

Quantitative Serum HBV DNA Levels During Different Stages of Chronic Hepatitis B Infection

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The goals of this retrospective study were to determine whether there is a threshold hepatitis B virus (HBV) DNA value associated with spontaneous or antiviral therapy-related hepatitis B e antigen (HBeAg) clearance. We also investigated whether there is an HBV DNA value that can be used for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis B. HBV DNA levels in sequential serum samples of 165 Chinese patients with different stages of chronic HBV infection were quantified by a polymerase chain reaction (PCR)-based assay. Our results showed that almost all of the patients (89%) who remained HBeAg-positive had HBV DNA levels that were persistently above 10^5 copies/mL. Serum HBV DNA levels decreased by a mean of $3 \log_{10}$ in patients with HBeAg loss, but 51% had levels above 10^5 copies/mL at the time HBeAg first became undetectable. Mean serum HBV DNA levels were significantly lower in HBeAg-negative patients. HBV DNA value above 10^5 copies/mL would exclude all inactive carriers, but 45% of patients with HBeAg-negative chronic hepatitis would also be excluded if testing were only performed at presentation and 30% would be excluded if testing were performed on 3 occasions. In conclusion, serum HBV DNA levels decreased significantly in patients with HBeAg loss, but there was no threshold HBV DNA level associated with HBeAg clearance. Given the fluctuating course of HBeAg-negative chronic hepatitis, it is not possible to define a single cutoff HBV DNA value for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis. (HEPATOLOGY 2002;36:1408-1415.)

The evaluation of patients with hepatitis B virus (HBV) infection has evolved from serologic to molecular diagnostic assays. Using polymerase chain reaction (PCR) assays, the vast majority of patients with chronic HBV infection, including those who are hepatitis B e antigen (HBeAg) negative and hepatitis B e antibody (anti-HBe) positive have detectable HBV DNA in serum.¹⁻⁷ The improvement in sensitivity of HBV

DNA assays has improved our understanding of the pathogenesis and natural history of HBV infection and facilitated the monitoring of response to treatment, but it also has generated new questions and dilemmas.⁸

Previous studies based on a single time point found that serum HBV DNA levels generally exceed 10^5 copies/mL among patients with HBeAg-positive chronic hepatitis and may be as high as 10^{10} copies/mL.¹⁻⁷ Serum HBV DNA levels tended to be lower (10^4 to 10^8 copies/mL) among patients with HBeAg-negative chronic hepatitis B (HBeAg-negative, anti-HBe-positive, elevated alanine aminotransferase [ALT]).^{3,4} and may be intermittently undetectable in non-PCR based assays.^{9,10} Until now, there have been very little data on what level of serum HBV DNA is associated with progressive liver disease. Consequently, there is no consensus on the level of serum HBV DNA when treatment is indicated.

A key question in the management of patients with HBeAg-positive chronic hepatitis B is to what level should serum HBV DNA be reduced to achieve sustained HBeAg seroconversion? To date, only 1 study had addressed this issue. In a study of 23 patients who received lamivudine therapy, 6 (50%) of 12 patients whose serum HBV DNA decreased to $<10^4$ copies/mL developed

Abbreviations: HBV, hepatitis B virus; PCR, polymerase chain reaction; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; IFN, interferon.

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HBeAg seroconversion, versus none of 11 whose serum HBV DNA remained $>10^4$ copies/mL.¹¹ This study suggested that there may be a threshold HBV DNA level associated with lamivudine-induced HBeAg seroconversion. However, this study was based on a very small number of patients. Thus, further studies are needed to validate this finding and to determine whether this threshold level applies to HBeAg seroconversion that occurs spontaneously or in association with other treatment.

An arbitrary serum HBV DNA level of 10^5 copies/mL has been proposed at the National Institutes of Health Workshop to differentiate chronic hepatitis B from an inactive carrier state (HBeAg-negative, persistently normal ALT).¹² A recent study in France found that 98% of the inactive carriers had HBV DNA levels $<10^5$ copies/mL at presentation and 97% had an HBV DNA level persistently below 10^5 copies/mL during a 1- to 6-year follow-up period.¹³ The investigators concluded that their results support the National Institutes of Health recommendation of 10^5 copies/mL as a cutoff HBV DNA value for differentiating inactive carriers from patients with chronic hepatitis B, but this study did not include patients with HBeAg-negative chronic hepatitis B for comparison. Another study in Greece found that a cutoff value of 10^5 copies/mL would lead to misclassification of 13% of their patients with HBeAg-negative chronic hepatitis B and possibly denial of treatment. They suggested that a cutoff HBV DNA level of 3×10^4 copies/mL is more appropriate for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis B.¹⁴ However, HBV DNA was tested at 1 time point only in this study. Thus, the appropriate HBV DNA value for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis B remains to be determined.

We performed this retrospective study to measure quantitative HBV DNA levels in sequential serum samples of patients with different stages of chronic HBV infection. Our goals were to determine (1) whether there is a threshold HBV DNA value associated with spontaneous or antiviral therapy-related HBeAg seroconversion, and (2) the HBV DNA value that should be used for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis B.

Patients and Methods

Patients

This was a retrospective study using stored sera from Chinese patients with chronic HBV infection seen in the Hepatitis Clinic, Queen Mary Hospital, Hong Kong, between 1984 and 1992. Patients were seen every 3 to 6 months, or more often if clinically indicated. At each visit, liver biochemistry and HBV serology, including hepatitis B surface antigen (HBsAg), HBeAg, and anti-HBe, were checked. Serum was collected and stored frozen at -20°C for HBV DNA testing. All patients who had had at least 3 serum samples 1 year apart were studied. A total of 165 patients were included. These patients were classified into 5 groups: (1) persistently HBeAg-positive; (2) transient HBeAg loss/seroconversion: spontaneous (A) or interferon (IFN)-related (B); (3) sustained HBeAg loss/seroconversion: spontaneous (A) or IFN-related (B); (4) HBeAg-negative with persistent or intermittent elevation in ALT level; and (5) HBeAg-negative with persistently normal ALT (Table 1). Except for patients in groups 2B and 3B, none of the patients received antiviral therapy during the study period.

Definition

The upper limit of normal for serum ALT was 45 IU/L. HBeAg loss was defined as disappearance of serum

Table 1. Timing of Blood Samples in the 5 Groups of Patients Studied

Group	Number of Patients	Interval Between 1st and 2nd Samples (mo)	2nd Sample	Interval Between 2nd and 3rd Samples (mo)	3rd Sample
1. Persistently HBeAg-positive	27	21 ± 2	Midway during FU	22 ± 2	Last FU
2. Transient HBeAg loss, spontaneous or IFN-related	27*	25 ± 3	First HBeAg-negative	8 ± 3	Reappearance of HBeAg
3A. Sustained HBeAg loss, spontaneous	25†	31 ± 3	First HBeAg-negative	31 ± 3	Last FU
3B. Sustained HBeAg loss, IFN-related	24‡	20 ± 3	First HBeAg-negative	40 ± 4	Last FU
4. HBeAg-negative with persistent or intermittently abnormal ALT	33	28 ± 2	Midway during FU	26 ± 2	Last FU
5. HBeAg-negative with persistently normal ALT	29	28 ± 2	Midway during FU	34 ± 2	Last FU

Abbreviations: HBeAg, hepatitis B e antigen; FU, follow-up; IFN, interferon; ALT, alanine aminotransferase.

*Fourteen spontaneous, 13 IFN-related, 13 developed anti-HBe.

†Eighteen developed anti-HBe.

‡Nineteen developed anti-HBe.

HBeAg in a patient who previously had been HBeAg-positive. HBeAg seroconversion was defined as disappearance of HBeAg accompanied by the development of anti-HBe. Sustained HBeAg loss/seroconversion was defined as maintenance of this phenomenon for at least 1 year and until the last visit, with HBeAg/anti-HBe retested at least twice per year. HBeAg loss/seroconversion that occurred within 1 year after stopping IFN therapy was defined as IFN-related. Sustained biochemical remission was defined as persistently normal serum ALT from 6 months after first HBeAg loss until the last visit.

Hepatitis B Serology

The hepatitis B serologic markers HBsAg, HBeAg, and anti-HBe were tested using commercially available enzyme-linked immunosorbent assay kits from Abbott Laboratories (North Chicago, IL).

Quantification of Serum HBV DNA Levels

Serum HBV DNA levels were quantified using the Cobas Amplicor HBV Monitor kits according to the manufacturer's instructions (Roche Molecular Systems, Inc., Branchburg, NJ).^{4,7,15-17} The lower limit of detection of this assay is 200 copies/mL (approximately 0.001 pg/mL) with a range of linearity up to 10^6 copies/mL. Samples from patients who were HBeAg-positive were initially tested after a 1 in 100,000 dilution; samples with undetectable HBV DNA were retested without dilution. Samples from patients who were HBeAg-negative were initially tested undiluted; samples with HBV DNA results $\geq 10^6$ copies/mL were retested after 1 in 100,000 dilution. An arbitrary value of 100 copies/mL was assigned to samples with undetectable HBV DNA for statistical comparisons.

HBV Genotyping

The first available sample from each patient was used for HBV genotyping using a line-probe assay (Inno-Lipa HBV genotyping assay; Innogenetics, Inc., Ghent, Belgium).¹⁸⁻²⁰ All necessary precautions to prevent cross-contamination were observed, and negative controls were included at each step. To validate the results of line probe assay, 10% randomly selected samples of genotypes B and C and all samples with genotype A or D were sent for bidirectional automated sequencing at the DNA sequencing core facility, University of Michigan Medical Center, using the standard protocol for the Applied Biosystems DNA sequencer 377 (Perkin Elmer Corp., Foster City, CA). Sequencing results were compared with published sequences to ascertain HBV genotypes.^{21,22} HBV genotype could be determined by line-probe assay in all patients who had detectable HBV DNA by PCR. Com-

parison between results of direct sequencing and the line-probe assay showed 100% concordance.

Precore Stop Codon Variant (G_{1896A}) and Core Promotor Variant (A_{1762T}, G_{1764A})

Serial samples from patients with sustained HBeAg seroconversion (group 3) and initial samples of HBeAg-negative patients (groups 4 and 5) were tested for precore (PC) stop codon (G_{1896A}) and core promotor (CP) (A_{1762T}, G_{1764A}) variants using Inno-Lipa precore/core promotor kits (Innogenetics, Inc.).^{19,20} Except for the primers, the procedure was similar to that for HBV genotyping. All samples with indeterminate results and 20% of randomly selected samples with PC and/or CP variant were sent for direct sequencing as described above. Indeterminate results were obtained in only 2% of samples in the PC and 9% in the CP region using the line-probe assay. Of the samples that could be typed, the concordance between the line-probe assay and sequencing was 99%. The line-probe assay was more sensitive in detecting mixed sequences than direct sequencing.

Statistical Analyses

Results were expressed as mean \pm standard error. Data were entered into an Excel database and analyzed using SPSS version 9.0 software package (SPSS, Inc., Chicago, IL). Statistical analyses were performed using χ^2 and Fisher's exact test for categorical variables. Paired or unpaired Student's *t* test or one-way ANOVA with Tukey test was used for continuous variables as appropriate. Results were considered statistically significant at $P < .05$.

Results

The demographics, baseline liver histology, results of HBV genotyping, and serial quantitative HBV DNA and ALT values are summarized in Tables 2 and 3.

Group 1: Persistently HBeAg-Positive, n = 27

Serum HBV DNA levels varied from 10^{5-10} copies/mL with a slight ($<1 \log_{10}$) decline during a mean follow-up of 43 ± 3 months (range, 24 to 88 months) (Fig. 1A). There was no correlation between serum HBV DNA and ALT levels. HBV DNA levels above 10^5 copies/mL were observed in 96% of the samples. None of the patients in this group had HBV DNA levels persistently below 10^5 copies/mL.

Group 2: Transient HBeAg Loss/Seroconversion: Spontaneous or IFN-Related, n = 27

Serum HBV DNA levels decreased by a mean of 3 \log_{10} to 10^{3-8} copies/mL when HBeAg first became undetectable and increased by a mean of 2 \log_{10} with the reap-

Table 2. Demographics, Baseline Histology, and HBV Genotypes of Patients Studied

Group	Gender (M/F)	Mean Age (yr)	Number (%) of Patients With Liver Biopsies	Histology (NS/CH/cirrhosis)	HBV Genotypes (A/B/C/D)
1	20/7	24 ± 1	13 (48)	5/6/2	0/8/19/0
2	22/5	32 ± 2	21 (78)	2/13/6	0/5/21/1
3A	12/13	29 ± 2	14 (56)	8/4/2	0/12/13/0
3B	17/7	29 ± 1	23 (96)	6/14/3	0/11/12/1
4	24/9	34 ± 2	6 (18)	0/5/1	0/12/19/0
5	9/20	39 ± 3	0 (0)	0/0/0	1/11/7/1

Abbreviations: HBV, hepatitis B virus; NS, nonspecific changes; CH, chronic hepatitis.

pearance of HBeAg (Fig. 1B). There was no difference in absolute value or degree of decline of the HBV DNA level between patients with spontaneous or IFN-related transient HBeAg loss.

Group 3: Sustained HBeAg Loss/Seroconversion: Spontaneous (A), n = 25, or IFN-Related (B), n = 24

HBV DNA Level and HBeAg Loss. Thirty-seven (76%) of these 49 patients developed anti-HBe, simultaneous with the loss of HBeAg in 26 patients and after a mean of 10 ± 3 months (range, 1 to 30 months) after HBeAg first became undetectable in the remaining 11 patients. Serum HBV DNA levels decreased by a mean of 3 log₁₀ to 10³⁻⁷ copies/mL when HBeAg first became undetectable (Fig. 1C). HBV DNA levels above 10⁶, 10⁵, and 10⁴ copies/mL were found in 38%, 51%, and 63% patients, respectively, at the time when HBeAg first became undetectable. After HBeAg loss, a further decline in serum HBV DNA levels by 1 to 1.5 log₁₀ was observed during a mean follow-up of 35 ± 3 months (range, 12 to 81 months).

Baseline HBV DNA levels were similar among the 3 groups of patients (1 to 3) who were HBeAg-positive at presentation (mean, 7.9 to 8.3 log₁₀ copies/mL). HBV DNA levels at the time when HBeAg first became undetectable were comparable in groups 2 and 3 patients, mean levels being 4.98 ± 0.21 and 4.99 ± 0.24 log₁₀ copies/mL, respectively. Within each of the 2 groups of

patients with HBeAg loss (2 and 3), HBV DNA levels at the time when HBeAg first became undetectable were similar in patients with spontaneous versus IFN-related HBeAg loss, and in patients with or without anti-HBe seroconversion (Table 4).

HBV DNA Level and Sustained Biochemical Remission. Thirty-eight (78%) patients had sustained biochemical remission after HBeAg loss. Patients with sustained biochemical remission had a lower HBV DNA level at the time of HBeAg loss (mean, 4.80 ± 0.26 vs. 5.67 ± 0.53 log₁₀ copies/mL) and at the last follow-up visit (mean, 3.74 ± 0.18 vs. 4.07 ± 0.56 log₁₀ copies/mL), but these differences were not statistically significant. Figure 2 shows a patient who had sustained biochemical remission after HBeAg seroconversion, and Fig. 3 shows a patient with persistently elevated ALT after HBeAg seroconversion.

HBV DNA Level and Selection of Precore or Core Promotor Variants. Of the 49 patients with sustained HBeAg loss/seroconversion, 16 (33%) had core promotor and 3 (6%) had precore variant as the dominant sequence at presentation, whereas 17 (35%) had core promotor and 16 (33%) had precore variant as the dominant sequence when HBeAg first became undetectable. Serum HBV DNA levels at the time of HBeAg loss were comparable between patients with and without precore variants (Table 5) and in patients with and without core promotor variants (4.87 ± 0.41 vs. 5.40 ± 0.32 log₁₀). The prevalence of precore (13 of 38

Table 3. Serial HBV DNA and ALT Levels of Patients Studied

Group	1st Sample			2nd Sample		3rd Sample	
	Mean Log ₁₀ HBV DNA (Copies/mL)	Number (%) With Undetectable HBV DNA	Mean ALT (IU/L)	Mean Log ₁₀ Change in HBV DNA From 1st Sample	Mean ALT (IU/L)	Mean Log ₁₀ Change in HBV DNA From 2nd Sample	Mean ALT (IU/L)
1	8.37 ± 0.22	0 (0)	149 ± 45	-0.39 ± 0.33	69 ± 19	-0.28 ± 0.28	93 ± 22
2	7.86 ± 0.32	0 (0)	210 ± 49	-2.87 ± 0.35	80 ± 18	1.59 ± 0.37	87 ± 14
3A	8.31 ± 0.28	0 (0)	105 ± 23	-3.10 ± 0.40	71 ± 19	-1.44 ± 0.32	27 ± 4
3B	7.74 ± 0.35	0 (0)	108 ± 15	-2.96 ± 0.34	69 ± 19	-0.91 ± 0.40	29 ± 3
4	5.17 ± 0.33	2 (6)	118 ± 23	0.39 ± 0.29	190 ± 35	-0.81 ± 0.33	85 ± 21
5	3.10 ± 0.17	9 (31)	18 ± 2	0.18 ± 0.17	17 ± 1	-0.16 ± 0.13	20 ± 1

Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase.

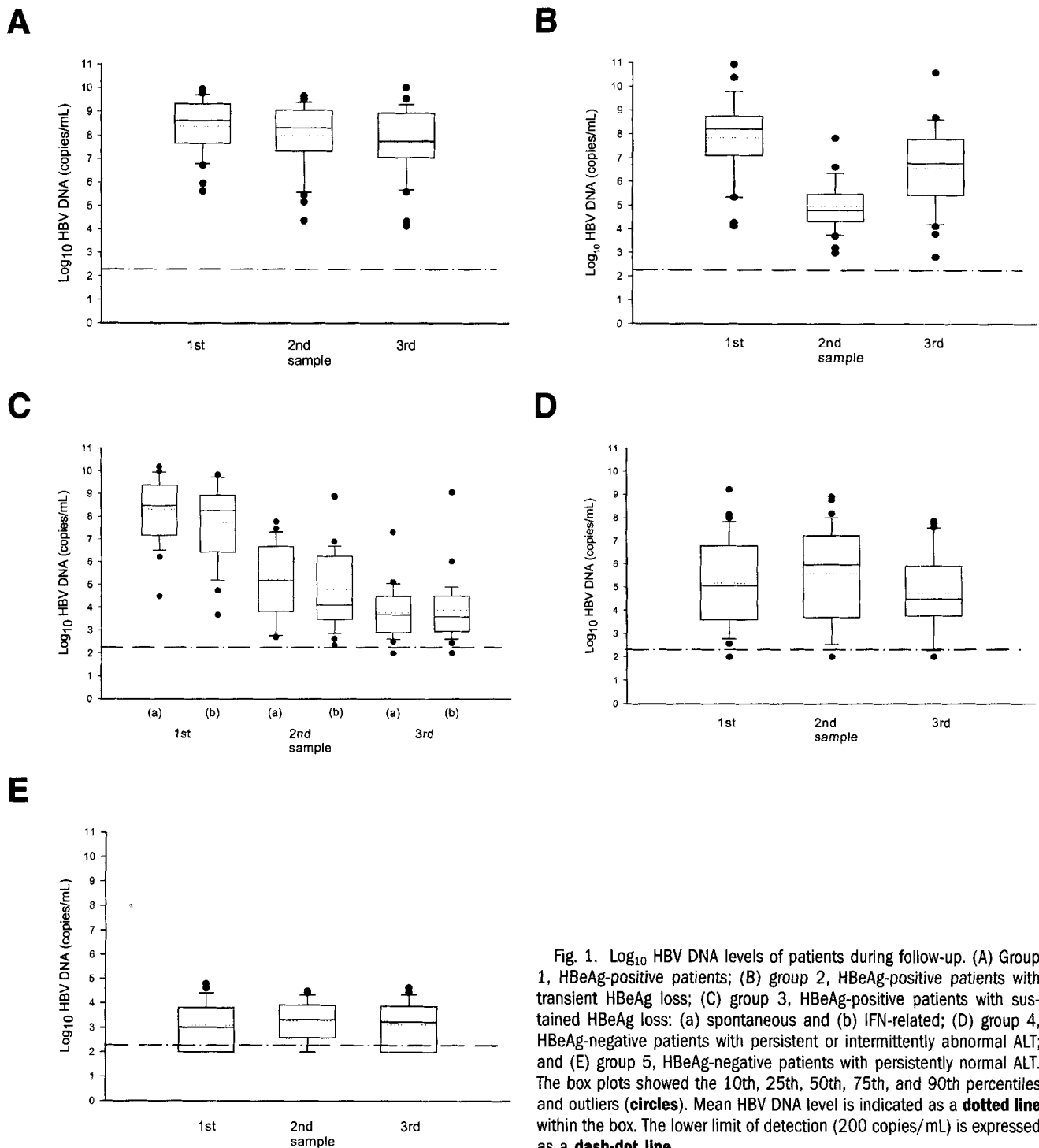


Fig. 1. Log₁₀ HBV DNA levels of patients during follow-up. (A) Group 1, HBeAg-positive patients; (B) group 2, HBeAg-positive patients with transient HBeAg loss; (C) group 3, HBeAg-positive patients with sustained HBeAg loss: (a) spontaneous and (b) IFN-related; (D) group 4, HBeAg-negative patients with persistent or intermittently abnormal ALT; and (E) group 5, HBeAg-negative patients with persistently normal ALT. The box plots showed the 10th, 25th, 50th, 75th, and 90th percentiles and outliers (circles). Mean HBV DNA level is indicated as a dotted line within the box. The lower limit of detection (200 copies/mL) is expressed as a dash-dot line.

vs. 3 of 11) and core promoter (12 of 38 vs. 5 of 11) variants at the time when HBeAg first became undetectable was similar between patients with and without sustained biochemical remission.

Group 4: HBeAg-Negative With Persistent or Intermittently Abnormal ALT, n = 33

Compared with patients who were HBeAg-positive, this group of patients had significantly lower serum

HBV DNA levels; mean, 5.2 log₁₀ versus 8.4 log₁₀ copies/mL ($P < .001$). There was a wide range in HBV DNA levels at presentation (10^3 - 10^8 copies/mL) (Fig. 1D) and during the course of follow-up (Fig. 4). About half (47%) of the samples had HBV DNA levels less than 10^5 copies/mL. HBV DNA levels persistently greater than 10^5 , 10^4 , and 10^3 copies/mL were found in 33%, 58%, and 82% patients, respectively. Twenty-

Table 4. Serial HBV DNA Levels in Group 3 Patients Who Lost HBeAg With and Without HBeAg Seroconversion

	HBeAg Seroconversion	HBeAg Loss Only	P Value
Number of patients	37	12	
Mean ± SEM HBV DNA (log ₁₀ copies/mL)			
At presentation	8.12 ± 0.23	7.68 ± 0.69	NS
At the time of HBeAg loss	5.11 ± 0.26	4.55 ± 0.55	NS
Last follow-up	3.78 ± 0.20	3.92 ± 0.46	NS

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; NS, not significant.

three (70%) patients had at least 1 sample with an HBV DNA level >10⁵ copies/mL.

Nine patients of this group had persistently abnormal ALT levels during the follow-up period. Patients with persistently abnormal ALT levels tend to have a higher HBV DNA level at presentation as compared with patients with intermittently abnormal ALT level (6.11 ± 0.76 log₁₀ copies/mL vs. 4.82 ± 0.34 log₁₀ copies/mL, P = .08). At presentation, the CP variant as the dominant sequence was found in 59% of patients and the PC variant was found as the dominant sequence in 36% of patients (Table 6). HBV DNA level was comparable between patients with and without the PC variant (5.52 ± 0.30 vs. 5.30 ± 0.48 log₁₀ copies/mL).

Group 5: HBeAg-Negative With Persistently Normal ALT, n = 29

The HBV DNA level in this group was significantly lower than that of patients in group 4, the mean HBV DNA level at presentation being 3.1 log₁₀ copies/mL (P < .001). At presentation, the CP variant as the dominant sequence was found in 30% of patients and the PC variant as the dominant sequence was found in 50% of patients (Table 6). HBV DNA levels remained stable during a mean follow-up period of 62 ± 3 months (range, 41 to 92 months). Twenty-six (90%) patients had detectable HBV

DNA in at least 1 of their 3 samples, and 15 (52%) had persistently detectable serum HBV DNA. All of the samples had HBV DNA levels lower than 10⁵ copies/mL. Only 2 (7%) patients had HBV DNA levels persistently higher than 10⁴ copies/mL.

Discussion

In accordance with previous reports,¹⁻⁷ we found that more than 90% of HBeAg-positive patients had serum HBV DNA levels that could be detected by non-PCR assays, which have detection limits of 10⁵⁻⁶ copies/mL. In this study, the correlation between HBV DNA and ALT levels in HBeAg-positive patients was poor, possibly related to immune tolerance secondary to perinatal infection in many of our patients.

A previous small-scale study on lamivudine-treated patients suggested that HBV DNA levels must be reduced to less than 10⁴ copies/mL for HBeAg seroconversion to occur.¹¹ Our results showed a wide range in HBV DNA levels (10³⁻⁷ copies/mL) at the time HBeAg first became undetectable, implying that there is no threshold HBV DNA level for HBeAg clearance/seroconversion. This observation was true for spontaneous as well as IFN-related HBeAg clearance/seroconversion. Because our samples were collected in the prelamivudine era, we cannot ascer-

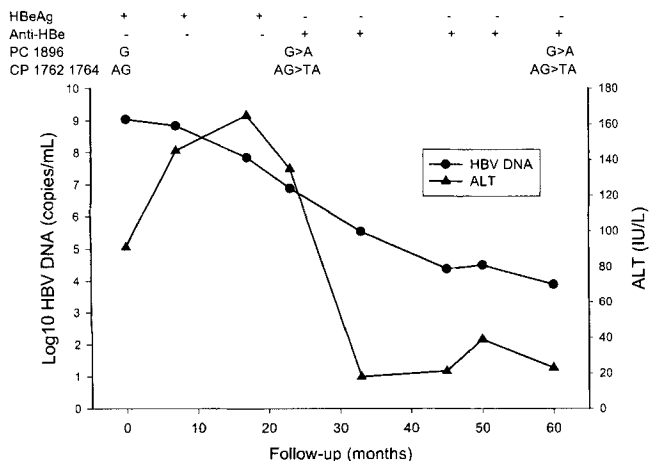


Fig. 2. HBV DNA and ALT levels in one patient with sustained biochemical remission after HBeAg seroconversion.

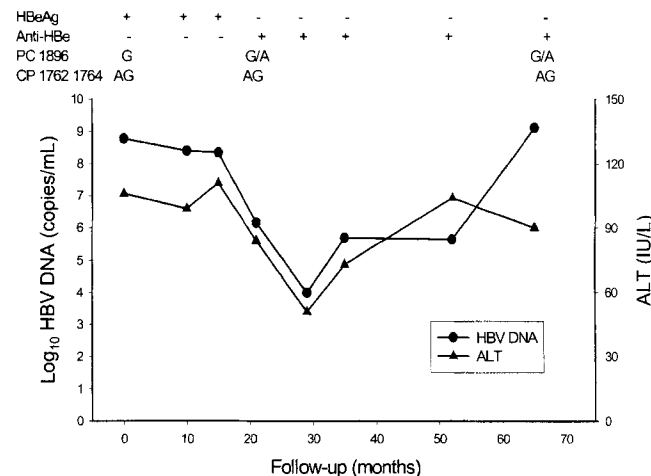


Fig. 3. HBV DNA and ALT levels in one patient without biochemical remission after HBeAg seroconversion.

Table 5. Serial HBV DNA Levels in Group 3 Patients Who Lost HBeAg With and Without Selection of Precore G₁₈₉₆A Mutation

	With Selection of PC Variant	Without Selection of PC Variant	P Value
Number of patients	16	33	
Mean \pm SEM HBV DNA (log ₁₀ copies/mL)			
Sample 1	8.41 \pm 0.29	7.82 \pm 0.35	NS
Sample 2	5.62 \pm 0.32	5.00 \pm 0.35	NS
Sample 3	4.20 \pm 0.48	3.64 \pm 0.15	NS

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; PC, precore; NS, not significant.

tain whether our results can be generalized to nucleos(t)ide therapy-related HBeAg clearance. All of our patients were ethnically Chinese, most of whom had perinatally acquired HBV infection. Thus, it is not clear whether our results can be generalized to patients with adult-acquired HBV infection.

Interestingly, we found no difference in HBV DNA levels at the time HBeAg first became undetectable between patients with transient and sustained HBeAg loss, indicating that serum HBV DNA level at this time point is not a good predictor of durability of HBeAg loss. In addition, our study showed that HBV DNA levels at the time of HBeAg loss were similar in patients with and without anti-HBe seroconversion. In patients with sustained HBeAg loss, there was a further reduction in HBV DNA levels during follow-up. However, HBV DNA remained detectable by PCR in 96% of patients 35 ± 3 months after sustained HBeAg loss.

As in our previous reports,^{9,20,23} we found that patients with sustained HBeAg loss were more likely to be infected with HBV genotype B, that HBeAg clearance can occur in the absence of detectable mutations that abolish or decrease HBeAg production, and that selection of precore or

Table 6. Core Promotor/Precore Sequence of Group 4 and 5 Patients at Presentation

	Group 4	Group 5
Number of patients	33	29
HBV DNA-positive by PCR	31 (94%)	20 (69%)
CP (1762, 1764) sequence		
WT only	6 (19%)	11 (55%)
WT > CP Var	6 (19%)	1 (5%)
CP Var > WT	2 (7%)	0 (0%)
CP Var only	16 (52%)	6 (30%)
Other mutations*	1 (3%)	2 (10%)
PC (1896) sequence		
WT only	15 (48%)	7 (35%)
WT > PC Var	4 (13%)	2 (10%)
PC Var > WT	3 (10%)	4 (20%)
PC Var only	8 (26%)	6 (30%)
Other point mutation	1 (3%)	1 (5%)
WT-dominant sequence in both CP and PC regions	7 (23%)	6 (30%)

Abbreviations: CP, core promotor; PC, precore; HBV, hepatitis B virus; PCR, polymerase chain reaction; WT, wild type; CP Var, A₁₇₆₂T G₁₇₆₄A; PC Var, G₁₈₉₆A.

*Including single mutation A₁₇₆₂T and G₁₇₆₄A, deletions, and other point mutations.

core promotor variants after HBeAg loss was not necessarily associated with a higher HBV DNA or ALT level. In accordance with recent literature,²⁴ not all patients with sustained HBeAg seroconversion had sustained biochemical remission. Although 36% of the patients without sustained biochemical remission had a transient ALT flare, the remaining 64% had intermittent or persistently abnormal ALT values. The latter pattern explains why some patients develop progressive liver disease despite sustained HBeAg seroconversion.

The National Institutes of Health workshop on management of hepatitis B proposed that a serum HBV DNA level of 10⁵ copies/mL be used to differentiate chronic hepatitis B from an inactive carrier state.¹² Our results showed that a HBV DNA value above 10⁵ copies/mL would exclude all inactive carriers but also 45% of patients with HBeAg-negative chronic hepatitis if testing were only performed at presentation and 30% if testing were performed on 3 occasions. Decreasing the cutoff value to 3 \times 10⁴ copies/mL would misclassify 7% of inactive carriers and 30% of patients with HBeAg-negative chronic hepatitis if testing were only performed at presentation. Given the variable course of HBeAg-negative chronic hepatitis B, retesting on more than one occasion helps in differentiating patients with HBeAg-negative chronic hepatitis from inactive carriers, but no single HBV DNA value reliably differentiates these 2 groups. In accordance with other reports,¹³ we found that serum HBV DNA remained detectable in the vast majority of inactive carriers, but HBV DNA levels tend to be lower and to remain stable over a 5-year follow-up period.

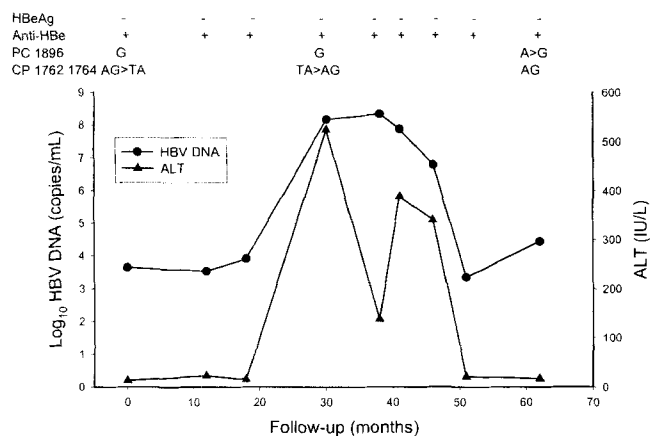


Fig. 4. HBV DNA and ALT levels in one patient with HBeAg-negative chronic hepatitis B.

In summary, we found that serum HBV DNA levels were very high (10^{5-10} copies/mL) in HBeAg-positive patients. Serum HBV DNA levels decreased by a mean of 3 \log_{10} in patients who had spontaneous or IFN-related HBeAg loss, but there was no threshold HBV DNA level associated with HBeAg clearance. Serum HBV DNA level at the time of HBeAg loss was not a predictor of durability of HBeAg loss. A further decline in serum HBV DNA levels was observed in patients with sustained HBeAg loss regardless of anti-HBe seroconversion. Serum HBV DNA levels were significantly lower in HBeAg-negative patients, but levels as high as 10^9 copies/mL were detected. Although all of the inactive carriers had HBV DNA levels that were persistently below 10^5 copies/mL, only 33% of patients with HBeAg-negative chronic hepatitis had HBV DNA levels that were persistently above 10^5 copies/mL. Given the fluctuating course of HBeAg-negative chronic hepatitis B, it is not possible to define a single cutoff HBV DNA value for differentiating inactive carriers from patients with HBeAg-negative chronic hepatitis.

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