Quantitative polymerase chain reaction assay for serum hepatitis B virus DNA as a predictive factor for post-treatment relapse after lamivudine induced hepatitis B e antigen loss or seroconversion


Background and aims: Lamivudine induces favourable virological and biochemical responses but post-treatment relapses are frequent, even in patients with hepatitis B e antigen (HBeAg) loss or seroconversion. The aim of this study was to determine whether extended lamivudine therapy for up to 12 months after HBeAg loss/seroconversion could decrease the risk of post-treatment virological relapse. In addition, we monitored serum hepatitis B virus (HBV) DNA levels using a quantitative polymerase chain reaction (PCR) assay during extended lamivudine therapy and analysed predictive factors for post-treatment relapse.

Patients and methods: A total of 49 patients who exhibited HBeAg loss/seroconversion during lamivudine therapy received extended lamivudine therapy for six months (group 1, n = 23) or 12 months (group 2, n = 26) after HBeAg loss/seroconversion. Serum HBV DNA levels were quantified by a PCR based assay at the time of HBeAg loss/seroconversion, and at cessation of therapy.

Results: Post-treatment virological relapse rates at two years were 59% in group 1 and 50% in group 2. Age, time interval to HBeAg loss/seroconversion, and serum HBV DNA levels at the time of cessation of therapy were independent predictive factors for post-treatment relapse. The post-treatment relapse rate was 37% at two years in patients with serum HBV DNA levels of <200 copies/ml but 73% in those with ≥10³ copies/ml.

Conclusions: Extended lamivudine therapy for up to 12 months did not decrease the rate of post-treatment virological relapse, and monitoring of serum HBV DNA by a quantitative PCR method was helpful in predicting post-treatment relapse.

Lamivudine safely induces favourable virological and biochemical responses in most patients within a short time of the initiation of therapy. Although hepatitis B e antigen (HBeAg) loss and/or seroconversion are generally regarded as end points of therapy, several studies from Korea and Taiwan have reported high post-treatment relapse rates after lamivudine induced HBeAg seroconversion. Two approaches can be considered in the reduction of the risk of post-treatment relapse. One approach is to extend the duration of lamivudine therapy after HBeAg loss/seroconversion. Our previous retrospective analysis suggested that extension of lamivudine therapy after HBeAg seroconversion might reduce the risk of post-treatment relapse. However, the optimal duration of additional therapy has not been determined. The second approach is to establish the predictive factors for relapse, and use these to discontinue treatment in low risk patients. Lee et al reported that the Digene ultrasensitive hybrid capture II assay (Digene Diagnostics, Beltsville, Maryland, USA), a more sensitive assay than conventional hybridisation methods, may be helpful in predicting relapse after lamivudine induced HBeAg seroconversion. However, only five of 42 patients were positive for hepatitis B virus (HBV) DNA by the Digene ultrasensitive hybrid capture II assay, and the post-treatment relapse rate was 51.4%, even in patients with serum HBV DNA levels that were not detectable by that assay. Therefore, this assay is not sufficient for predicting post-treatment relapse in clinical practice.

In the present study, we prospectively analysed the relationship between duration of extended lamivudine therapy and the post-treatment relapse rate in patients with lamivudine induced HBeAg loss/seroconversion. We also analysed changes in HBV DNA titres during extended lamivudine therapy using a quantitative polymerase chain reaction (PCR) assay and predictive factors for post-treatment relapse in patients with lamivudine induced HBeAg loss/seroconversion.

PATIENTS AND METHODS

Patients
Between June 1999 and May 2000, a total of 49 consecutive patients who exhibited HBeAg loss/seroconversion during lamivudine therapy and agreed to receive extended lamivudine therapy were enrolled into the study. All patients were positive for serum hepatitis B surface antigen, HBeAg, and HBV DNA, and had elevated serum alanine aminotransferase (ALT) levels for more than six months prior to lamivudine therapy. HBeAg loss was defined as loss of serum HBeAg and HBV DNA in two consecutive tests performed two months apart. HBeAg seroconversion was defined as loss of serum HBeAg and HBV DNA, and the development of antibodies to hepatitis B e antigen (anti-HBe).

Table 1 lists the baseline demographic and clinical characteristics of these 49 patients. Written informed consent...
was obtained from all patients participating in this study, which was approved by the Investigation and Ethics Committee for Human Research at the Asan Medical Centre.

Study protocol
Lamivudine was given at a dose of 100 mg per day. During lamivudine therapy, serum HBeAg, anti-HBe (as measured by an immunoradiometric assay kit; DiaSorin, Vercelli, Italy), HBV DNA (as measured by the Digene hybrid capture II (DHCI11 assay; Digene Diagnostics; lower limit of detection 0.5 pg/ml), and ALT levels were tested every 2–3 months.

Once patients achieved HBeAg loss/seroconversion on two consecutive tests during lamivudine therapy, they were allocated to one of two groups: (1) 23 patients allocated to the six month extended lamivudine therapy group received additional lamivudine therapy for six months from the time when HBeAg first became negative (group 1), and (2) 26 patients received 12 months of additional lamivudine therapy after HBeAg loss/seroconversion (group 2). Allocation was performed according to the patient’s own preference because medical insurance in South Korea covers the use of lamivudine for only one year. Post-treatment monitoring continued every 2–3 months for at least 12 months after discontinuation of therapy.

The end point for analysis was virological relapse, which was defined as post-treatment reappearance of serum HBV DNA, as measured by the DHCI11 assay, and/or HBeAg in two consecutive tests. Virological breakthrough was defined as reappearance of serum HBV DNA in two consecutive tests, as measured by the DHCI11 assay, during extended lamivudine therapy.

Quantification of serum HBV DNA levels
Serum HBV DNA levels were quantified at the time of HBeAg loss/seroconversion and discontinuation of lamivudine therapy using Cobas Amplicor HBV monitor kits (Roche Molecular Systems, Pleasanton, California, USA) according to the manufacturer’s instructions. This assay is linear from 200 to 10⁸ copies/ml.

Detection of mutations in the YMDD motif and precore/core promoter region of HBV DNA by direct sequencing
Serum samples were analysed for the presence of mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif at the time of virological breakthrough and discontinuation of lamivudine therapy. Mutations in precore or core promoter regions were determined prior to treatment, and at the time of HBeAg loss/seroconversion and discontinuation of therapy. To determine mutations in the YMDD motif, or precore and core promoter regions, PCR and direct sequencing were performed according to the methods of our previous study. However, to increase the sensitivity of PCR, in the current study HBV DNA was isolated from serum using a high pure viral nucleic acid kit (Roche Molecular Systems).

Statistical analysis
Data analyses were performed using the statistical package SPSS for PC (version 9.0; SPSS, Chicago, Illinois, USA). Data are expressed as mean (SD) or median (range). The Student’s t test, Mann-Whitney U test, Fisher’s exact test, and the χ² test were used, as appropriate. The cumulative relapse rate was calculated by the Kaplan-Meier method, and the difference was determined by the log rank test. Predictors for post-treatment relapse were determined by multivariate analysis using Cox’s proportional hazard model. A p value < 0.05 was considered to be statistically significant.

RESULTS
Virological breakthrough during extended lamivudine therapy and post-treatment relapse
Serum HBV DNA was positive in the DHCI11 assay in three of 49 (6.1%) patients during extended lamivudine therapy after HBeAg loss/seroconversion. The cumulative virological breakthrough rates were 4.1% at six months and 8.1% at 12 months during extended lamivudine therapy. Therefore, a total of 46 patients (22 in group 1, 24 in group 2) completed extended lamivudine therapy successfully, and 38 were positive for anti-HBe at cessation of therapy (90.9% in group 1, 75.0% in group 2). Three patients (one in group 1, two in group 2) were lost to follow up after virological relapse at six, six, and nine months, respectively, while two patients in group 1 were lost to follow up without virological relapse at 13 and 18 months after cessation of lamivudine therapy, respectively. The remaining 41 patients were followed for at least 25 months (median 33 months (range 6–42)).

The overall cumulative post-treatment relapse rates were 48% and 54% at six and 12 months of follow up, respectively. The post-treatment virological relapse rates were 50% at six months, 59% at one year, and 59% at two years in group 1, and 46% at six months, 50% at one year, and 50% at two years in group 2 (fig 1).

Most relapses (96%) occurred within 12 months after discontinuation of lamivudine and were accompanied by elevation in serum ALT levels (89%). One patient relapsed after 26 months of follow up. Five of the 26 relapers (19.2%) were negative for HBeAg at the time of relapse. There was no difference in post-treatment relapse rate between 38 patients with anti-HBe (51% at six months and 60% at 12 months) and eight patients without anti-HBe (33% at six months and 33% at 12 months) at the time of cessation of lamivudine therapy.

### Table 1 Baseline characteristics of the study patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total patients (n = 49)</th>
<th>Group 1 (n = 23)†</th>
<th>Group 2 (n = 26)‡</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39.2 (11.0)</td>
<td>38.6 (11.0)</td>
<td>39.9 (11.3)</td>
<td>0.688</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>41.8</td>
<td>21.2</td>
<td>20.6</td>
<td>0.254</td>
</tr>
<tr>
<td>Disease (CHB:LC)</td>
<td>34.15</td>
<td>18.5</td>
<td>16.10</td>
<td>0.205</td>
</tr>
<tr>
<td>ALT (IU/l)*</td>
<td>146 (80–1764)</td>
<td>160 (80–1764)</td>
<td>124 (80–530)</td>
<td>0.403</td>
</tr>
<tr>
<td>ALT = 400 IU/l</td>
<td>8 (16.3%)</td>
<td>5 (21.7%)</td>
<td>3 (11.5%)</td>
<td>0.448</td>
</tr>
<tr>
<td>HBV DNA (pg/ml)*</td>
<td>295 (5–11 748)</td>
<td>295 (5–11 748)</td>
<td>265 (8–8791)</td>
<td>0.628</td>
</tr>
<tr>
<td>History of previous IFN-α therapy</td>
<td>9 (18.4%)</td>
<td>2 (8.7%)</td>
<td>7 (26.9%)</td>
<td>0.145</td>
</tr>
<tr>
<td>Time to HBeAg loss (months)*</td>
<td>4 (2–18)</td>
<td>3 (2–12)</td>
<td>4 (2–18)</td>
<td>0.348</td>
</tr>
</tbody>
</table>

CHB, chronic hepatitis B; LC, liver cirrhosis; ALT, alanine aminotransferase; HBV, hepatitis B virus; IFN, interferon; HBeAg, hepatitis B e antigen.

*Median (range).
†Group 1, patients who received lamivudine for an additional six months after HBeAg loss/seroconversion.
‡Group 2, patients who received lamivudine for an additional 12 months after HBeAg loss/seroconversion.
Serum HBV DNA levels by quantitative PCR assay at the time of HBeAg loss/seroconversion and discontinuation of lamivudine therapy

The mean serum HBV DNA level at the time when HBeAg first became undetectable was 2.91 (1.07) log₁₀ copies/ml (range 0–4.41). Only four (9%) patients were negative for serum HBV DNA by the quantitative PCR assay, and 11 (25%) had levels <200 copies/ml (lower limit of linearity of this assay) at the time of HBeAg loss/seroconversion. The mean serum HBV DNA level changed from 2.99 (1.19) log₁₀ copies/ml at the time of HBeAg loss/seroconversion to 2.37 (1.79) log₁₀ copies/ml at six months in group 1, and from 2.83 (0.96) log₁₀ copies/ml to 2.26 (1.68) log₁₀ copies/ml at 12 months in group 2. The proportion of patients with serum HBV DNA levels <200 copies/ml increased from 22.7% (5/22) to 36.4% (8/22) at six months in group 1 and from 25% (6/24) to 45.8% (11/24) at 12 months in group 2. Serum HBV DNA levels declined by more than 1 log₁₀ in 14 of 28 patients with serum HBV DNA levels ≥10⁴ copies/ml at the time of HBeAg loss/seroconversion. Serum HBV DNA levels remained stable or decreased to levels <200 copies/ml in 16 (89%) of 18 patients with serum HBV DNA levels <10⁴ copies/ml at the time of HBeAg loss/seroconversion. Serum HBV DNA levels increased more than 10-fold in 20% of patients during extended lamivudine therapy.

Emergence of mutants in the YMDD motif during extended lamivudine therapy

Mutants in the YMDD motif were detected in six of the 49 initially enrolled patients. All three patients with virological breakthrough during extended lamivudine therapy had mutants in the YMDD motif (YIDD variant in one patient; YIDD and YVDD variants in two patients). PCR amplification and direct sequencing was possible in 32 of the remaining 46 patients at the time of cessation of lamivudine therapy. Three of the 32 patients (one in group 1, two in group 2) harboured mutants in the YMDD motif (YVDD variant in one patient; YIDD variant in one patient; YIDD and YVDD variants in one patient). Therefore, the cumulative incidences of mutants in the YMDD motif during extended lamivudine therapy were 6.1% at six months and 18.1% at one year. Serum HBV DNA levels increased more than 10-fold in four of the six patients.

Mixed infection with precore stop codon variants and serum HBV DNA levels at the time of HBeAg loss/seroconversion

All patients were positive for serum HBeAg before treatment but precore stop codon variants (G1896A) were detected in 34.7% and core promoter variants (A1762T/G1764A) in 77.6% of patients at that time. Mean serum HBV DNA levels tended to be higher in patients coinfected with precore stop codon variants (3.28 (0.72) ν 2.88 (1.18) log₁₀ copies/ml) but the difference was not statistically significant (p = 0.17). Three patients who had wild-type HBV before treatment acquired precore stop codon mutations at the time of HBeAg loss/seroconversion while three others converted from mixed infection with precore stop codon variants to wild-type HBV infection. Four of five relapsers who were negative for HBeAg at the time of relapse were infected with precore stop codon variants. Post-treatment relapse rates at two years were 60% in patients with wild-type HBV infection and 56.2% in those coinfected with precore stop codon variants.

Post-treatment virological relapse according to serum HBV DNA levels by quantitative PCR at the time of discontinuation of lamivudine therapy

Cumulative relapse rates differed significantly with serum HBV DNA levels at the time of discontinuation of lamivudine therapy despite the absence of differences in baseline characteristics (p = 0.017). Cumulative relapse rates in patients (n = 19) with HBV DNA levels <200 copies/ml (lower limit of linearity of this assay) were 26% at six months and 37% at one year after treatment. Cumulative relapse rates in patients with HBV DNA levels of 200–1000 copies/ml (n = 12) were 50% at six months and 58% at one year. Cumulative relapse rates in those with HBV DNA levels >1000 copies/ml (n = 15) were 67% at six months and 73% at one year (fig 2).
Predictive factors for post-treatment virological relapse

Univariate analyses revealed that age, presence of cirrhosis, and serum HBV DNA levels by quantitative PCR at the time of discontinuation of lamivudine therapy were significant predictive factors for post-treatment relapse. The time interval from the initiation of lamivudine therapy to HBeAg loss/seroconversion, and the total duration of lamivudine therapy was marginally significant (table 2).

Multivariate analyses revealed that older age (odds ratio (OR) 1.06 (95% confidence interval (CI) 1.01–1.10); p = 0.008), shorter time interval to HBeAg loss/seroconversion (OR 1.12 (95% CI 1.01–1.25); p = 0.032), and higher HBV DNA levels at the time of cessation of lamivudine therapy (OR 1.79 (95% CI 1.10–2.91); p = 0.019) were three independent predictive factors for post-treatment virological relapse. The post-treatment relapse rate was 30% at two years in patients younger than 30 years and 61% in those 30 years of age or older.

DISCUSSION

Dienstag et al reported a sustained response after lamivudine induced HBeAg loss/seroconversion\(^a\) but all the studies performed in Korea and Taiwan and a recent meta-analysis reported frequent post-treatment relapse.\(^2\)\(^–\)\(^4\)\(^9\)\(^11\) In contrast, a durable HBeAg response was reported in phase II and phase III trials in Hong Kong.\(^1\)\(^2\)

The reason for this discrepancy is still unknown. Interestingly, in all of the studies\(^2\)\(^–\)\(^4\)\(^9\)\(^11\) that reported frequent post-treatment relapse, the HBeAg seroconversion rates at one year of treatment were much higher (30–35%) and the time intervals from initiation of therapy to HBeAg seroconversion were shorter than in phase II and phase III trials.\(^2\)\(^–\)\(^4\)\(^9\)\(^11\)

In the current study, a shorter time interval from initiation of therapy to HBeAg loss/seroconversion was an independent predictor of post-treatment relapse. Therefore, there may be contributing factors for early HBeAg seroconversion during lamivudine therapy and the high post-treatment relapse.

In contrast with phase II and phase III trials, which excluded patients with ALT levels greater than 10 times the upper limit of normal, those with acute exacerbation were included in our present and previous studies.\(^2\)\(^–\)\(^4\)\(^9\)\(^11\) However, the high post-treatment relapse rate could not be attributed to those with acute exacerbation and possible spontaneous seroconversion as only eight patients (16.3%) had pretreatment ALT levels \(\geq 400\) U/L in the current study and pretreatment ALT level was not a predictive factor for relapse in all of these studies. Furthermore, the reported HBeAg reappearance rate after spontaneous HBeAg seroconversion was 30–35% over 18 months to five years of follow up.\(^1\)\(^3\)\(^4\)

Another possible factor may be variants of HBV. In the current study, we focused on precore stop codon variants and core promoter variants because patients coinfected with these variants may experience early seroconversion or be mistaken for those with HBeAg loss/seroconversion serologically during lamivudine therapy owing to the low levels of HBeAg production. However, serum HBV DNA levels at the time of HBeAg loss/seroconversion and post-treatment relapse rates were not different between those with and without these variants, although a quantitative analysis was not possible. Recently, one study performed in Taiwan suggested that genotype C is associated with frequent post-treatment relapse after lamivudine induced HBeAg seroconversion.\(^10\) This observation supports the high post-treatment relapse rate in Korean patients because all patients were infected with genotype C in the current study (data not shown).

In the current study, we included not only patients with HBeAg seroconversion but also those with HBeAg loss without acquisition of anti-HBe. This was because there were patients with fluctuating serum anti-HBe status, and hence the duration of extended lamivudine therapy could not be determined from the time of HBeAg seroconversion in these patients. Therefore, we determined the duration of extended lamivudine therapy from the time when HBeAg first became undetectable. In contrast with a previous suggestion,\(^7\) there was no difference in post-treatment relapse rate between patients with anti-HBe and those without anti-HBe.

In contrast with our previous retrospective study, extended lamivudine therapy for up to 12 months after HBeAg loss/seroconversion did not reduce the rate of post-treatment virological relapse in this prospective study. Although the results may have been influenced by the larger proportions of patients with cirrhosis and/or a history of previous interferon therapy in group 2, these factors were not independent predictive factors for virological relapse in the multivariate analysis. In addition, relapse rates were not also different from those in previous studies.\(^2\)\(^–\)\(^4\)\(^9\)\(^11\)

One possible explanation for this discrepancy is that lamivudine therapy was extended to those with fluctuating serum anti-HBe status, and hence the duration of extended lamivudine therapy could not be determined from the time of HBeAg seroconversion in these patients. Therefore, we determined the duration of extended lamivudine therapy from the time when HBeAg first became undetectable. In contrast with a previous suggestion, there was no difference in post-treatment relapse rate between patients with anti-HBe and those without anti-HBe.

In contrast with our previous retrospective study, extended lamivudine therapy for up to 12 months after HBeAg loss/seroconversion did not reduce the rate of post-treatment virological relapse in this prospective study. Although the results may have been influenced by the larger proportions of patients with cirrhosis and/or a history of previous interferon therapy in group 2, these factors were not independent predictive factors for virological relapse in the multivariate analysis. In addition, relapse rates were not also different from those in previous studies.\(^2\)\(^–\)\(^4\)\(^9\)\(^11\)

One possible explanation (table 2).

### Table 2 Univariate analysis for predictive factors for virological relapse

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.05 1.01–1.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex (male vs female)</td>
<td>1.51 0.57–4.04</td>
<td>0.41</td>
</tr>
<tr>
<td>Disease (CHB vs CHB)</td>
<td>2.03 0.93–4.46</td>
<td>0.08</td>
</tr>
<tr>
<td>Pretreatment ALT (U/L)</td>
<td>1.00 0.99–1.00</td>
<td>0.29</td>
</tr>
<tr>
<td>Pretreatment log(_10) HBV DNA (pg/ml)</td>
<td>1.07 0.69–1.66</td>
<td>0.76</td>
</tr>
<tr>
<td>Positive anti-HBe at the end of therapy</td>
<td>1.96 0.59–6.33</td>
<td>0.27</td>
</tr>
<tr>
<td>Treatment HBeAg loss (months)</td>
<td>0.92 0.83–1.02</td>
<td>0.12</td>
</tr>
<tr>
<td>Total duration of lamivudine therapy (months)</td>
<td>0.95 0.89–1.01</td>
<td>0.12</td>
</tr>
<tr>
<td>Group (I:2)(^a)</td>
<td>0.77 0.35–1.66</td>
<td>0.50</td>
</tr>
<tr>
<td>HBV DNA levels at the end of therapy (A:B:C)(^†)</td>
<td>1.85 1.15–2.98</td>
<td>0.01</td>
</tr>
<tr>
<td>A (&lt;200 copies/ml)</td>
<td>1.67 0.59–4.78</td>
<td>0.34</td>
</tr>
<tr>
<td>B (200–1000 copies/ml)</td>
<td>3.38 1.31–8.71</td>
<td>0.01</td>
</tr>
<tr>
<td>C (&gt;1000 copies/ml)</td>
<td>3.38 1.31–8.71</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are odds ratios (OR) with 95% confidence interval (CI).

CHB, chronic hepatitis B; LC, liver cirrhosis; ALT, alanine aminotransferase; HBV, hepatitis B virus; anti-HBe, antibodies to hepatitis B e antigen; HBeAg, hepatitis B e antigen; \(\leq\)400 IU/L.

\(^a\)Groups 1 and 2 represent patients who received additional lamivudine therapy after HBeAg loss/seroconversion for six months and 12 months, respectively.

\(^\dagger\)Groups A, B, and C represent patients whose serum HBV DNA levels were <200 copies/ml, 200–1000 copies/ml, and >1000 copies/ml, respectively, at the time of discontinuation of therapy.
is that the duration of extended lamivudine therapy was insufficient because the minimum half life of cccDNA containing hepatocytes was estimated to exceed 10–100 days.\textsuperscript{15, 16} The observation that serum HBV DNA decreased by more than 1 log\textsubscript{10} only in 50% of patients with serum HBV DNA levels $\geq 10^5$ copies/ml at the time of seroconversion also suggested the insufficient duration of extended lamivudine therapy. However, the optimal duration of extended lamivudine therapy cannot be determined because mutants in the YMDD motif still emerged even after HBeAg loss/seroconversion.

The post-treatment relapse rate in patients with serum HBV DNA levels $< 200$ copies/ml at the end of therapy was 37% at two years, which is similar to that in patients with spontaneous HBeAg seroconversion.\textsuperscript{13, 14} Although the relapse rate was still correlated with HBV DNA titre above this level, more than 60% of patients relapsed after cessation of lamivudine therapy. Therefore, serum HBV DNA monitoring by a quantitative PCR assay may be useful in determining when lamivudine therapy should be discontinued. However, 45% of sustained responders still had serum HBV DNA levels $\geq 200$ copies/ml. In addition, despite extended lamivudine therapy for six or 12 months, serum HBV DNA levels declined by more than 1 log\textsubscript{10} in only 50% of patients. These findings suggest that prolonged immunological suppression of HBV or clearance of infected cells may be essential in maintaining the virological response after HBeAg loss/seroconversion. Low HBV DNA levels during the course of lamivudine therapy may reflect more vigorous immunological clearance of infected cells.

In the current study, age was another significant predictive factor for post-treatment relapse, which is consistent with the results of previous studies.\textsuperscript{4, 11} This phenomenon may also reflect a more competent immune response in younger adults or the selection of refractory cases in older patients.

In conclusion, extended lamivudine therapy for up to 12 months after HBeAg loss/seroconversion did not decrease the risk of post-treatment virological relapse in Korean patients. It is advisable to continue lamivudine therapy in patients older than 30 years until serum HBV DNA levels decrease to 200 copies/ml or less, even after HBeAg loss/seroconversion. Further studies are needed to determine the predispoding factors to the frequent post-treatment relapse in this endemic area.

ACKNOWLEDGEMENTS

This work was supported by a grant (2002-238) from the Asan Institute for Life Sciences, Seoul, Korea.

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Gut 2003 52: 1779-1783
doi: 10.1136/gut.52.12.1779

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