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ORIGINAL ARTICLE

A comparison of hepatitis B viral markers of patients in different clinical stages of chronic infection

Myron John Tong · Leeyen Hsu · Carlos Hsien · Jia-Horng Kao · Francisco Antonio Durazo · Sammy Saab · Lawrence Mitchell Blatt

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Abstract
Purpose Hepatitis B viral markers may be useful for predicting outcomes such as liver-related deaths or development of hepatocellular carcinoma. We determined the frequency of these markers in different clinical stages of chronic hepatitis B infection.
Methods We compared baseline hepatitis B viral markers in 317 patients who were enrolled in a prospective study and identified the frequency of these tests in immune-tolerant (IT) patients, in inactive carriers, and in patients with either hepatitis B e antigen (HBeAg)-positive or HBeAg-negative chronic hepatitis or cirrhosis.
Results IT patients were youngest (median age 27 years) and HBeAg-negative patients with cirrhosis were oldest (median age 58 years) ($p = 0.03$ to <0.0001). The male to female ratio was similar both in IT patients and in inactive carriers, but there was a male preponderance both in patients with chronic hepatitis and in patients with cirrhosis ($p < 0.0001$). The A1896 precore mutants were most prevalent in inactive carriers (36.4%) and HBeAg-negative patients with chronic hepatitis (38.8%; $p < 0.0001$), and the T1762/A1764 basal core promoter mutants were most often detected in HBeAg-negative patients with cirrhosis (65.1%; $p = 0.02$). Genotype A was detected only in 5.3% of IT patients, and genotype B was least often detected in both HBeAg-Positive patients with chronic hepatitis and cirrhosis ($p = 0.03$). The hepatitis B viral DNA levels were lowest in inactive carriers (2.69 $\log_{10}$ IU/mL) and highest in IT patients (6.80 $\log_{10}$ IU/mL; $p = 0.02$ to <0.0001). At follow-up, HBeAg-positive and HBeAg-negative patients with cirrhosis accounted for 57 of 64 (89.1%) liver-related deaths ($p < 0.0001$).
Conclusion Differences in baseline hepatitis B viral markers were detected in patients in various clinical stages of hepatitis B virus infection. HBeAg-positive and HBeAg-negative patients with cirrhosis accounted for the majority of the liver-related fatalities.

Keywords HBeAg · Precore mutant · Basal core promoter mutant · Genotype · HBV DNA · Immune tolerant · Inactive carriers · Chronic hepatitis · Cirrhosis

Abbreviations

Anti-HBe	Hepatitis B e antibody
BCP	Basal core promoter
CH	Chronic hepatitis
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B viral DNA
HCC	Hepatocellular carcinoma
IC	Inactive carrier

M. J. Tong · F. A. Durazo · S. Saab · L. M. Blatt
Division of Digestive Diseases, The Pfleger Liver Institute, David Geffen School of Medicine, University of California in Los Angeles, Los Angeles, CA, USA

M. J. Tong (✉) · L. Hsu · C. Hsien
The Liver Center, Huntington Medical Research Institutes, 660 S. Fair Oaks Ave, Pasadena, CA 91105, USA
e-mail: myrontong@hmri.org

J.-H. Kao
Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan

L. M. Blatt
Alios Biopharma, South San Francisco, CA, USA

IT	Immune tolerant
PC	Precore

Introduction

Patients with chronic hepatitis (CH) B have different clinical presentations. On initial evaluation in the clinic, routine liver, hematologic, and virologic tests are obtained and a liver biopsy may be required for diagnosis. Thereafter, patients may be placed into one of several clinical categories. Those with chronic hepatitis will have elevated serum aminotransferase levels and liver biopsy findings that show grade 1–3 inflammation and stage 1–3 fibrosis, whereas patients with cirrhosis may have clinical evidence of chronic liver disease, decreased platelet counts, and histologic findings of grade 4 inflammation and stage 4 fibrosis [1]. More difficult to categorize are hepatitis B surface antigen (HBsAg)-positive patients who are asymptomatic, have normal liver tests, and whose liver biopsy results may not be available to assist with diagnosis. In these patients, those who are hepatitis B e antigen (HBeAg) negative with low or undetectable levels of hepatitis B viral DNA (HBV DNA) are considered inactive carriers, whereas others who are HBeAg positive with high levels of HBV DNA are categorized as "immune tolerant (IT) [2]. In a clinical setting, these latter patients are virologic "active" carriers since histologic findings show little or no fibrosis [3].

Recently, the availability of other hepatitis B viral markers has shed further light on their potential roles in identifying patients who develop liver complications. The presence of high levels of HBV DNA has been associated with progression to cirrhosis and to the development of hepatocellular carcinoma (HCC) [4, 5]. Also, hepatitis B virus (HBV) genotype C and specific alleles of the basal core promoter (*BCP*) and precore (*PC*) genes were associated with a high risk of developing HCC [6]. The T1762/A1764 BCP mutants have been associated with progressive liver disease, hepatic decompensation, and HCC [7–9]. The role of A1896 PC mutants is less clear since reports from Asia showed that there was no difference in its prevalence in patients with or without liver complications, and its selection may actually lead to the inactivation of ongoing liver disease [8, 9]. However, reports from Europe showed that the A1896 PC mutants were associated with an aggressive form of HBeAg-negative chronic hepatitis [10, 11].

A recent report from the Agency for Healthcare Research and Quality on management of CHB suggested that patients in different clinical stages of CHB needed to be more clearly defined, since antiviral treatment guides for hepatitis B are based on specific categories of patients [12]. Accordingly, the report herein describes differences in the baseline laboratory tests and HBV viral markers of patients in different clinical stages of CHB. These observations may be useful in identifying patients who will have liver disease progression and therefore be the main targets for antiviral therapy.

Methods

Patients

From January 1989 to March 1998, HBsAg-positive patients who presented at our clinic in Pasadena, CA, were enrolled in a prospective follow-up study [1]. Patients who were positive for anti-HCV or HIV, or had a history of alcoholism or other chronic liver diseases such as hemochromatosis and autoimmune hepatitis, were excluded. During the first visit, routine liver and hematologic tests and HBeAg seroconversion rates were obtained. Serum was collected from each patient, stored at −70°C, and later tested for HBV DNA, BCP mutants, PC mutants, and HBV genotypes in one research laboratory [7]. For the present analysis, HBsAg-positive patients were stratified into six clinical categories: (1) immune tolerant, (2) inactive carriers, (3) HBeAg-positive chronic hepatitis, (4) HBeAg-negative chronic hepatitis, (5) HBeAg-positive cirrhosis, and (6) HBeAg-negative cirrhosis. By definition, IT patients were HBeAg positive, had normal alanine aminotransferase (ALT) levels, and had HBV DNA values of more than 20,000 IU/mL, whereas inactive carriers were HBeAg negative, had normal ALT values, and their HBV DNA levels were 20,000 IU/mL or less [13, 14]. At baseline, patients in the latter two categories were asymptomatic, and liver biopsies were not requested. One hundred forty-four HBsAg-positive patients had elevated serum aminotransferase levels, and all had liver biopsies (grades 1–3 and stages 1–3), confirming the diagnosis of chronic hepatitis. One hundred thirty patients had abnormal liver tests and had clinical stigmata of chronic liver disease [15]. Liver biopsies were obtained from 109 of these patients, which showed the presence of cirrhosis. The remaining 21 patients had platelet counts below 75,000 mm^3 and liver biopsies were not performed because of the risk of bleeding. The subsequent clinical outcomes in these patients have been reported [1].

Statistical analysis

The baseline data were descriptively summarized and the assessment of differences was completed using the analysis of variance with post hoc pairwise Student t tests for parametric data. Categorical data were summarized using frequencies and analyzed by Chi-squared analyses for the assessment of differences. All statistical significance values were assessed at $p < 0.05$.

Results

Baseline characteristics

The median age of the 317 HBsAg-positive patients was 49 (6, 80) years, 76% were men and 76% were Asian (Table 1). One hundred eighty-one (57%) patients were HBeAg positive, 53% had genotype C, 28% had A1896 PC mutants, and 45% had T1762/A1764 BCP mutants. The median HBV DNA level was 5.2 (0, 9.2) $\log_{10}$ IU/mL.

Baselines characteristics and laboratory tests by clinical status

Compared with others, IT patients were significantly younger in age, and HBeAg-negative patients with cirrhosis were older in age ($p = 0.03$ to <0.0001; Table 2). The male to female ratios were similar in the IT patients and inactive carriers, but there was an increase in male preponderance in the chronic hepatitis and cirrhosis groups ($p < 0.0001$; Table 3). Compared with others, the HBeAg-positive and HBeAg-negative patients with cirrhosis had the lowest albumin values and platelet counts ($p = 0.04$ to <0.0001).

Table 1 Baseline characteristics of 317 HBsAg-positive patients

Age (years)[a]	49 (6, 80)
Males	240 (76%)
Asians	240 (76%)
Albumin (g/dL)[a]	4.2 (1.9, 5.1)
Bilirubin (mg/dL)[a]	0.7 (0.1, 3.9)
AST (U/L)[a]	43 (8, 980)
ALT (U/L)[a]	50 (1, 620)
Platelets ($\times 10^3$ mm^3)[a]	188 (34, 527)
HBeAg	
+	181 (57%)
−	136 (43%)
Genotype (273)[b]	
A	49 (18%)
B	73 (27%)
C	145 (53%)
Mixed	6 (2%)
Precore (311)[b]	
A1896 mutant	88 (28%)
Wild	223 (72%)
Basal core promoter (246)[b]	
T1762/A1764 mutant	110 (45%)
Wild	136 (55%)
HBV DNA ($\log_{10}$ IU/mL)[a]	5.2 (0, 9.2)

[a] Median (minimal, maximal)

[b] Number detectable in 317 patients

Virologic tests by clinical status

Compared with others, IT patients had the highest median HBV DNA levels (6.81 $\log_{10}$ IU/mL, $p = 0.02$ to <0.0001), and inactive carriers had the lowest HBV DNA values (2.96 $\log_{10}$ IU/ml, $p = 0.02$ to <0.0001) compared with other groups (Table 2). Genotype A was least often detected in IT patients, and genotype B was least often detected in both HBeAg-positive patients with chronic hepatitis and cirrhosis ($p = 0.03$; Table 3). The A1896 PC mutants were more frequently detected in inactive carriers (36.4%) and in HBeAg-negative patients with chronic hepatitis (38.8%, $p < 0.0001$). The T1762/A1764 BCP mutants were most often detected in HBeAg-negative patients with cirrhosis (65.1%, $p = 0.02$). A scheme on the progression of CHB, which includes our findings in each clinical category, is shown in Fig. 1.

Liver-related deaths

During a mean follow-up of 84 months, 30 developed HCC and 37 died from non-HCC liver-related complications [1]. Most deaths (89%) occurred in the HBeAg-positive and HBeAg-negative patients with cirrhosis ($p < 0.0001$; Table 4).

Discussion

The natural history of CHB consists of four phases [2]. The first is the immune tolerance phase, which is mainly observed in younger patients who acquire HBV infection either at birth from HBsAg-positive mothers or by exposure during early childhood. These individuals have no symptoms, liver tests are normal, HBeAg is positive, and high circulating levels of HBV DNA (>20,000 IU/mL) are observed. The second is the immune clearance phase, which occurs in the second or third decade of life during which time patients are still HBeAg positive and have high HBV DNA levels, but the serum ALT levels are elevated. In this phase, liver biopsy findings show active inflammation and fibrosis. There are three potential sequelae to the second phase. Patients may either (1) remain HBeAg positive with future episodes of HBV reactivation, (2) undergo seroconversion to hepatitis B e antibody (anti-HBe) positivity and enter into the IC phase, or (3) progress to HBeAg-negative chronic hepatitis. Phase 3 is the IC phase characterized by anti-HBe positivity, normal ALT values, and low or undetectable serum HBV DNA levels. Phase 4 is reactivation of HBV after HBeAg seroconversion and is referred to as HBeAg-negative chronic hepatitis. In this phase, ALT levels are elevated, HBV DNA levels are high, and histologic features on liver biopsy

Table 2 Bivariate analysis of continuous variables in 317 HBsAg-positive patients

	Immune-tolerant HBeAg+ (no. 19)	Inactive carrier HBeAg− (no. 24)	Chronic hepatitis		Cirrhosis		*p* value
			HBeAg+ (no. 93)	HBeAg− (no. 51)	HBeAg+ (no. 69)	HBeAg− (no. 61)	
Age (years)	27 (9, 63)	45 (16, 64)	44 (6, 77)	49 (16, 75)	54 (22, 78)	58 (28, 80)	0.03 to <0.0001
Albumin (mg/dL)	4.4 (3.5, 4.8)	4.4 (2.8, 5)	4.3 (1.9, 5)	4.4 (3.4, 4.9)	3.9 (2.2, 5.1)	3.9 (1.9, 5)	<0.0001
Bilirubin (mg/dL)	0.5 (0.2, 1.4)	0.6 (0.1, 1)	0.6 (0.1, 3.2)	0.6 (0.3, 1.4)	0.8 (0.2, 2.8)	1.0 (0.3, 3.9)	0.46
ALT (U/L)	27 (1, 45)	23 (2, 45)	58 (2, 436)	45 (1, 98)	67 (1, 500)	52 (9, 620)	0.05 to <0.000
AST (U/L)	28 (11, 45)	21.5 (15,45)	43 (8, 239)	39 (12, 126)	55 (18, 270)	51 (15, 980)	0.04–0.001
Platelets ($\times 10^3$ mm^3)	268 (160, 527)	200 (103, 334)	202 (87, 376)	205 (79, 314)	140 (34, 336)	113 (44, 311)	0.0002 to <0.0001
HBV DNA ($\log_{10}$ IU/mL)	6.81 (4.31, 8.49)	2.69 (0, 4.26)	5.55 (0, 8.97)	4.98 (0, 9.02)	4.91 (0, 9.20)	5.51 (0, 8.97)	0.02 to <0.0001

Values represent median (minimal, maximal)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

Table 3 Bivariate analysis of discrete variables in HBsAg-positive patients

	Immune-tolerant HBeAg+ (no. 19)	Inactive carrier HBeAg− (no. 24)	Chronic hepatitis		Cirrhosis		*p* value
			HBeAg+ (no. 93)	HBeAg− (no. 51)	HBeAg+ (no. 69)	HBeAg− (no. 61)	
Sex							<0.0001
Male	9 (47.4%)	12 (50%)	75 (80.7%)	33 (64.7%)	56 (81.2%)	55 (90.2%)	
Female	10 (52.6%)	12 (50%)	18 (19.3%)	18 (39.3%)	13 (18.8%)	6 (9.8%)	
Race							0.007
Asian	18 (94.7%)	18 (75%)	73 (78.5%)	40 (78.4%)	41 (59.4%)	50 (82%)	
Non-Asian	1 (5.3%)	6 (25%)	20 (21.5%)	11 (21.6%)	28 (40.6%)	11 (18%)	
Genotype (no. 273)							0.03
A	1 (5.3%)	3 (18.7%)	19 (21.6%)	5 (12.8%)	16 (26.2%)	5 (10%)	
B	7 (36.8%)	6 (37.5%)	17 (19.3%)	15 (38.5%)	8 (13.1%)	20 (40%)	
C	11 (57.9%)	7 (43.7%)	50 (56.8%)	19 (48.7%)	34 (55.7%)	24 (48%)	
Mixed	0	0	2 (2.3%)	0	3 (4.9%)	1 (2%)	
Precore (no. 311)							<0.0001
A1896 mutant	4 (21%)	8 (36.4%)	11 (11.8%)	19 (38.8%)	17 (24.6%)	29 (29.1%)	
Wild	15 (79%)	14 (63.6%)	82 (88.2%)	30 (61.2%)	52 (75.4%)	30 (50.8%)	
Basal core promoter (no. 246)							0.02
T1762/A1764 mutant	7 (43.7%)	6 (54.5%)	26 (32.1%)	16 (44.4%)	27 (45.8%)	28 (65.1%)	
Wild	9 (56.2%)	5 (45.5%)	55 (67.9%)	20 (55.6%)	32 (54.2%)	15 (34.9%)	

show progressive inflammation and fibrosis. Although of value for understanding the natural history of hepatitis B, the four phases are neither helpful in identifying the clinical stages of patients presenting to the physician with CHB nor useful when evaluating patients for potential antiviral therapy.

There are few studies identifying prognostic baseline characteristics in patients in the four phases of CHB infection, but reports are available on the prevalence of hepatitis B viral markers in various clinical settings of CHB. A recent study from Hong Kong described the course of 57 patients considered to be in the IT phase of CHB [3]. By definition, all patients were HBeAg positive, 32 had genotype B and 25 had genotype C, and the mean HBV was 9.1 $\log_{10}$ IU/mL. Liver biopsy findings showed that 19 had fibrosis stage 0 and 38 had fibrosis stage 1. A report from Taiwan showed that 71% of asymptomatic carriers were genotype B compared with 15–23% with genotype C [9, 16]. Genotype C was associated with lower rates of HBeAg seroconversion, which occurred at an older age, and these patients were more likely to have worse clinical outcomes than genotype B patients [17, 18]. Also,

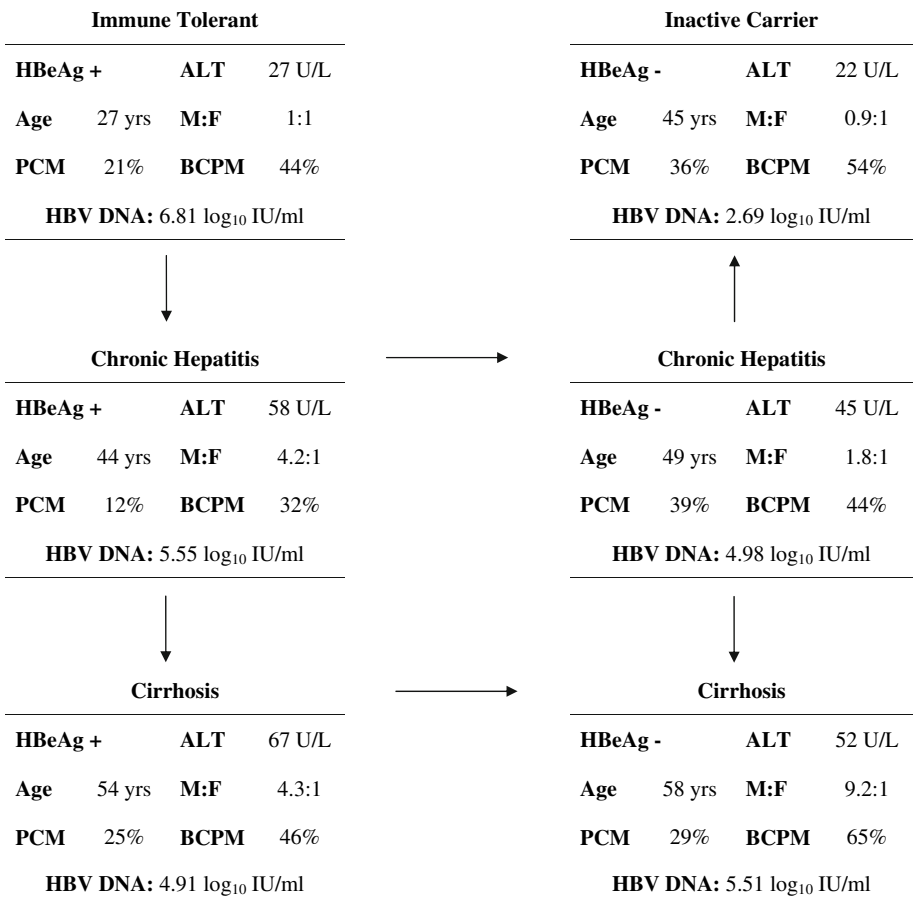

Fig. 1 A scheme on the progression of chronic hepatitis B infection. ALT, age and HBV DNA expressed as medians; HBV DNA in $\log_{10}$ IU/mL; *ALT* alanine aminotransferase, *PCM* precore mutant, *BCPM* basal core promoter mutant

Table 4 Liver-related deaths in 317 patients with chronic hepatitis B

	No. of deaths from liver-related complications (%)
Immune tolerant (no. 19)	0
Inactive carriers (no. 24)	0
Chronic hepatitis[a]	
HBeAg+ (no. 93)	5 (5.4%)
HBeAg− (no. 51)	2 (3.9%)
Cirrhosis[b]	
HBeAg+ (No. 69)[c]	29 (42%)
HBeAg− (No. 61)[d]	28 (45.9%)
Total	64 (20.2%)

[a] All deaths from HCC

[b] HBeAg-positive and HBeAg-negative cirrhosis patients versus others: $p < 0.0001$

[c] Seven deaths from HCC; 22 deaths from non-HCC liver complications

[d] 13 deaths from HCC; 15 deaths from non-HCC liver complications

genotype C was more prevalent in patients with cirrhosis than in asymptomatic carriers, and genotype B patients were less apt to progress to cirrhosis [9]. In an 11-year follow-up study from Taiwan, elevated HBV DNA levels at baseline were associated with progression to cirrhosis [5]. This was evident even in patients who were HBeAg negative and had normal ALT levels at baseline. Another report from China indicated that compared with HBV carriers with low or undetectable viral loads, those with high viral loads tended to have adverse clinical outcomes [19]. Reports from Europe indicated that mutations in the nucleotide 1896 and nucleotide 1762/1764 regions of the hepatitis B core genome were associated with progressive HBeAg-negative chronic hepatitis [11, 20]. Liver biopsy findings in these latter patients showed that more than 5% had moderate to severe inflammation and up to 40% had cirrhosis [21, 22]. Also, the risk of cirrhosis was reported to be higher in the HBeAg-negative patients with chronic hepatitis than in the HBeAg-positive patients with chronic hepatitis [23]. However, reports from Asia indicated that acquisition of A1896 PC mutants may lead to inactive liver disease and that similar percentages of A1896 PC mutations were detected in asymptomatic carriers and patients with cirrhosis and HCC [9, 24]. At present, the role of A1896 PC mutants in the progression of liver disease remains unclear. On the other hand, the T1762/A1764 BCP mutants have been associated with liver disease

progression, hepatic decompensation, and HCC development [7–9].

In the report herein, we classified our patients into six clinical categories. Age differences were noted, with IT patients being the youngest and HBeAg-negative patients with cirrhosis being the oldest. Gender differences were also noted, with an equal male to female ratio in IT patients and inactive carriers but a male preponderance in the patients with chronic hepatitis and cirrhosis. The highest HBV DNA levels were in the IT patients, and the lowest levels in inactive carriers. There did not appear to be significant differences in A1896 PC mutations since patients with cirrhosis had similar prevalence as IT patients and inactive carriers. HBeAg-negative patients with cirrhosis had the highest prevalence of T1762/A1764 BCP mutants, but our HBeAg-positive patients with cirrhosis had a similar prevalence of T1762/A1764 BCP mutants as IT patients and inactive carriers. These findings indicate that basal core mutants may appear in HBeAg-positive patients and may be independent of HBeAg seroconversion. Further investigations of hepatitis B viral markers in different categories of patients with hepatitis B relating to their eventual clinical outcomes are warranted.

There are several limitations to our study. Liver biopsies were not obtained for 96 clinically asymptomatic HBsAg-positive patients who had normal liver tests [1]. By definition, 19 of these patients were classified as immune tolerant since they were positive for HBeAg and their HBV DNA levels were more than 20,000 IU/mL. Also, by definition, 24 patients were classified as inactive carriers since they were negative for HBeAg, had normal baseline ALT levels, and their HBV DNA levels were equal to or less than 20,000 IU/mL or undetectable. This level of HBV DNA was chosen to identify patients with hepatitis B who were inactive and nonprogressive since the lower limits of detection for HBV DNA levels at that time period was 5 $\log_{10}$ IU/mL [13]. However, it has been recently reported that 21–37% HBeAg-negative patients with persistently normal ALT levels and HBV DNA levels of less than 5 $\log_{10}$ IU/mL may have liver biopsy findings of significant inflammation and fibrosis [25, 26]. In addition, anti-HBeAg-positive patients with HBV DNA levels of less than 5 $\log_{10}$ IU/mL may still progress to cirrhosis, experience liver decompensation, and develop HCC [4, 5, 27, 28]. The level of HBV DNA that includes inactive carriers may be less than 5 $\log_{10}$ IU/mL [29], but a cutoff level of less than 4 $\log_{10}$ IU/mL may be more appropriate since patients with HBV DNA levels above this value may still develop liver-related complications [30]. In our inactive carriers, the median HBV DNA level was 2.69 $\log_{10}$ IU/mL. Also, serial measurements of ALT levels are necessary to reaffirm the IC state if liver biopsies are not available [14]. In our 24 inactive carriers, the serum ALT levels have remained persistently normal over a 10-year period (Fig. 2). In addition, there were 53 of the 96 remaining patients who did not fit into these 2 categories. Of these, 47 were HBeAg negative and had normal ALT and HBV DNA levels of 8.0 $\log_{10}$ IU/mL. It is possible that these patients were in the quiescent phase of HBeAg-negative chronic hepatitis. The remaining six patients were HBeAg positive, had normal ALT and HBV DNA levels of 2.3 $\log_{10}$ IU/mL, and they may have been in the process of HBeAg seroconversion. Up to the present, none of the "no fit" patients have developed liver complication and are still currently followed in our clinic. Another limitation is that we analyzed only hepatitis B viral markers at baseline, and it is possible that HBeAg seroconversion with subsequent viral mutations as well as changes in HBV DNA levels may have influenced the course of the disease.

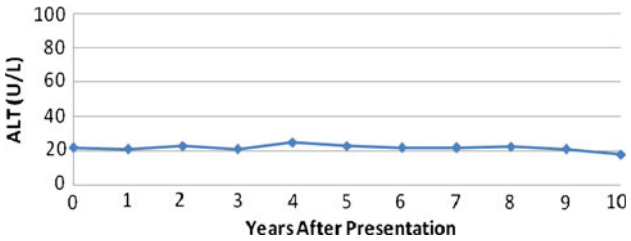

Fig. 2 The median ALT levels in inactive carriers over a 10-year follow-up period. *ALT* alanine aminotransferase

In dividing our patients with hepatitis B into six clinical categories, we were able to determine which categories of patients eventually died from complications of hepatitis B infection. Hepatitis B viral markers must be used in conjunction with routine laboratory tests for clinical assessment of patients and together the information will be useful for the identification of patients who will require close monitoring and who will need antiviral therapy.

Conflict of interest statement There is no conflict of interest to disclose.

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