; Sex; Viral Genotype; Viral Load; Viral Mutants.

The natural course of chronic hepatitis B virus (HBV) infection constitutes 3 chronologic phases: an initial immune tolerance phase during which the patients are positive for hepatitis B e antigen (HBeAg) and have normal alanine aminotransferase (ALT) levels, followed by an immune clearance phase during which the HBeAg–positive patients have increased ALT levels, and, finally, the inactive carrier state during which HBeAg seroconverts to its antibody (anti-HBe) and ALT levels normalize.[1] The third phase may remain stably inactive in a lifetime. On the other hand, HBV also can reactivate either by reversion back to the previous HBeAg–positive phase or, much more frequently, by progression to HBeAg–negative chronic hepatitis.[2,3]

Among these phases, the inactive carrier state may be a retrospective–prospective diagnosis because inactive carriers show some propensity to reactivation. However, there is no single value of HBV DNA above which future reactivation is likely to occur and below which the disease is likely to be quiescent.[4] A HBV DNA level threshold of 10^4 copies/mL has been used to discriminate active HBV infection from its inactive form.[1,5–9] However, several studies have shown that between 7% and 55% of so-called inactive carriers with persistently normal ALT levels for variable duration had HBV DNA levels greater than 10^4 copies/mL,[10–13] and even in 2 studies from India and Taiwan, 35% and 32%, respectively, had levels greater than 10^5 copies/mL.[12,13] According to the long-term observations of 1965 anti-HBe–positive hepatitis B surface antigen (HBsAg) carriers with normal ALT levels from Taiwan, reactivation of hepatitis B most often occurred during the first 5 to 10 years of follow-up evaluation and became extremely rare thereafter.[14] However, in most previous studies the so-called inactive carriers had persistently normal ALT levels for a relatively short period of only 1 to 2 years; therefore, future reactivation can be anticipated in some of these carriers.[15] Clearly, viral load status of the inactive carrier state needs to be defined further.

Because reactivation of hepatitis B in anti-HBe–positive carriers became extremely rare 10 years after entry[14] and virtually none of the anti-HBe–positive carriers with persistently normal ALT levels over 10 years died of liver disease,[16] anti-HBe–positive carriers with persistently normal ALT levels over 10 years can be considered as real inactive carriers. Serum HBV DNA levels in such carriers may represent the viral load status of inactive carrier state. In this investigation, we first studied HBV DNA levels in 250 anti-HBe–positive HBsAg carriers with persistently normal ALT levels for more than 10 years. Our results showed that 90 (36%) of them had HBV DNA levels of 10^4 copies/mL or more, a level being used to indicate active hepatitis. To further identify factors that correlate with inactive carrier state among anti-HBe–positive carriers with HBV DNA levels of 10^4 copies/mL or more, we next compared the clinical and virologic features between inactive carriers with HBV DNA levels of 10^4 copies/mL or more and age-matched patients with HBeAg–negative chronic hepatitis.

Abbreviations used in this paper: ALT, alanine aminotransferase; anti-HBe, antibody against hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

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Materials and Methods

Inactive Carriers and Patients With Hepatitis B e Antigen–Negative Chronic Hepatitis

Asymptomatic adults who were identified incidentally as HBsAg carriers during blood donation or health check-ups received regular clinic follow-up evaluation at the carrier clinic of Chang Gung Memorial Hospital in Taipei, Taiwan.[14,17] The subjects were considered inactive carriers if they fulfilled the following criteria: (1) positive HBsAg, negative HBeAg, positive anti-HBe, and persistently normal ALT levels (≤36 U/L) at least once every 6 to 12 months for at least 10 years until the last visit; (2) no evidence of cirrhosis or hepatocellular carcinoma based on clinical assessment and liver ultrasonographic findings[18]; (3) no concomitant infection with hepatitis C virus or hepatitis D virus; and (4) no antiviral or immunomodulatory therapy before enrollment. Patients who underwent HBsAg seroclearance during the follow-up period[17] also were excluded.

Patients were diagnosed as having HBeAg–negative chronic hepatitis if they were HBsAg positive, HBeAg negative, and anti-HBe positive, had persistently abnormal ALT levels more than twice the upper limit of normal for at least 2 years, and HBV DNA levels were 10^4 copies/mL or more. Patients who had concomitant infection with hepatitis C virus or hepatitis D virus, autoimmune or metabolic liver disease, who consumed alcohol or drugs that might be potential etiologic agents of hepatitis, or who had ever received antiviral or immunomodulatory therapy were excluded.

Methods

HBsAg, HBeAg, anti-HBe, and antibody against hepatitis D virus were assayed using radioimmunoassay kits (Abbott Diagnostics, North Chicago, IL). Antibodies against hepatitis C virus were assayed using a second- or third-generation enzyme immunoassay (Abbott Diagnostics). Serum HBV DNA levels were assayed using the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Branchburg, NJ; lower limit of detection, 200 copies/mL). The conversion in IU/mL (1 IU is equivalent to 5.26 HBV DNA copies) was made according to the manufacturer's instructions. HBV genotypes were determined using the polymerase chain reaction–restriction fragment length polymorphism of the surface gene of HBV, as previously described.[19] Precore A1896 mutant was detected by amplification-created restriction site method, as described before.[20] Basal core promoter genes were amplified by polymerase chain reaction, and nucleotide sequences of the amplified products were determined directly by using an automatic sequencer, as described by others.[13]

Statistical Analyses

Data are presented as mean ± standard deviation, median (range), or number (%). To compare characteristics between groups, either the chi-square test or the Fisher exact test was used to analyze categoric variables and the Student *t* test or the Mann–Whitney *U* nonparametric test was used to analyze continuous variables. HBV DNA levels were correlated with ALT levels or age of patients by using Spearman rank correlation. Univariate and multivariate logistic regression analyses were performed to identify the factors that correlated with active disease among anti-HBe–positive HBsAg carriers with HBV DNA levels of 10^4 copies/mL or more. Variables with *P* values less than .1 in the univariate models were tested in a multivariate setting. Significant associations identified in multivariate analysis are presented as odds ratio (95% confidence interval). Statistical procedures were performed using the SPSS statistical software (version 13.0; SPSS, Chicago, IL). *P* values less than .05 were considered significant.

Results

Clinical and Demographic Features of Inactive Carriers

From January 2007 to December 2007, there were 250 consecutive anti-HBe–positive HBsAg carriers who had had persistently normal ALT levels for at least 10 years who received regular follow-up evaluation at our hepatitis carrier clinic. They were considered inactive carriers and were enrolled in this study. The demographic and clinical data are summarized in Table 1. There were 84 men and 166 women. The mean age at baseline was 34.4 years (range, 18–64 y) and the mean age at enrollment was 50.6 years (range, 31–81 y). The mean number of ALT measurements for each carrier was 28.4 (range, 12–48) during a mean period of 16.1 years (range, 10–25 y) before enrollment. The mean maximal ALT level before enrollment was 26.3 U/L (range, 9–36 U/L). Maximal ALT levels were 19 U/L or less in 26 patients (10.4%), 20 to 30 U/L in 159 patients (63.6%), and 31 to 36 U/L in 65 patients (26%). ALT levels were significantly higher in male carriers than female carriers. A total of 52 male carriers and 23 female carriers had persistently normal ALT levels according to the strict criteria of ALT of 30 U/L or less in males and 19 U/L or less in females, as suggested by Prati et al.[21]

Table 1. Clinical and Demographic Data of Inactive Carriers

Data	Total (n = 250)	Male (n = 84)	Female (n = 166)	*P*
Age at baseline, *y*	34.4 ± 8.8	35.6 ± 9.3	33.9 ± 8.6	.16
Male:female ratio	84:166			
Duration of persistently normal ALT levels before enrollment, *y*	16.1 ± 4.7	16.6 ± 4.9	15.9 ± 4.5	.27
Number of ALT determinations before enrollment	28.4 ± 8.9	29.8 ± 9.6	27.6 ± 8.4	.093
Maximal ALT levels before enrollment, *U/L*	26.3 ± 5.6	28.1 ± 5.0	25.3 ± 5.7	.0002
≤19 U/L	26 (10)	3 (4)	23 (14)	.012
20–30 U/L	159 (64)	49 (58)	110 (66)	.22
30–36 U/L	65 (26)	32 (38)	33 (20)	.0019
Age at enrollment, *y*	50.6 ± 9.6	52.2 ± 10.1	49.8 ± 8.4	.069

NOTE. Data are given as mean ± SD or number (%).