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# Long-Term Presence of HBV in the Sera of Chronic Hepatitis B Patients with HBsAg Seroclearance

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**Key Words**
Chronic hepatitis B · Hepatitis B virus DNA · Seroclearance, hepatitis B surface antigen

**Abstract**
***Objects:*** The aim of this study was to elucidate the presence of serum hepatitis B virus (HBV) DNA at a prolonged time after seroclearance of hepatitis B surface antigen (HBsAg). ***Methods:*** Seventy Japanese patients who had been observed for >5 years after HBsAg seroclearance were included in this study. Anti-HBs, anti-HBe and anti-HBc antibodies were measured 0, 5 and 10 years after HBsAg seroclearance. Serum HBV DNA was measured using nested polymerase chain reaction (PCR) at 0, 5 and 10 years after HBsAg seroclearance. The PCR detection of serum HBV DNA using the X gene and core gene primers was done. The HBV DNA was regarded as positive when PCR detection of HBV DNA using either or both the X gene and core gene primers was positive. A multivariate regression analysis was used to assess the factors contributing to the positivity of serum HBV DNA 5 years after HBsAg seroclearance: the factors examined included age, gender, histological findings, HBV genotype, aminotransferase, total protein and interferon administration. ***Results:*** The titers of 200-fold diluted serum anti-HBc were 6.5 ± 4.0 at 0 year after HBsAg seroclearance, 1.8 ± 1.4 at 5 years and 0.9 ± 0.7 at 10 years. The titers of 200-fold diluted serum anti-HBc decreased 5 and 10 years after HBsAg seroclearance with statistical significance. The positive rate of HBV DNA by the nested PCR was 71.4% (50/70) at 0 year after HBsAg seroclearance, 21.4% (15/70) at 5 years and 14.3% (3/21) at 10 years. However, there were no significant factors contributing to the positivity of serum HBV DNA 5 years after HBsAg seroclearance. ***Conclusion:*** Our results suggest that serum HBV DNA disappears with an incidence of 10–20% 5 and 10 years after HBsAg seroclearance.



## Introduction

Chronic hepatitis B is a serious liver disease with significant mortality. In patients with chronic hepatitis B virus (HBV) infection, persistent viral replication is associated with ongoing necroinflammation in the liver and progressive liver damage [1–3]. However, in patients with seroclearance of hepatitis B envelope antigen (HBeAg) and marked reduction of HBV DNA, the prognosis of the disease is generally improved [4–6]. Moreover, hepatitis B surface antigen (HBsAg) seroclearance has probably been associated with a good prognosis [7–12].

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An explosion of papers argue that some patients with seroclearance of HBsAg showed positive HBV DNA at the time of HBsAg seroclearance or within 1 year of HBsAg seroclearance [13–17]. However, it is not clear how long serum HBV DNA could be detected after prolonged observation after HBsAg seroclearance. Moreover, it is still a question whether the patients with seroclearance of HBsAg could be really cleared of serum HBV DNA or not. To further investigate these issues, we performed the present study on the long-term virological outcome after HBsAg seroclearance in Japanese patients.

## Materials and Methods

*Patients*

From 1972 to 2002, a total of 5,055 chronic HBsAg carriers, who were known to be seropositive for HBsAg for at least 6 months, were studied in Toranomon Hospital in Tokyo, Japan. After a mean follow-up period of 4 years (range 0.5–30 years), 231 patients were noted to have delayed HBsAg seroclearance, which is defined as persistent absence of HBsAg antigenemia by radioimmunoassay for at least 1 year until the last examination. Of these 70 patients had the following criteria: (1) laparoscopy and liver biopsy taken before HBsAg seroclearance showed histological features of chronic active hepatitis or liver cirrhosis; (2) the follow-up period was more than 5 years after seroclearance of HBsAg.

We excluded from the study all the patients: (1) with concurrent hepatitis C virus and hepatitis D virus; (2) with a history of alcohol abuse or autoimmune liver disease; (3) with clinical evidence of hepatocellular carcinoma at entry into the study on the basis of ultrasonography, α-fetoprotein levels (<200 ng/ml) and/or histology; (4) with a history or clinical evidence of complications of decompensated cirrhosis at enrollment (that is ascites, encephalopathy or icterus).

Thirty-seven of 70 patients had spontaneous seroclearance of HBsAg, 20 patients had been given interferon (IFN) therapy for 1–16 months, 9 had been given steroid withdrawal monotherapy and 4 had been treated with combination therapy of steroid + IFN. The total median dose of IFN therapy was 336 mega units (range, 168–624 mega units). The patients treated with steroids were generally given prednisolone for 4 weeks, in a single dose of 40 mg/day for 1 week, 30 mg/day for 1 week, 20 mg/day for 1 week and then 10 mg/day for 1 week until it was abruptly withdrawn (total dose 700 mg).

*Methods*

The serums were stored at –80° until enzyme assays and measurement of HBV DNA level by the nested PCR method could be performed on all the samples for 70 patients at one time. Serum samples had been conserved at 0, 5 and 10 years after seroclearance of serum HBsAg. Serum HBV DNA was determined using the nested PCR independently by an experienced technician (J.S.), who had no clinical information or knowledge of each patient. The sensitivity of HBV DNA according to the manufacturer is about 50–100 copies/ml in the nested PCR method. Two kinds of primers in the core and X gene of HBV were used in the nested PCR method. First of all, primers used for the detection of HBV were Cof1 (sense, 5′-CTGCCTTACTTTTGGAGAGA-3′) and Cer1 (antisense, 5′-ACTTTACTGGGCTTTATTA-3′) for the first PCR and core sense (sense, 5′-GAGTGTGGATTCGCACTCC-TC-3′) and anticore (5′-GATTGAGATCTTCTGCGACGC-3′) for the second PCR in the core gene. Second, primers used for detection of HBV were P2 (sense, 5′-GTCCCGTCGGCGCTGAAT-CCC-3′) and Br102 (antisense, 5′-GCAGATGAGAAGGCACA-GAC-3′) for the first PCR and X sense (sense, 5′-CTGGATCCT-GCGCGG GACGTCCTT-3′) and anti-X (5′-GTTCACGGTGGT-CTCCAT-3′) for the second PCR in the X gene. In the first PCR and the second PCR, amplification was performed over 35 cycles (94 for 1 s; 55° for 1 s; 72° for 1 s) after initial denaturing at 94° for 4 min and a final extension at 72° for 7 min. Negative and positive controls confirmed the HBV DNA band in parallel. Ten healthy volunteers without HBsAg and anti-HCV were selected for negative HBV DNA controls. Ten patients with chronic hepatitis B and with HBsAg were selected for positive controls. The HBV DNA was considered positive when PCR detection of HBV DNA using either or both the X gene and core gene primers showed positivity. On the other hand, the HBV DNA was considered negative when PCR detection of HBV DNA using both the X gene and core primers showed negativity.

When serum samples showed positive HBV DNA by the nested PCR, we also examined the serum HBV DNA level. It was measured by a transcription-mediated amplification and hybridization-protection assay (Chugai Diagnostics, Tokyo, Japan), and the results were expressed as log genome equivalents (LGE) per milliliter. The lower detection limit of this assay is 3.7 LGE/ml, which is equivalent to 5,000 copies/ml.

HBsAg, anti-HBs, HBeAg, anti-HBe and antibody to HDV were all assayed using commercially available radioimmunoassay kits. Anti-HBc was assayed by chemiluminescent enzyme immunoassay. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay (Ortho Diagnostic Japan, Tokyo). The HBV genotype was determined using a previously reported method [18]. Biochemical tests were made using routine automated techniques and carried out in the laboratories of Toranomon Hospital. This study was approved by the institutional review board of our hospital. The physicians in charge explained the purpose and method of this clinical trial to each patient, who gave their informed consent for participation.

*Liver Histology*

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan).

*Statistical Analysis*

We used Fisher's exact test (two-tailed) or the Wilcoxon rank sum test to compare differences between groups. Moreover, we used univariate analysis and multivariate analysis (multiple logistic regression analysis) to establish which factors contributed to the positivity of HBV DNA 5 years after HBsAg seroclearance. Results for each variable were transformed into categorical data consisting of two simple original numbers for univariate and multivariate analyses. Variables that achieved statistical significance

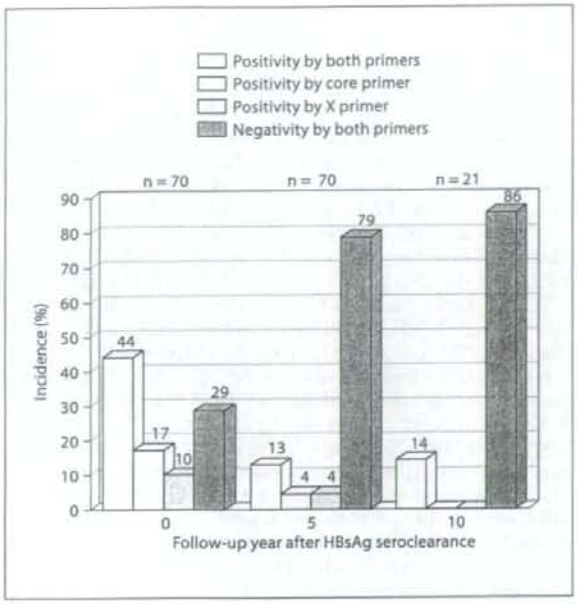


**Fig. 1.** Changes of detection pattern of serum HBV DNA after seroclearance of HBsAg. Negative controls: 10 healthy volunteers; positive controls: 10 patients with chronic hepatitis B; core (X): positivity indicates positive HBV DNA by nested PCR using core (X) gene primers, negativity indicates negative HBV DNA by nested PCR using core (X) gene primers.

($p < 0.05$) were subjected to multiple logistic regressions to identify significant independent predictors. The SPSS software package (SPSS 10.0 for Windows; SPSS Inc., Chicago, Ill., USA) was used for analyses.

## Results

### *Clinical Profiles*

Table 1 shows the characteristics of the 70 patients who had seroclearance of HBsAg. The median age of the 70 patients (male 55, female 15) was 53 years. Thirty-seven patients had spontaneously cleared HBsAg. At the time of HBsAg seroclearance, 30 patients showed liver cirrhosis.

Sixty-three of 70 (87.9%) patients had normal alanine aminotransferase levels after HBsAg seroclearance. Seven patients with elevated alanine aminotransferase had 4 fatty infiltrations of the liver and 3 cases of alcohol abuse.

**Table 1.** Characteristics of subjects at the time of seroclearance of HBsAg

| | |
|---|---|
| Number | 70 |
| Sex (male/female) | 55/15 |
| Age, years | 53 (30–82) |
| HBV genotype (A/B/C/D/F) | 3/7/45/2/6 |
| US (non-LC/LC) | 40/30 |
| Total protein, g/dl | 7.4 (6.6–8.8) |
| Albumin, g/dl | 4.2 (3.4–5.1) |
| Total bilirubin, g/dl | 0.7 (0.1–1.7) |
| AST, IU/l | 21 (11–71) |
| ALT, IU/l | 16 (6–101) |
| Hb, g/dl | 15.2 (12.9–17.1) |
| Platelets, $\times 10^4/mm^3$ | 17.3 (8.4–32.5) |
| Follow-up period after disappearance of HBs antigen, years | 8.3 (5.3–23.6) |

Data are numbers of patients or medians, with ranges in parentheses. ALT = Alanine aminotransferase; AST = aspartate aminotransferase; Hb = hemoglobin; US = ultrasonographic findings; LC = liver cirrhosis.

**Table 2.** Change of anti-HBc antibody after HBsAg seroclearance

| | Follow-up year of HBsAg seroclearance | | |
|---|---|---|---|
| | 0 | 5 | 10 |
| Anti-HBc antibody | 14.2 ± 2.7 | 13.9 ± 2.2 | 13.3 ± 3.6 |
| Anti-HBc antibody (200-fold dilution) | 6.5 ± 4.0 | 1.8 ± 1.4 | 0.9 ± 0.7 |

The serum was diluted 1:200 with saline. The titer of anti-HBc antibody was determined by the chemiluminescent immunoassay method.

### *Changes of Anti-HBs, Anti-HBe and Anti-HBc*

Table 2 shows the titers of serum anti-HBc. As regards the titer of nondiluted anti-HBc, there was no difference between the time of HBsAg seroclearance, 5 years and 10 years after HBsAg seroclearance. The titers of 200-fold diluted serum anti-HBc decreased 5 and 10 years after HBsAg seroclearance with statistical significance.

### *Serum HBV DNA after HBsAg Seroclearance*

The detection pattern of serum HBV DNA based on the difference of HBV primers by the nested PCR is shown in figure 1. The negative controls of healthy volunteers showed negative HBV DNA with both primers. On the

**Table 3.** Predictive factors for the positivity of HBV DNA 5 years after HBsAg seroclearance

| Factor | Category | Odds ratio | 95% CI | p value |
|---|---|---|---|---|
| IFN therapy | −/+ | 1/0.58 | 0.14–2.32 | 0.438 |
| Age, years | <60/≥60 | 1/1.99 | 0.56–7.07 | 0.287 |
| Total protein, g/dl | <8/≥8 | 1/1.84 | 0.90–3.76 | 0.096 |
| Liver histology | non-LC/LC | 1/2.00 | 0.28–5.48 | 0.781 |
| HBV genotype | B/C | 1/0.254 | 0.05–1.36 | 0.109 |
| Sex | male/female | 1/0.23 | 0.03–1.88 | 0.169 |
| AST, IU/l | ≥38/<38 | 1/1.59 | 0.57–4.43 | 0.375 |
| Platelets, $\times 10^4/mm^3$ | ≤20/>20 | 1/1.20 | 0.700–2.06 | 0.504 |
| ALT, IU/l | ≥50/<50 | 1/1.28 | 0.46–3.55 | 0.634 |

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; LC = liver cirrhosis.

other hand, the positive controls with chronic hepatitis B showed positive HBV DNA with both primers. The consistent rate of PCR detection of HBV DNA using both the X gene and core primers was 84.5% (136/161) in all. The positive rate of HBV DNA was 71.4% (50/70) at year 0, 21.4% (15/70) at 5 years and 14.3% (3/21) at 10 years by using both the X gene and core primers.

A multivariate regression analysis was used to assess the factors contributing to the positivity of serum HBV DNA: the factors examined included age, gender, histological findings, HBV genotype and IFN administration. However, there were no significant factors contributing to the positivity of serum HBV DNA (table 3).

In total 68 serum samples which showed positive HBV DNA by the nested PCR were examined by the transcription-mediated amplification and hybridization-protection assay. All the samples showed serum HBV DNA of less than 3.7 LGE/ml.

## Discussion

In the present study, the detection rate of serum HBV by the nested PCR after HBsAg seroclearance was about 70% at the time of HBsAg seroclearance and about 10–20% at 5 and 10 years after seroclearance of HBsAg. The positive rate of HBV DNA decreased 5 and 10 years after HBsAg seroclearance compared to the time of HBsAg seroclearance. Moreover, the titer of anti-HBc antibody by the 200-fold dilution gradually decreased after HBsAg seroclearance. This suggests that HBV may ultimately be cleared from the serum after a long time of more than 10 years. However, this present study showed that about 10–20% of patients had serum HBV DNA levels of 50–100 copies/ml at 5 and 10 years after seroclearance of HBsAg. The remaining patients might have low levels of HBV DNA of <50–100 copies/ml. Yuen et al. [14] have reported that HBV remains in the liver even if serum HBV is shown to be negative in some patients. These findings mean that a trace of HBV remains for a prolonged period after HBsAg seroclearance.

HBV DNA replications sometimes occur after administration of steroids and/or immunosuppressive agents in patients with a small amount of residual HBV [19–21]. Our previous study suggests that steroid withdrawal therapy for HBeAg-positive patients with chronic hepatitis induces an elevation of serum HBV DNA and acute exacerbation of liver function [10]. Five of 230 HBeAg-positive patients treated with steroid withdrawal therapy showed acute exacerbation of liver function and icterus. Therefore, when the patients with serum HBsAg are given steroids and/or immunosuppressive agents, they should be carefully followed up by monitoring serum levels of HBV DNA and liver function. The present study shows that a trace of HBV remains during a prolonged period after HBsAg seroclearance. This suggests the following point: when patients with HBsAg seroclearance are treated with steroids and/or immunosuppressive agents, they should be carefully followed up by monitoring the serum level of HBV DNA and/or liver function to prevent acute exacerbation of liver impairment.

Seventy patients enrolled in the present study were not treated with steroids and/or immunosuppressive agents, so they did not show acute exacerbation during the follow-up. Moreover, these 70 patients did not show progression to decompensated liver cirrhosis and/or death due to hepatocellular carcinoma. Thus, our results suggest that even if patients with HBsAg seroclearance have a trace of

HBV DNA, they have generally a good prognosis concerning liver function.

In conclusion, as some patients also had a trace of serum HBV DNA 5 and/or 10 years after seroclearance of HBsAg, they should be carefully followed concerning administration of steroids and/or immunosuppressive agents.


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Journal of Gastroenterology


# Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine

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***Background.*** The aim of this study was to investigate the factors associated with the response of lamivudine-resistant hepatitis B virus (HBV) during combination therapy with adefovir dipivoxil plus lamivudine. ***Methods.*** Sixty-three patients with breakthrough hepatitis received a 10-mg once-daily dose of oral adefovir dipivoxil. ***Results.*** The rates of undetectable serum HBV-DNA were 49.2% after 24 weeks, 61.9% after 48 weeks, and 67.2% after 72 weeks. The cumulative hepatitis B e antigen (HBeAg) loss rates in patients with alanine aminotransferase (ALT) levels of more than twice the upper limit of normal (ULN) were significantly higher than in patients with ALT less than twice the ULN ($P = 0.0145$). Multivariate analysis revealed that baseline ALT level ($P = 0.003$) and HBeAg status ($P = 0.049$) were associated with early virological response. ***Conclusions.*** Baseline ALT level was associated with HBeAg loss and seroconversion, and baseline ALT level and HBeAg status were associated with the virological response of lamivudine-resistant HBV during combination therapy with adefovir dipivoxil plus lamivudine.

**Key words:** adefovir dipivoxil, lamivudine-resistant mutant, hepatitis B virus, HBeAg seroconversion

## Introduction

Chronic hepatitis B is a common disease and leads to progressive liver disease and hepatocellular carcinoma. Lamivudine, a nucleoside analog administered orally, inhibits the replication of hepatitis B virus (HBV), and is used worldwide for the treatment of chronic hepatitis B.[1-3] Lamivudine improves alanine aminotransferase (ALT) levels and liver histological findings, causes hepatitis B e antigen (HBeAg) loss and seroconversion, and is well tolerated.[4,5] However, the emergence of lamivudine-resistant HBV strains in patients on long-term lamivudine therapy has been observed; the mutation that results in such resistance occurs in the HBV DNA polymerase gene in the YMDD motif.[6-8] The emergence of such mutant viruses results in the reelevation of HBV DNA and ALT levels and causes clinical and histologic progression.[9] Resistance was recently reported to develop in 12.5% of patients after 1 year of lamivudine therapy, in 43.8% after 3 years, and in 62.5% after 5 years.[10]

Adefovir dipivoxil (ADV) is a nucleotide analog that selectively inhibits viral polymerases and reverse transcriptases.[11-14] ADV has antiviral activity against not only wild-type HBV but also lamivudine-resistant HBV mutants in vitro and in vivo.[15,16] Worldwide clinical trials of ADV monotherapy or ADV plus lamivudine combination therapy for lamivudine-resistant mutants have been recently reported in cases of compensated or decompensated liver disease and in liver transplant recipients.[17,18] These studies reported virological and biochemical improvements in lamivudine-resistant chronic hepatitis B after 48–52 weeks of ADV therapy: 20%–35% of patients showed a negative serum HBV DNA level by polymerase chain reaction (PCR) assay; 15%–17% showed negative HBeAg; and 53%–61% showed normalized serum ALT levels.[17,18] We also recently reported the result of a pilot study on the efficacy of combination therapy with ADV plus lamivudine for treating lamivudine-resistant chronic hepatitis B.[19] We found that by week 24, 55.6% of patients showed a negative serum HBV DNA level by PCR assay and 75% showed normalized serum ALT levels. Although these data are encouraging, clinical factors associated with the virological and biochemical responses to this treatment are unknown. In this study, we investigate the factors associated with the virological response of

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lamivudine-resistant HBV during combination therapy with ADV plus lamivudine.

## Patients and methods

### *Patients*

Adefovir dipivoxil was administered to 72 adult patients at Toranomon Hospital, Tokyo, Japan; the patients had received ongoing lamivudine treatment for chronic hepatitis B for more than 72 weeks since 2002. Serum HBV DNA and ALT levels increased again despite the continuation of lamivudine, indicating breakthrough hepatitis, in all patients, who then received ADV along with the lamivudine. Of the 72 patients treated with ADV, 63 were enrolled in this retrospective study. Enrollment in this study and the start of ADV treatment were determined by the following criteria: (1) Increase of serum HBV DNA levels was ≥1 log copies/ml during lamivudine treatment on at least two consecutive occasions, compared with the nadir of initial antiviral efficacy (≥1 log decrease in serum HBV DNA). (2) Serum aspartate transaminase (AST) and/or ALT levels were greater than the upper limit of normal (ULN) before the start of ADV treatment (ULN: AST = 38 IU/l, ALT = 50 IU/l). (3) Mutations of the YMDD motif were detected before the start of ADV treatment by the PCR-based method described later. (4) Other nucleoside analogs such as famciclovir and entecavir had not been previously administered. The exclusion criteria were as follows: (1) serum creatinine levels ≥1.5 mg/dl; (2) interferon (IFN) added to ADV and lamivudine to treat severe acute exacerbation; and (3) infection with hepatitis A, hepatitis C, delta viruses, or human immunodeficiency virus, or a history of other liver diseases such as autoimmune hepatitis, alcoholic liver disease, or metabolic liver disease.

### *Methods*

Patients received a 10-mg once-daily dose of oral ADV. Lamivudine treatment was continued in all patients. Blood samples were obtained at least once every month before, during, and after treatment with ADV, and analyzed for virological markers, biochemical markers associated with liver function and renal function, and complete blood cell counts every visit. The diagnosis of cirrhosis was based on liver biopsy histology and/or on clinical criteria, including image studies and signs of portal hypertension. The primary efficacy measures of low hepatitis activity were undetectable HBV DNA level by PCR assay and normalization of the ALT level; the secondary efficacy measures were HBeAg loss and seroconversion. The rate of each measure was evaluated 24, 48, and 72 weeks after the start of treatment. Adverse effects were monitored clinically by careful interview and medical examination at least once every month. Patient compliance with treatment was evaluated by questionnaire.

Serum HBV DNA levels were evaluated by quantitative PCR assay (Amplicor HBV Monitor test, Roche Molecular Systems, Pleasanton, CA, USA). The detection range of this assay was 2.6–7.6 log copies/ml (400 to $4 \times 10^7$ copies/ml). Antibodies to hepatitis B s and e antigens were determined by commercially available radioimmunoassay systems (Abbott Japan, Tokyo, Japan). Confirmation of mutation in the HBV DNA polymerase gene (rtM204 of the YMDD motif) was determined using the PCR-based method of Chayama et al.[20] The HBV genotype was determined by enzyme-linked immunosorbent assay (HBV Genotype EIA, Institute of Immunology, Tokyo, Japan) based on the method of Usuda et al.[21]

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments, and informed consent was obtained from every patient. This study was approved by the local ethics committee of Toranomon Hospital.

### *Statistical analysis*

Analysis of efficacy was performed on an intention-to-treat basis. The $\chi$-squared test was used to compare efficacies. The cumulative rate of HBeAg loss and seroconversion were calculated using the Kaplan-Meier method, and differences between the curves were tested using the log-rank test. Exploratory analyses with covariates of interest were used to evaluate potential predictors of early virological response. Early virological response was defined by an undetectable serum HBV DNA level using the Amplicor monitor assay at week 24. The baseline factors included were patient age, sex, ALT level, HBeAg status, HBV DNA level, YMDD mutant status, HBV genotype, and the presence of cirrhosis. Initially, univariate analyses were conducted using logistic regression analysis. Next, all factors found to be at least marginally associated with early virological response ($P < 0.15$) were tested by multivariate analysis using a stepwise logistic model. A $P$ value of less than 0.05 was considered statistically significant. Changes in serum HBV DNA and ALT levels were plotted in a graph format using the median values. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

## Results

### *Study population*

The clinical and virological profiles of the 63 patients at the start of ADV treatment are shown in Table 1. At

baseline, 19 patients (30.2%) had cirrhosis, 42 patients (66.7%) were positive for HBeAg, and all 63 patients had detectable YMDD mutants. The median serum HBV DNA level at baseline was 7.3 log copies/ml, and the median serum ALT level was 3.9 × ULN.

*Efficacy of ADV plus lamivudine treatment*

Table 2 shows the point prevalence with respect to the efficacy measures in the intention-to-treat population analysis and summarizes the virological response 24, 48, and 72 weeks from the start of ADV treatment. The rates of undetectable serum HBV DNA levels (<2.6 log copies/ml) in the total study population were 49.2% (31/63) after 24 weeks, 61.9% (39/63) after 48 weeks, and 67.2% (39/58) after 72 weeks. The undetectable rates in the HBeAg-negative group were significantly higher than those in the HBeAg-positive group at week 24 ($P = 0.05$), week 48 ($P = 0.006$), and week 72 ($P = 0.001$).

Normalized rates of serum ALT levels are summarized in Table 2. The rates in the total study population were 73.0% (47/63) after 24 weeks, 81.0% (51/63) after 48 weeks, and 93.1% (54/58) after 72 weeks. The normalized rates of serum ALT levels in HBeAg-positive and HBeAg-negative groups were similar. No patient had a virological relapse of serum HBV DNA elevation of more than 2 log copies/ml from the lowest values or a biochemical relapse of serum ALT elevation over 2 × ULN.

**Table 1.** Baseline characteristics at commencement of adefovir dipivoxil ($n = 63$)

| | |
|---|---|
| Treatment period (weeks)[a] | 108 (73–165) |
| Age[a] | 48 (26–73) |
| Sex (Male : Female) | 52 : 11 |
| Presence of cirrhosis (%) | 30.2 |
| HBV genotype (A : B : C) | 4 : 3 : 56 |
| HBeAg positive (%) | 66.7 |
| HBV DNA (log copies/ml)[a] | 7.3 (4.0 to >7.6) |
| rtM204 mutant (I : V : I + V)[b] | 28 : 10 : 25 |
| AST (IU/l)[a] | 116 (42–331) |
| ALT (IU/l)[a] | 188 (24–892) |
| ALT / ULN[a] | 3.8 (0.5–17.8) |
| Total bilirubin (mg/dl)[a] | 0.8 (0.3–15.5) |
| Serum creatinine (mg/dl)[a] | 0.8 (0.5–1.2) |

HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; AST, aspartate transaminase; ALT, alanine aminotransferase; ULN, upper limit of normal; T-bil, total bilirubin
[a]Median (range)
[b]I → YIDD, V → YVDD, I + V → YIDD + YVDD mix

*Cumulative rate of HBeAg loss and seroconversion*

At baseline, 42 of 63 patients (66.7%) were HBeAg-positive. Among the 42 HBeAg-positive patients, the cumulative rates of HBeAg loss were 7.3% after 24 weeks, 19.5% after 48 weeks, and 29.7% after 72 weeks (Fig. 1). In this same group, the cumulative rates of HBeAg seroconversion (HBeAg-negative to anti-HBe-positive) were 4.9% after 24 weeks, 9.8% after 48 weeks, and 14.8% after 72 weeks (Fig. 2).

Patients were categorized into two groups by serum ALT levels at baseline: ALT levels ≥ 2 × ULN in one group and ALT levels < 2 × ULN in the other. The rates of HBe loss in the ALT ≥ 2 × ULN group were significantly higher than those in the ALT < 2 × ULN group ($P = 0.0145$) (Fig. 1). The rates of HBeAg seroconversion in the ALT ≥ 2 × ULN group were higher than those in the ALT < 2 × ULN group ($P = 0.1065$) (Fig. 2). No patient experienced a reappearance of HBeAg or reverse seroconversion to HBeAg-positive status. Patients who achieved HBeAg loss or seroconversion had a sustained response during this treatment.

*Factors associated with early virological response after 24 weeks*

Univariate analysis of individual baseline factors showed that the baseline ALT level ($P = 0.001$) and HBeAg status ($P = 0.040$) were each predictive factors of early virological response. There was no association with the other factors: patient age, sex, HBV DNA level, YMDD mutant status, HBV genotype, or the presence of cirrhosis. As shown in Table 3, multivariate analysis revealed that baseline ALT level ($P = 0.003$) and HBeAg status ($P = 0.049$) were associated with early virological response.

**Table 2.** Undetectable rate of HBV-DNA by Amplicor monitor assay and normalized rates of ALT levels

| | | 24 weeks | 48 weeks | 72 weeks |
|---|---|---|---|---|
| Undetectable rate of HBV-DNA | HBeAg-positive | 40.5% (17/42)* | 50.0% (21/42)** | 51.3% (19/37)*** |
| | HBeAg-negative | 66.7% (14/21)* | 85.7% (18/21)** | 95.2% (20/21)*** |
| | Overall | 49.2% (31/63) | 61.9% (39/63) | 67.2% (39/58) |
| Normalized rates of ALT | HBeAg-positive | 73.8% (31/42) | 76.2% (32/42) | 89.2% (33/37) |
| | HBeAg-negative | 76.2% (16/21) | 90.5% (19/21) | 100% (21/21) |
| | Overall | 73.0% (47/63) | 81.0% (51/63) | 93.1% (54/58) |

* $P = 0.032$
** $P = 0.006$
*** $P < 0.001$

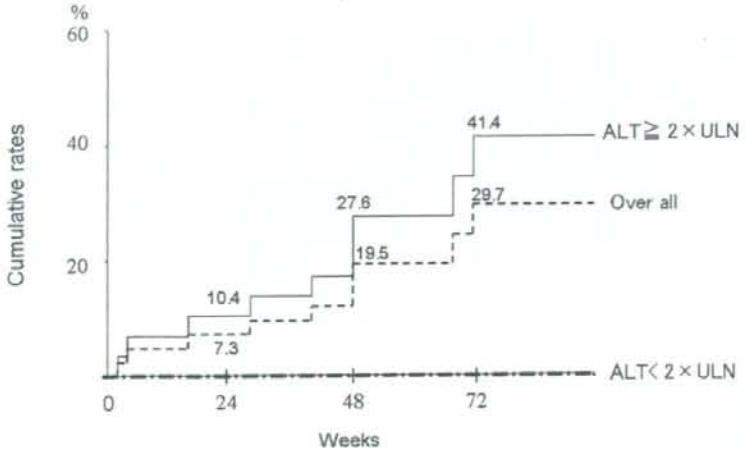


**Fig. 1.** Overall cumulative rate of hepatitis B e antigen loss during combination therapy with adefovir dipivoxil plus lamivudine: overall (*broken line*), alanine aminotransferase (*ALT*) greater than or equal to twice the upper limit of normal (*ULN*; *thick line*), and ALT less than twice the ULN (*dotted line*)

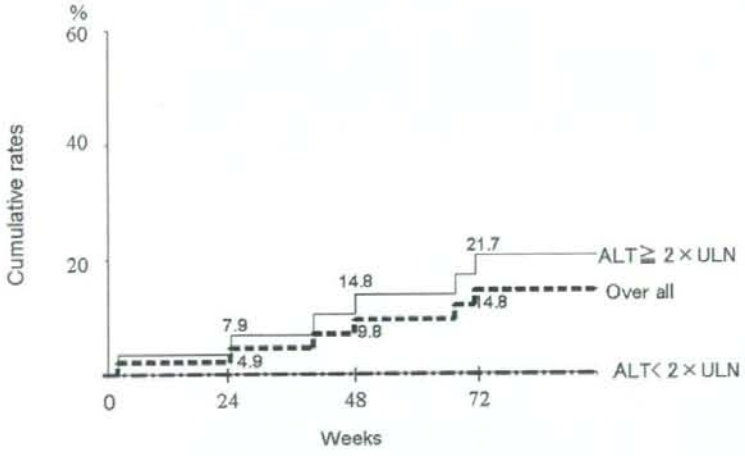


**Fig. 2.** Cumulative rate of hepatitis B e antigen seroconversion during combination therapy with adefovir dipivoxil plus lamivudine: overall (*broken line*), ALT greater than or equal to twice the ULN (*thick line*), and ALT less than twice the ULN (*dotted line*)

**Table 3.** Multivariate analysis of factors associated with early virological response[a] at week 24

| Factors | Category | Odds ratio | 95% CI | *P*[b] |
|---|---|---|---|---|
| Baseline ALT | 1: <2 × ULN | 1 | | |
| | 2: ≥ 2 × ULN | 8.924 | 2.131–37.38 | 0.003 |
| Baseline HBeAg status | 1: negative | 1 | | |
| | 2: positive | 0.300 | 0.091–0.993 | 0.049 |

CI, confidence interval
[a] Early virologic response: undetectable by Amplicor monitor assay
[b] *P* value by logistic regression analysis

Figure 3 shows the change in median serum HBV DNA level by baseline ALT level and HBeAg status. Patients were categorized into four groups: group A, ALT < 2 × ULN and HBeAg+; group B, ALT < 2 × ULN and HBeAg–; group C, ALT ≥ 2 × ULN and HBeAg+; and group D, ALT ≥ 2 × ULN and HBeAg–. Median values of the baseline HBV DNA level were 7.4 log copies/ml in group A, 7.4 log copies/ml in group B, 7.3 log copies/ml in group C, and 7.1 log copies/ml in group D. Although the HBV DNA level in group A declined similarly to levels in the other three groups during the first 4 weeks, the HBV DNA level in group

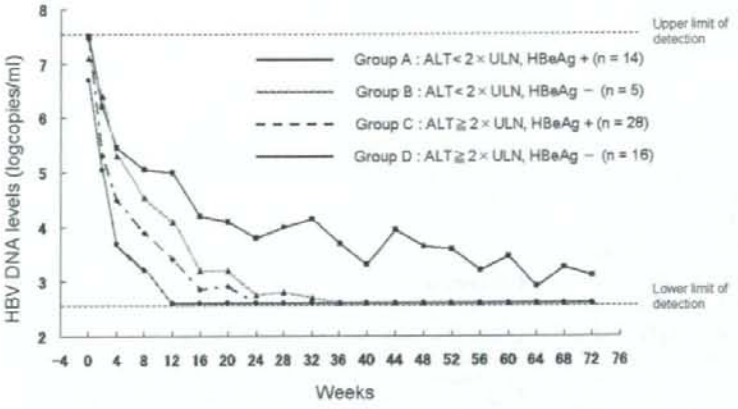


**Fig. 3.** Median values of hepatitis B virus DNA level by baseline ALT level and hepatitis B e antigen (*HBeAg*) status: group A (*thick line*), group B (*light gray line*), group C (*broken line*), and group D (*dark gray line*)

A decreased more slowly than in the other three groups over the first 4 weeks. The median values of the HBV DNA levels after 12 weeks were 5.0 log copies/ml in group A, 4.1 log copies/ml in group B, 3.4 log copies/ml in group C, and 2.6 log copies/ml in group D. The median values of the HBV DNA levels after 24 weeks were 3.8 log copies/ml in group A, 2.8 log copies/ml in groups B, and 2.6 log copies/ml in groups C and D.

*Biochemical response*

The median values of serum ALT level at baseline were 170 IU/l (3.4 × ULN) in HBeAg-positive patients and 184 IU/l (3.7 × ULN) in HBeAg-negative patients. In the first 12 weeks, there was an initial rapid reduction in serum ALT level of 124 IU/l in HBeAg-positive patients and 141 IU/l in HBeAg-negative patients. The median values of serum ALT level declined to less than the ULN after 12 weeks in HBeAg-positive patients and after 8 weeks in HBeAg-negative patients. These values remained less than the ULN thereafter.

## Discussion

Previous studies have shown that HBeAg seroconversion induced by interferon therapy is associated with pretherapy ALT levels.[22] Some previous lamivudine studies also showed that an initially high ALT level was a predictor of sustained HBeAg seroconversion and/or HBV DNA suppression.[3,23-25] In this combination therapy of ADV plus lamivudine for the treatment of breakthrough hepatitis resulting from lamivudine-resistant mutants, our results also indicated that the pretherapy ALT level was associated with HBeAg seroconversion and serum HBV DNA suppression. In particular, rates of early virological response in the first 24 weeks showed a direct relation with baseline serum ALT levels. This observation may suggest that the host's endogenous immune response and the potent direct antiviral activity of ADV also caused the marked decline in serum HBV DNA level against lamivudine-resistant HBV.

The current study showed encouraging results regarding the efficacy of combination therapy with ADV plus lamivudine for the treatment of lamivudine-resistant HBV. However, two previous studies reported that the rates of HBV DNA negativity demonstrated by PCR assay were 20%–35% and serum ALT level normalization rates were 37%–61% after 48–52 weeks.[17,18] However, in this study, the overall rate of HBV DNA negativity was 61.9% and that of serum ALT level normalization was 81.0% after 48 weeks. The differences in these results seem to have been caused by a difference in baseline ALT levels; the median value of baseline serum ALT level in this study (188 IU/l) was higher than those in previous studies (74–94.5 IU/l). On the other hand, the rates of HBeAg loss were 19.5% in this study and 15–17% in previous studies.[17,18] With respect to the rates of HBeAg loss, our results are similar to previous results. Although baseline serum ALT level was associated with HBeAg loss and marginally associated with HBeAg seroconversion by the current univariate analysis (Fig. 2), there was no independent factor associated with HBeAg loss or seroconversion, including baseline serum ALT level, by multivariate analysis (data not shown). We considered that baseline ALT levels were more associated with a decrease of HBV DNA levels than with HBeAg loss or seroconversion because the rates of HBeAg loss were similar but the rates of HBV DNA negativity and ALT normalization were different between this study and previous studies.

On the basis of the current results, we consider that the favorable timing of ADV added to ongoing lamivu-

dine treatment for lamivudine-resistance is ALT elevation of more than 2 × ULN in patients without cirrhosis. Although starting ADV when serum ALT level is <2 × ULN is not too favorable in terms of subsequent sustained HBV DNA suppression and HBeAg seroconversion, ADV treatment should be begun when low-grade elevation of ALT levels is continuous. However, if decompensation is present and bilirubin is elevated, treatment should be begun immediately. In patients with cirrhosis, the timing of the start of ADV should be earlier than in those without cirrhosis because decompensation is sometimes present before ALT levels elevate to >2 × ULN. This concept supports the therapeutic recommendations of several consensus panels for the treatment of chronic hepatitis B.[26-28]

It was recently reported in multicenter studies from Asia and Japan that prolonged lamivudine treatment might delay disease progression and reduce the risk of hepatocellular carcinoma in cases of chronic hepatitis B.[29,30] Our colleagues indicated that persistently low HBV DNA levels might save patients from hepatocellular carcinogenesis in cases of HBV-related cirrhosis.[31] Some previous reports indicated that long-term lamivudine treatment led to a favorable prognosis if YMDD mutants did not emerge.[24,32,33] These reports support the idea that sustained HBV DNA suppression and serum ALT level normalization benefit patients. At present, many patients with chronic hepatitis B worldwide are taking lamivudine. Although YMDD mutants may emerge and cause biochemical relapse in patients who receive ongoing prolonged lamivudine, starting ADV at the earliest indication of virological and biochemical relapse and continuing to suppress HBV replication may extend the favorable prognosis in patients with YMDD mutants. The current results suggested the optimal timing of ADV addition to ongoing lamivudine treatment.

Combination therapy of ADV plus lamivudine was well tolerated in the 72 weeks of the current study. Other studies have already shown that 10 mg/day ADV is safe for a 1-year treatment period.[17,18] In the present study, no patient discontinued this treatment because of adverse events. It was recently reported that nephrotoxicity, as defined by an increase in serum creatinine level of at least 0.5 mg/dl from baseline or a serum phosphorus value of less than 1.5 mg/dl on two consecutive occasions, was not observed in patients treated with 10 mg/day ADV for a median follow-up period of approximately 64 weeks, but mild nephrotoxicity was demonstrated with the dose of 30 mg/day.[34] A major advantage of ADV is the very low emergence rate of drug-resistant mutants.[35] In the current study, drug resistance was not observed. The remaining issue regarding ADV is its long-term safety.

In summary, the current study shows that the baseline serum ALT level is associated with HBeAg loss and seroconversion and that the baseline serum ALT level and HBeAg status are associated with the virological response of lamivudine-resistant HBV during combination therapy with ADV plus lamivudine. The current findings may enhance the treatment of virological and biochemical relapse resulting from the emergence of YMDD mutants during prolonged lamivudine treatment.

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# Efficacy of lamivudine therapy in elderly patients with chronic hepatitis B infection

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***Background.*** The aim of this study was to evaluate the efficacy of lamivudine therapy in elderly patients with chronic HBV infection. ***Methods.*** Patients aged ≥60 years ($n$ = 40) received lamivudine monotherapy between February 1995 and September 2005 at Toranomon Hospital. We compared the efficacy of lamivudine therapy in these patients and in 639 patients aged <60 years, including 80 patients aged <60 years matched for sex, hepatitis B e antigen (HBeAg) status, and hepatitis B virus (HBV) DNA level. ***Results.*** The rates of normalization of alanine aminotransferase (ALT) level in 40 patients aged ≥60 years and 639 patients aged <60 years were 85% versus 76%, and 86% versus 73% at 1 and 3 years, respectively. The respective rates of loss of HBV-DNA were 74% versus 74%, and 76% versus 68% at 1 and 3 years. The respective cumulative emergence rates of the YMDD mutant were 16% and 17% at 1 year, and 46% and 49% at 3 years. In 80 patients <60 years old matched for sex, HBeAg status, and HBV-DNA level, the rates of normalization of the ALT level and loss of HBV-DNA were similar to those in the 639 patients aged <60 years. The emergence rate of YMDD mutants in patients aged ≥60 years were similar to those in matched patients aged <60 years. Multivariate analyses identified low serum bilirubin (<1 mg/dl) as an independent factor associated with the emergence of the YMDD motif mutation in patients aged ≥60 years. ***Conclusions.*** Our results suggest that treatment with lamivudine is both well tolerated and efficacious in elderly patients with chronic HBV infection.

**Key words:** HBV, elderly patients, lamivudine, YMDD mutant

## Introduction

Chronic infection with hepatitis B virus (HBV) affects as many as 350 to 400 million people worldwide and 1.5 million people in Japan.[1] Vaccination is mainly used in Japan to prevent HBV infection via mother-to-infant transmission and to reduce the number of HBV carriers. However, there are still many patients with HBV infection. Moreover, elderly patients with chronic hepatitis are on the increase, and the potential for development of cirrhosis or hepatocellular carcinoma in such patients is real. Hence, treatment of elderly patients with HBV is an important issue.

Lamivudine is an oral cytosine nucleoside analog that potently inhibits HBV replication by interfering with HBV reverse transcriptase activity.[2–5] Several studies have reported the effectiveness of lamivudine in the suppression of HBV replication, improvement of transaminase levels and liver histology, and enhancement of the rate of loss of hepatitis B e antigen (HBeAg).[3,5–12] In this regard, a major problem with the long-term use of lamivudine is the development of viral resistance, associated with increases in HBV-DNA and serum transaminase levels.[13–15] We already reported the efficacy of lamivudine therapy and factors associated with the emergence of resistance in chronic HBV infection in Japan.[15] However, to our knowledge, there are no reports that describe the efficacy of lamivudine treatment in elderly patients (≥60 years) with chronic hepatitis B.

The aims of the present study were (1) to assess the benefits of lamivudine therapy for elderly patients (≥60 years) with chronic hepatitis B, and (2) to determine differences in the emergence rate of YMDD mutants and the appearance of breakthrough hepatitis between patients <60 years old and those ≥60 years old.

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**Table 1.** Characteristics of unmatched patients at commencement of lamivudine therapy

| | Patients aged <60 ($n = 639$) | Patients aged ≥60 ($n = 40$) | $P$ value |
|---|---|---|---|
| Age (years)[a] | 42 (23–58) | 63 (60–76) | <0.001 |
| Sex (male/female) | 523/116 | 23/17 | 0.001 |
| Liver cirrhosis (%) | 103 (16.1%) | 7 (17.5%) | NS |
| Median duration of treatment (months)[a] | 25 (6–135) | 44 (6–69) | NS |
| Platelet count ($\times 10^4/\mu l$)[a] | 18.5 (4.1–47.3) | 13.3 (5.2–25.8) | 0.018 |
| Aspartate aminotransferase (IU/l)[a] | 75 (15–2718) | 81 (27–1309) | NS |
| Alanine aminotransferase (IU/l)[a] | 106 (12–2437) | 123 (16–928) | NS |
| Serum bilirubin (mg/dl)[a] | 0.8 (0.3–20.2) | 0.8 (0.3–3.6) | NS |
| Serum albumin (g/dl)[a] | 3.9 (1.0–4.5) | 3.6 (1.8–4.4) | 0.01 |
| HBV-DNA (LGE/ml)[a] | 7.2 (<3.7–8.7) | 6.8 (<3.7–8.7) | NS |
| HBeAg-positive (%) | 376 (58.8%) | 15 (37.5%) | 0.01 |
| HBV genotype (A/B/C/other) | 20/38/536/45 | 0/4/32/4 | NS |

NS, not significant; HBV, hepatitis B virus; LGE, $\log_{10}$ genome equivalents; HBeAg, hepatitis B e antigen
[a]Values are median (range)

**Table 2.** Characteristics of patients matched for sex, HBeAg status, and HBV-DNA level at commencement of lamivudine therapy

| | Patients aged <60 ($n = 80$) | Patients aged ≥60 ($n = 40$) | $P$ value |
|---|---|---|---|
| Age (years)[a] | 44 (23–58) | 63 (60–76) | <0.001 |
| Sex (male/female) | 46/34 | 23/17 | Matched |
| Liver cirrhosis (%) | 13 (16.0%) | 7 (17.5%) | NS |
| Median duration of treatment (months)[a] | 44 (6–118) | 44 (6–69) | NS |
| Platelet count ($\times 10^4/\mu l$)[a] | 16.5 (4.1–47.3) | 13.3 (5.2–25.8) | 0.017 |
| Aspartate aminotransferase (IU/l)[a] | 61 (21–1656) | 81 (27–1309) | NS |
| Alanine aminotransferase (IU/l)[a] | 80 (12–1854) | 123 (16–928) | NS |
| Serum bilirubin (mg/dl)[a] | 0.7 (0.3–12.2) | 0.8 (0.3–3.6) | NS |
| Serum albumin (g/dl)[a] | 3.8 (1.0–4.5) | 3.6 (1.8–4.4) | 0.05 |
| HBV-DNA (LGE/ml)[a] | 6.7 (<3.7–8.7) | 6.8 (<3.7–8.7) | Matched |
| HBeAg positive (%) | 30 (37.5%) | 15 (37.5%) | Matched |
| HBV genotype (A/B/C/other) | 2/9/66/3 | 0/4/32/4 | NS |

[a]Values are median (range)

## Patients and methods

### *Patients*

Between February 1995 and September 2005, 40 consecutive Japanese patients aged ≥60 years were enrolled in this study at Toranomon hospital, Tokyo. All patients fulfilled the following criteria: (1) presence of hepatitis B surface antigen (HBsAg) in serum (positive for HBsAg for >6 months); (2) HBV-DNA positivity by quantitative assay; (3) absence of hepatoma; (4) absence of coinfection with hepatitis C virus (HCV); and (5) no previous treatment with any nucleoside analog.

The baseline characteristics of the 40 patients included in the study are listed in Table 1. All patients were Japanese; 23 were men and 17 were women; 15 were HBeAg-positive and 25 were HBeAg-negative; 32 patients had genotype C, four had genotype B, and the genotype was unknown in four. To determine the efficacy of lamivudine therapy and emergence rate of YMDD mutants, we compared the 40 patients aged ≥60 years with another group of 639 patients aged <60 years with HBV-related chronic infection on lamivudine therapy in our hospital. The baseline characteristics of the 679 patients are also listed in Table 1.

Since sex and HBeAg status were significantly different between the two age groups, we selected 80 patients from the 679 patients aged <60 years with HBV-related chronic infection on lamivudine therapy in our hospital who were matched to the ≥60 age group with respect to sex, HBV-DNA level, and HBeAg status (Table 2). The median duration of lamivudine therapy in the 40 patients aged ≥60 years was 44 months (range, 6–69 months). All comparisons described in this study pertain to two age groups of patients matched for sex, HBeAg status, and HBV-DNA levels unless otherwise stated.

One patient requested termination of lamivudine therapy and died from hepatocellular carcinoma during the observation period. He received lamivudine therapy for 16 months. Baseline platelet count and serum albumin concentrations differed between the ≥60-year-old and <60-year-old groups. Although the numbers of patients with genotypes A and B were small, the distribution of the HBV genotype was similar in patients with chronic HBV infection who had received care in our hospital over a follow-up period of more than 2 years.[16] Other factors were not significantly different between the two age groups.

*Methods*

All patients were treated with lamivudine at a dose of 100 mg/day orally, given continuously for at least 6 months, after providing informed consent. Clinical and laboratory assessments were performed once a month. Chronic hepatitis or cirrhosis was confirmed by needle biopsy, peritoneoscopy, or clinical criteria before treatment. The clinical criteria for chronic hepatitis included elevated alanine aminotransferase (ALT) levels over 6 months; absence of clinical evidence of portal hypertension, such as esophageal varices, ascites, or hepatic encephalopathy; and imaging features suggestive of cirrhosis on ultrasonography.[15] The diagnosis of chronic hepatitis and cirrhosis was established in 33 and 7 patients, respectively. HBeAg loss was defined as undetectable HBeAg. After the emergence of the YMDD mutant, we added adefovir dipivoxil (ADV) to ongoing lamivudine therapy. Three (5%) patients aged >60 years were treated with ADV in addition to lamivudine. On the other hand, 17 (28.3%) patients aged <60 years were treated with ADV in addition to lamivudine.

*Laboratory and virological tests*

Routine biochemical tests were performed at least once a month, before and during therapy, using standard procedures. Serial blood samples were taken before and during therapy every month and stored at −80°C until used for HBV mutant analysis. HBeAg and antibody to HBeAg (anti-HBe) were determined by radioimmunoassay kits (Abbott Diagnostics, Chicago, IL, USA). HBV-DNA was measured by a transcription-mediated amplification and hybridization protect assay (TMA-HPA; Chugai Diagnostics Science, Tokyo, Japan). Mutations in the YMDD motif in the polymerase gene were determined using polymerase chain reaction and restriction fragment length polymorphism, by a method described previously.[14] Lamivudine resistance was determined annually before the development of mutations, and, if a mutation appeared, the time of appearance of resistance was confirmed by monthly measurement.

*Data analysis*

Kaplan-Meier analysis and the log-rank test were used to estimate and compare the rates of viral resistance and appearance of breakthrough hepatitis between the ≥60-year-old and <60-year-old groups. A two-tailed $P$ value of less than 0.05 was considered statistically significant. Differences between groups were examined for statistical significance using the Mann-Whitney $U$ test and $\chi$-squared test where appropriate. Independent risk factors associated with emergence of YMDD motif mutation were studied using stepwise Cox regression analysis. Potential risk factors for emergence of the YMDD motif mutation that were assessed included the following ten variables: sex, degree of liver disease (cirrhosis or not), platelet count, aspartate aminotransferase (AST), ALT, serum bilirubin, serum albumin, HBV-DNA level, HBeAg, and HBV genotype. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with emergence of mutation of the YMDD motif ($P < 0.15$) were entered into the multivariate Cox proportional hazard model. A $P$ value less than 0.05 was considered statistically significant. All analyses described above were performed using the SPSS program (version 7.5, SPSS, Chicago, IL, USA).

## Results

*Serum HBV-DNA and ALT concentrations*

The rates of normalization of ALT level following lamivudine treatment for the 40 patients aged ≥60 years and the 639 patients aged <60 years were 85% versus 76%, 89% versus 75%, and 86% versus 73% at 1, 2, and 3 years, respectively. Furthermore, the respective rates of loss of HBV-DNA were 74% versus 74%, and 68% versus 71%, and 76% versus 68% at 1, 2, and 3 years, respectively. The cumulative emergence rates of YMDD mutant in patients aged ≥60 years and those <60 years were 16% and 17% at 1 year, 32% and 36% at 2 years, and 46% and 49% at 3 years. The rates of normalization of ALT level, loss of HBV-DNA, and emergence of YMDD mutants in patients aged ≥60 years were similar to those in the younger age group.

Figures 1 and 2 show the rates of normalization of ALT level and nondetection of HBV-DNA at 6 months and 1, 2, and 3 years in the patients matched for sex, HBeAg status, and HBV-DNA level during lamivudine therapy. The ALT normalization rate of the <60-year-old group tended to decrease year by year. On the other hand, the rate of the ≥60-year-old group tended to be higher than that of <60-year-old group at 2 and 3 years, although the difference was not significant (Fig. 1).

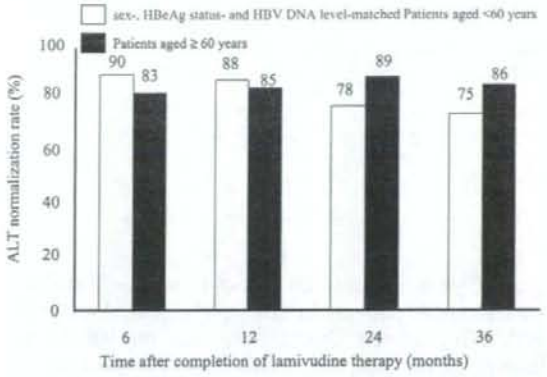


**Fig. 1.** Alanine aminotransferase (*ALT*) normalization rate. *Numbers above the bars* represent the values of normalization rates in patients of the two age groups matched for sex, hepatitis B e antigen (*HBeAg*) status, and hepatitis B virus (*HBV*)-DNA level

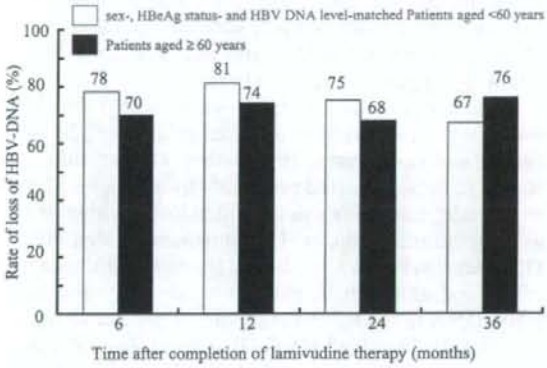


**Fig. 2.** HBV-DNA loss rate. *Numbers above the bars* represent the values of loss rates in patients of the two age groups matched for sex, HBeAg status, and HBV-DNA level

Figure 2 shows HBV-DNA loss rates in both groups. In patients aged <60 years, HBV-DNA and ALT levels at 2 and 3 years were lower than those at 6 months and 1 year, reflecting the development of viral resistance. In patients aged ≥60 years, the HBV-DNA loss rate was almost stable at all time points. HBeAg seronegative rates were 36% versus 37% at 1 year, 46% versus 41% at 2 years, and 46% versus 55% at 3 years for patients aged ≥60 years and <60 years, respectively (differences not significant).

### *Emergence of YMDD motif mutant and appearance of breakthrough hepatitis*

The cumulative emergence rates of the YMDD mutant in patients aged ≥60 years and <60 years were 16% and

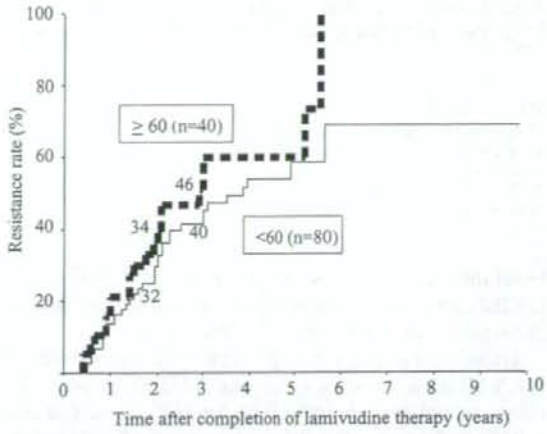


**Fig. 3.** Cumulative percentages of sex-, HBeAg status-, and HBV-DNA level-matched patients (<60 and ≥60) who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

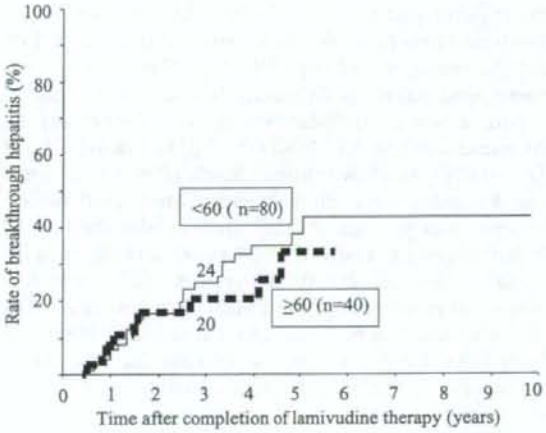


**Fig. 4.** Cumulative percentages of sex-, HBeAg status-, and HBV-DNA level-matched patients (<60 and ≥60) who showed breakthrough hepatitis during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

17% at 1 year, 32% and 34% at 2 years, and 46% and 40% at 3 years, respectively (Fig. 3). The emergence rates of YMDD mutants in patients aged ≥60 years were similar to those in patients aged <60 years at all three time intervals. The cumulative breakthrough hepatitis appearance rates in patients aged ≥60 and <60 years were 14.0% and 12.0% at 1 year, 12.0% and 12.0% at 2 years, and 20% and 26.0% at 3 years, respectively (not significant, Fig. 4). However, the appearance rate of

**Table 3.** Laboratory data for patients matched for sex, HBeAg status, and HBV-DNA level after emergence of YMDD mutant during lamivudine therapy

| | <60 ($n$ = 36) | ≥60 ($n$ = 21) | $P$ value |
|---|---|---|---|
| Maximum ALT (IU/l)[a] | 69 (15–727) | 24 (12–1669) | 0.049 |
| Maximum bilirubin (mg/dl)[a] | 0.9 (0.3–3.4) | 0.7 (0.3–1.2) | NS |
| Maximum HBV-DNA (LGE/ml)[a] | 6.5 (2.6–8.7) | 4.6 (2.6–8.7) | NS |

ALT, alanine aminotransferase
[a] Values are median (range)

breakthrough hepatitis in patients aged ≥60 years tended to be lower than in those <60 years, although the difference was not statistically significant.

These characteristics of patients with emergence of the YMDD mutant (sex, type of YMDD mutant, cirrhosis, duration of therapy, HBeAg, HBV genotype and HBV-DNA) were not different between the ≥60-year-old and the <60-year old groups. Table 3 summarizes the laboratory data of patients after emergence of the YMDD mutant. The maximum ALT in patients aged ≥60 years was significantly lower than that in patients aged <60 years ($P$ = 0.049).

Twenty-two (55%) patients among patients aged ≥60 years developed mutations in the YMDD motif during lamivudine therapy. We then explored the risk factors for the emergence of the YMDD motif mutation in patients aged ≥60 years. In univariate analyses, the following three factors significantly influenced the emergence of resistance: low AST level ($P$ = 0.045), low ALT level ($P$ = 0.028), and low bilirubin levels ($P$ = 0.001). Since the variables were mutually correlated, multivariate analysis was performed. That analysis identified a low bilirubin level (hazard ratio, 14.40; 95% confidence interval, 2.89–71.82; $P$ = 0.001) to be a significant determinant of emergence of the YMDD motif mutation. On the other hand, in patients aged <60 years, we identified high HBV-DNA level as a significant determinant of emergence of the YMDD motif mutation.

## Discussion

The benefits of lamivudine in patients with compensated HBV-related liver disease have been suggested by several groups.[3–7] Our study is the first to show long-term efficacy by lamivudine monotherapy in older patients. Our study demonstrated that lamivudine therapy was well tolerated by elderly Japanese patients and led to reductions in levels of transaminases and HBV-DNA prior to the emergence of YMDD mutants.

The emergence of YMDD mutants is known to be associated with high HBV-DNA levels, high ALT levels, and HBeAg-positivity at baseline, especially among patients with chronic hepatitis.[15,17–21] In our study, sex and HBeAg status were significantly different between the ≥60-year-old and <60-year-old groups. Therefore, we selected 80 patients from 639 patients aged <60 years with HBV-related chronic hepatitis on lamivudine therapy in our hospital, matched with respect to sex, HBV-DNA level, and HBeAg status.

In patients aged <60 years, the normalization rate of transaminases tended to decrease year by year; for example, the rates were 78% at 2 years and 75% at 3 years, because breakthrough hepatitis appeared in some patients. However, compared with patients aged <60 years, the high normalization rate of transaminases was sustained during follow-up in patients aged ≥60 years. Moreover, HBV-DNA loss rates were sustained during follow-up in patients aged ≥60 years.

In elderly patients with HBV, we identified low AST levels, low ALT levels, and low bilirubin levels by univariate analysis as associated factors. These results are similar to those reported previously by others.[19,22] Moreover, multivariate analysis identified low bilirubin levels as a significant predictor. In comparison, a high HBV-DNA level was not a predictive factor in patients aged ≥60 years, although in patients aged <60 years, high HBV-DNA levels were identified as a predictive factor ($P$ = 0.029). That high HBV-DNA level was not a predictive factor in patients aged ≥60 years may be due to the small number of patients.

The emergence rate of YMDD mutants in patients aged ≥60 years was similar to that in patients aged <60 years. On the other hand, the appearance rate of breakthrough hepatitis tended to be lower in patients aged ≥60 years than in those <60 years of age, although the difference was not statistically significant. The reason for the low frequency of breakthrough hepatitis in patients aged ≥60 years in spite of the same emergence rate of the YMDD mutant remains uncertain. The characteristics of patients with emergence of the YMDD mutant were not significantly different between the two age groups. Moreover, the maximum ALT in patients aged ≥60 years tended to be lower than that in patients aged <60 years. Nevertheless, one explanation may be that the immune response was lower in the elderly,[23] probably owing to a decrease in T helper cell function and in the responsiveness of B cells with age.[24,25] Further virological and/or immunological studies are necessary to investigate this issue.

Taken together, our results suggest that lamivudine therapy is safe in elderly persons. However, some of these patients with HBV could not receive interferon therapy because of the risk of potentially life-threatening complications. Therefore, the indications for therapy in elderly patients with breakthrough hepatitis must be carefully considered. These patients are in need of other antiviral agents with anti-HBV activity. Recent studies suggest that adefovir dipivoxil and entecavir may effectively suppress YMDD mutants.[20,21,26,27] However, the efficacy and safety of these agents in the elderly with HBV with YMDD mutants have not yet been established. A combination therapy with lamivudine and other anti-HBV agents may induce a decrease in the frequency of drug resistance and delay progression in elderly patients with HBV.

In conclusion, our results suggested that lamivudine therapy improved the clinical course in elderly patients. Lamivudine therapy for elderly Japanese patients was well tolerated and resulted in reduction of transaminases and HBV-DNA levels. The rate of breakthrough hepatitis in patients aged ≥60 years was lower than that in patients aged <60 years in spite of the frequent emergence rate of the YMDD mutant. Moreover, breakthrough hepatitis tended to occur in patients aged <60 years more than in patients aged ≥60 years. Further studies are needed to evaluate, under careful virological monitoring, whether new antiviral agents such as adefovir dipivoxil and entecavir are useful in elderly patients with HBV.

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