

만성B형간염에서 바이러스 돌파현상의 지연 예측인자로서 24주 이내의 HBV DNA 소실

전성란, 장재영, 정승원, 박승훈, 이세환, 김상균, 천영국, 김영석, 조영덕, 문종호, 김홍수, 이준성, 김부성
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HBV DNA Loss within 24 Weeks Predicts Late Viral Breakthrough in Chronic Hepatitis B

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Background/Aims: Sustained HBV DNA reduction is necessary for biochemical remission, histological improvement, and prevention of complications. We analyzed the time taken from HBV DNA loss to viral breakthrough after antiviral treatment in patients with chronic hepatitis B (CHB). The early fall of the HBV DNA level to undetectable levels assessed really whether it is related to late breakthrough.

Methods: A total of 91 patients whose HBV DNA levels dropped below undetectable levels were chosen from lamivudine-treated 306 patients and were analyzed retrospectively. The patients were divided into 4 groups (A: $A \leq 12$, $12 < B \leq 24$, $24 < C \leq 48$, $D > 48$ wk) according to the time taken for the HBV DNA to decrease below undetectable levels. HBV DNA level was determined every 3 months.

Results: The mean time taken for loss of HBV DNA was 34 ± 28 wk. The baseline ALT differed significantly among groups (A: 382 ± 274 , B: 340 ± 30 , C: 166 ± 92 , D: 54 ± 100 IU/L) ($p=0.007$). Fifty nine of the 91 patients (64.8%) experienced viral breakthrough. The mean interval between HBV DNA loss and viral breakthrough was 65 ± 40 wk and differed significantly between group A, B (82 ± 43 wk) and group C, D (56 ± 28 wk) ($p=0.015$). In multivariate analysis, only HBV DNA loss within 24 wk, was found to be independently associated with late viral breakthrough ($p=0.035$). Undetectable HBV DNA after 24 wk was associated with high odd ratio of 3.24 (95% CI, 1.09-9.67).

Conclusions: HBV DNA loss within 24 wk after antiviral treatment could predict the late breakthrough. (Korean J Gastroenterol 2011;58:25-30)

Key Words: HBV DNA; Virologic breakthrough

INTRODUCTION

Chronic HBV infection currently affects about 400 million people worldwide, and 15-40% of those who develop chronic HBV infection progress to cirrhosis and end-stage liver disease; of these, 25% will develop hepatocellular carcinoma

(HCC).¹⁻³ Despite the availability of safe, effective vaccines against hepatitis B for almost 20 yr, it remains among the ten most common causes of death worldwide.⁴

Serum HBV DNA is a marker of viral replication, and persistent HBV replication increases the risk of cirrhosis, HCC, and liver failure.^{5,6} Sustained HBV DNA reduction is necessary for

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biochemical remission, histological improvement, and the prevention of complications.⁴ Thus, reducing serum HBV DNA to undetectable levels or as low a level as possible appears to be the most important determinant of therapeutic outcome.⁷ Keeffe et al.⁸ proposed the roadmap concept for on-treatment monitoring and management of chronic hepatitis B (CHB). They reported that lower serum HBV DNA levels at 24-week after antiviral treatment were associated with higher rates of maintained HBV DNA loss.^{2,8}

We analyzed the time taken from HBV DNA loss to viral breakthrough after antiviral treatment in patients with CHB. The early fall of the HBV DNA level to undetectable levels assessed really whether it is related to late breakthrough.

SUBJECTS AND METHODS

1. Patients and study design

This was a retrospective study of 306 patients with CHB or compensated cirrhosis, with no prior history of treatment with immunomodulatory or antiviral drugs, who had been treated with 100 mg/day lamivudine (ZeffixTM, GlaxoSmithKline Korea, Ahnsan, Korea) daily for at least 6 months between May 2002 and August 2008 at Soonchunhyang University Hospital. Patients with CHB were started on lamivudine if they met the following criteria for the treatment: (a) HBeAg-positive patients with HBV DNA levels $> 10^5$ copies/mL and (i) serum AST or ALT levels $>$ twice the upper limit of normal (ULN) or (ii) a liver biopsy showing moderate/severe inflammation or significant fibrosis if the serum AST/ALT levels < 2 ULN; and (b) HBeAg-negative patients with HBV DNA levels $> 10^4$ copies/mL and (i) serum AST or ALT levels > 2 ULN or (ii) a liver biopsy showing moderate/severe inflammation or significant fibrosis if the serum AST or ALT levels < 2 ULN. And patients with compensated cirrhosis were started on lamivudine if they met the following criteria: regardless HBeAg status, HBV DNA levels $> 10^4$ copies/mL and serum AST or ALT levels $>$ ULN. Exclusion criteria were (a) sustained HBV DNA positive, (b) HBV DNA follow-up loss, (c) accompanying diseases, (d) lack of data, (e) decompensated cirrhosis with clinical evidences or history of complications such as ascites, jaundice, hepatic encephalopathy, or variceal bleeding, (f) patients treated without meeting stated inclusion criteria, and (g) cessation of antiviral treatment in less than 24 wk. Ultimately, 91 patients whose HBV DNA levels became undetectable were

identified from the 306 patients.

Late viral breakthrough was defined as when the time from HBV DNA loss to viral breakthrough was longer than 60 wk. Patients were divided into four groups according to the time elapsed when the HBV DNA levels became undetectable (A ≤ 12 wk, 12 $< B \leq 24$ wk, 24 $< C \leq 48$ wk, D > 48 wk); the characteristics of the groups were compared. At baseline, the AST/ALT, total bilirubin, albumin, platelets, prothrombin time (PT), HBsAg/Ab, HBeAg/Ab, anti-HBc IgM, and HBV DNA were determined. These were repeated at viral breakthrough, together with the test of mutation to lamivudine and we investigated the factors that affected different viral response rate.

2. Assay methodology

Serum HBV DNA levels were quantified using the Cobas AmpliCor HBV Monitor testTM (Roche Diagnostics, USA; lower limit of detection=300 copies/mL) and were measured every 3 months (mean \pm SD, 13 \pm 3.6 wk). HBV DNA loss or undetectable HBV DNA was defined as less than 300 copies/mL. We measured the times for HBV DNA loss and viral breakthrough following lamivudine treatment. Viral breakthrough was defined as a confirmed increase in the HBV DNA level of more than 1 log₁₀ IU/mL, compared with the nadir HBV DNA level on therapy.⁴

3. Statistical analyses

Data are expressed as mean \pm standard deviation (SD). Quantitative and qualitative variables were compared between four groups using the Kruskal-Wallis test. To compare two continuous variables, the Mann-Whitney *U* test was used. Comparisons of categorical endpoints were assessed by the Chi² test. Multivariate analysis was conducted using logistic regression analysis. The odds ratio (OR) with 95% confidence interval (95% CI) was presented. A *p*-value < 0.05 was deemed to be statistically significant. Statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Study population

Of the 91 patient, 59 (64.8%) experienced viral breakthrough; 15 patients (16.5%) had sustained undetectable HBV DNA, and 17 patients (18.7%) were lost to follow-up (Fig.

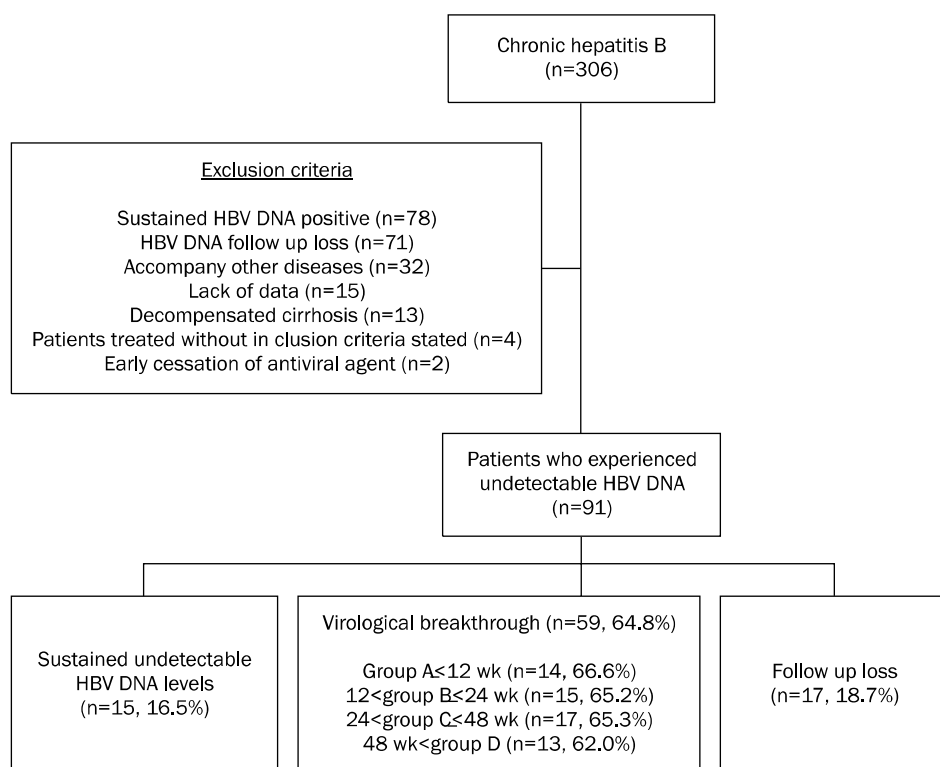


Fig. 1. Schematic representation of the long term follow-up of the chronic hepatitis B.

Table 1. Characteristics of the Patients with Undetectable HBV DNA

Characteristics (n=91)	
Undetectable HBV DNA (wk)	34±28
Follow up duration (wk)	152±82
Duration of therapy (wk)	129±83
Age (yr) Mean±SD	44±12
Male sex, n (%)	28 (69.2%)
AST (IU/L) Mean±SD	170±141
ALT (IU/L) Mean±SD	243±220
Total bilirubin (mg/dL) Mean±SD	1.1±0.6
Albumin (g/dL) Mean±SD	3.9±0.5
Platelet (×10 ³ /m ³) Mean±SD	169±67
Prothrombin time (INR) Mean±SD	1.1±0.23
HBeAg, n (%)	36 (39.5%)
Anti-HBc IgM, n (%)	20 (22%)
HBV DNA (log ₁₀ copies/mL) Mean±SD	6.18±1.11
Cirrhosis, n (%)	5 (5.5%)

1).

The baseline characteristics of all 91 patients are summarized in Table 1. The mean age of the subjects was 44±12 yr. The mean follow-up duration was 152±82 wk and the mean duration of lamivudine therapy was 129±83 wk. The mean time taken for HBV DNA loss was 34±28 wk.

2. Predictors of HBV DNA loss

The 91 patients were divided into groups A, B, C, and D ac-

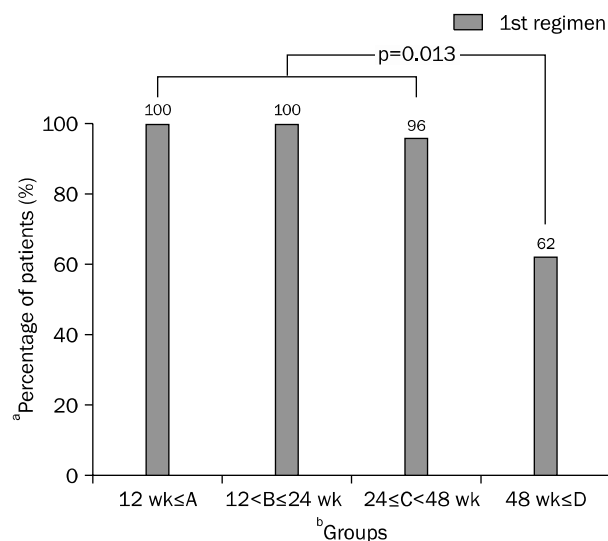


Fig. 2. Initial lamivudine treatment or change in regimen compared with time to undetectable HBV DNA. Twenty one of the 21 patients (100%), 23 of the 23 patients, (100%), 25 of the 26 patients (96%), 13 of the 21 patients (62%) had undetectable viral loads without change of initial antiviral agent and there was a significant difference between groups A, B, C and group D (p=0.013).

^aPercentage of patients without change of initial lamivudine regimen.
^bGroups according to the time to undetectable HBV DNA.

ording to the time elapsed when the HBV DNA levels became undetectable (A ≤ 12, 12 < B ≤ 24, 24 < C ≤ 48, D > 48 wk), including 21 (23%), 23 (25.5%), 26 (28.5%) and 21 (23%) pa-

tients, respectively. The viral load became undetectable in 21/21 (100%), 23/23 (100%), 25/26 (96%) and 13/21 (62%) patients in groups A, B, C, and D, respectively, without changing the initial lamivudine treatment. In other words, one of the 26 (4 %) and 8 of the 21 (38%) patients in group C and D experienced undetectable HBV DNA after adding or switching to adefovir. There was a significant difference between groups A, B, C and group D ($p=0.013$, Fig. 2). The baseline AST/ALT levels in groups A, B, C, and D were $232\pm195/382\pm274$, $197\pm121/340\pm306$, $156\pm144/166\pm92$, and $119\pm89/154\pm100$ IU/L, respectively, and differed significantly among the groups ($p=0.006/p=0.007$; Fig. 3). Gender, age, cirrhosis, pretreatment HBeAg status, and serum HBV DNA levels did not differ significantly between groups (Table 2).

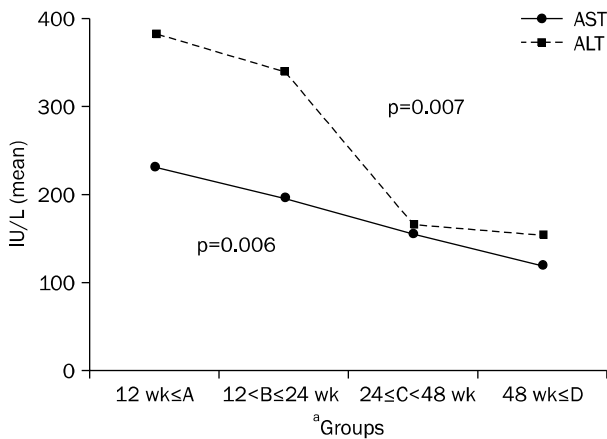


Fig. 3. Comparison of baseline AST/ALT levels among 4 groups. Baseline AST/ALT levels showed $232\pm195/382\pm274$ IU/L in group A, $197\pm121/340\pm306$ IU/L in group B, $156\pm144/166\pm92$ IU/L in group C and $119\pm89/154\pm100$ IU/L in group D and showed a significant difference between each group ($p=0.006/p=0.007$).
^aGroups according to the time to undetectable HBV DNA

Table 2. Baseline Characteristics between 4 Groups (A ≤ 12, 12 < B ≤ 24, 24 < C ≤ 48, and D > 48 wk)

Characteristics	Group A (n=21)	Group B (n=23)	Group C (n=26)	Group D (n=21)	p-value
Age (yr)	41±11	42±12	46±13	45±12	0.689
Male sex, n (%)	17 (81%)	14 (60.8%)	16 (61.5%)	16 (76%)	0.845
AST (IU/L)	232±195	197±121	156±144	119±89	0.006
ALT (IU/L)	382±274	340±306	166±92	154±100	0.007
Total bilirubin (mg/dL)	1.1±0.5	1.2±1.0	1.0±0.5	1.1±0.4	0.345
Albumin (g/dL)	4.0±0.4	4.0±0.4	3.9±0.5	3.8±0.6	0.647
Platelet ($\times 10^3/m^3$)	174±86	180±67	164±67	156±57	0.787
Prothrombin time (INR)	1.14±0.21	1.11±0.17	1.11±0.1	1.02±0.46	0.512
HBeAg, n (%)	11 (52.3%)	9 (39%)	11 (42%)	5 (24%)	0.46
Anti-HBc IgM, n (%)	7 (33.3%)	7 (30.4%)	3 (11.5%)	3 (14.2%)	0.67
HBV DNA (\log_{10} copies/mL)	5.76±1.09	5.94±1.18	6.37±1.15	6.47±0.96	0.076
Cirrhosis, n (%)	2 (9.5%)	1 (4.3%)	1 (3.8%)	1 (4.8%)	0.834

p-values were calculated by Kruskal-Wallis test.

3. Viral breakthrough and resistance

The characteristics of the 59 patients with viral breakthrough are summarized in Table 3. Viral breakthrough occurred in 14 (66.6%), 15 (65.2%), 17 (65.3%), and 13 (62%) patients in groups A, B, C, and D, respectively, and the mean interval from HBV DNA loss to viral breakthrough was 65 ± 40 wk. The time taken from HBV DNA loss to viral breakthrough was 89 ± 42 , 72 ± 54 , 63 ± 33 , and 42 ± 19 wk in groups A, B, C, and D, respectively, and differed significantly between A, B (82 ± 43 wk) and C, D (56 ± 28 wk) groups ($p=0.009$, Fig. 4). In HBeAg positive patients who achieved undetectable HBV DNA within 24 wk, the time taken until viral breakthrough differed significantly between groups A, B (77 ± 44 wk) and groups C, D (49 ± 19 wk) ($p=0.025$). The outcome for HBeAg negative patients who achieved undetectable HBV DNA within 24 wk was also significantly different between groups A, B (89 ± 43 wk) and groups C, D (59 ± 22 wk) ($p=0.05$).

Multivariate analysis included five parameters that independently influenced the late viral breakthrough. Factor that were entered into the multivariate analysis included basal AST or ALT level (≤ 150 vs. > 150 IU/L), pretreatment HBeAg status, basal serum HBV DNA level (≤ 5 vs. > 5 log copies/mL), and the time to undetectable HBV DNA (≤ 24 vs. > 24 wk). Only HBV DNA loss within 24 wk was found to be independently associated with late viral breakthrough ($p=0.035$, Table 3). Achievement of undetectable HBV DNA longer than 24 wk was associated with high OR of 3.24 (95% CI, 1.09-9.67).

At viral breakthrough, the mean HBV DNA level was $3.8\pm1.5 \log_{10}$ copies/mL; Thirty six of 59 patients had HBV

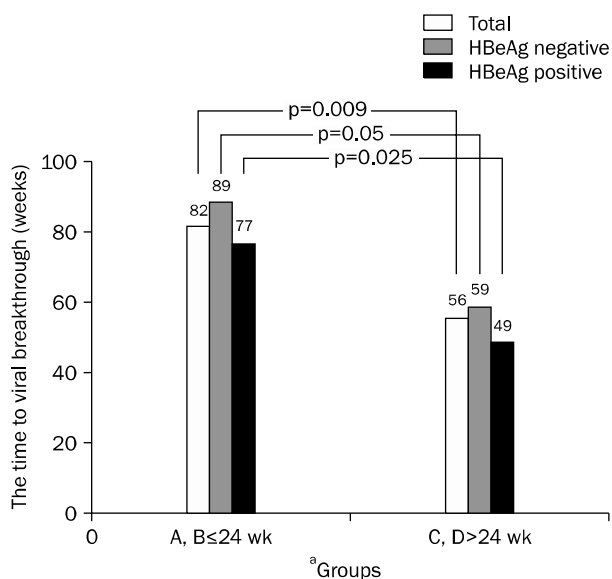


Fig. 4. The time taken until viral breakthrough according to pretreatment HBeAg status. In HBeAg positive, the mean time period between HBV DNA loss and viral breakthrough was 77±44 wk in groups A and B and 49±19 wk in groups C and D (p=0.025). In HBeAg negative, the mean time period between HBV DNA loss and viral breakthrough was 89±43 wk in groups A and B and 59±22 wk in groups C and D (p=0.05).

^aGroups according to the time to undetectable HBV DNA.

mutation, 13 of 59 patients did not perform the HBV DNA mutation test, and 10 of 59 patients did not show HBV mutation because they had low HBV viral load. HBV DNA mutation was M204I (25%), M204I/L180M (58.3%), and M204V/L180M (16.7%), respectively. The types of the HBV DNA mutation were not different significantly among the groups.

DISCUSSION

Some studies represents that lower 24-week serum HBV DNA levels after antiviral treatment were associated with higher rates of maintained HBV DNA loss.^{4,8} In telbivudine study, undetectable serum HBV DNA at treatment week 24 was reported the strongest predictor for optimal outcome at 2 yr.² Based on these studies, we have identified the time taken from HBV DNA loss to viral breakthrough after antiviral treatment. And the early fall of the HBV DNA level to undetectable levels assessed really whether it is related to maintain HBV DNA loss. In our study, the mean time until the HBV DNA became undetectable was 34±28 wk. Lamivudine therapy suppressed the serum HBV DNA to undetectable levels in 69 (75.8%) patients within 48 wk which was similar to pre-

Table 3. Multivariate Analysis of Factors Associated with Late Viral Breakthrough

Factors	p-value	OR (95% CI)
The time to undetectable HBV DNA (wk)	0.035	3.24 (1.09-9.67)
AST (IU/L)	0.291	
ALT (IU/L)	0.645	
HBeAg status	0.062	
HBV DNA (log copies/ml)	0.661	

p-values were calculated by logistic regression analysis.

vious results.⁹

There are many factors that affect treatment response. The predictors of treatment outcome include serum initial ALT and HBV DNA level, and viral response after antiviral treatment.^{8,10-12} In patients with normal ALT levels at baseline, including patients with cirrhosis, ALT levels may not be helpful for assessing the response to therapy.^{8,11,13} In our study, the baseline ALT differed significantly among the groups and the level decreased continuously, from the highest in group A to the lowest in group D. In other words, the higher the baseline ALT, the earlier the HBV DNA level was reduced to undetectable levels. This means that robust immune response result in good viral response. The mean HBV DNA level was 6.0±1.1 log₁₀ copies/mL in this study. We thought that HBV DNA levels did not influence the treatment response, because the baseline HBV DNA levels were not significantly different among the groups.

Fifty nine of 91 patients (64.8%) experienced viral breakthrough, and the mean interval between HBV DNA loss and viral breakthrough was 65±40 wk. After HBV DNA loss following lamivudine treatment, viral breakthrough occurred within approximately 2 yr. At viral breakthrough, the mean HBV DNA level was 3.8±1.5 log₁₀ copies/mL. Prolonged use of lamivudine therapy has been associated with increased emergence of lamivudine-resistant HBV. Summarizing published reports, the clinical frequency of lamivudine-resistant mutants was 18.6% for M204I, 1.4% for M204V, 11.4% for M204I/L180M, and 64.3% for M204V/L180M.^{14,15} Although the HBV mutation for lamivudine resistance did not differ significantly among the groups in our study, M204I/L108M was dominant (in 58.3%), which slightly differed from previous results.

When divided into each group, the time taken from HBV DNA loss to viral breakthrough was 82±43 wk in group A and B and 56±28 wk in group C and D, and differed significantly.

In this study, we found that achievement of undetectable HBV DNA within 24 wk played an important role in determining the risk of late viral breakthrough. Individuals achieved HBV DNA loss after longer than 24 wk of treatment had 3-fold increase in risk of early viral breakthrough. As with the previous studies, this suggests that HBV DNA loss within 24 wk during lamivudine therapy results in a longer persistent viral response. This effect may be applied to the patients with CHB treated with other antiviral agents.

This study has several limitations. First, this study was relatively small and conducted in a single tertiary-care academic referral center. Another set of limitations is inherent to retrospective study. A relatively many patients dropped out of the study due to lack of data or follow up loss. And, rates of patients experienced viral breakthrough from a medical record review was remarkably high compared to the results of previously published literature. We believed this was caused by poor drug adherence of patients, but could not identify detailed information just from medical records. Finally, our study included patients who took to lamivudine, lamivudine treatment is not the best option in patients with chronic HBV infection in current situation. But, lamivudine was one of the most commonly used during this study period. The aim of study was to identify the pattern of viral response rather than not to evaluate the effects of particular antiviral drugs.

In conclusion, a fall of the HBV DNA to undetectable levels within 24 wk after antiviral treatment could predict the late viral breakthrough.

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