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Prior Exposure to Lamivudine Increases Entecavir Resistance Risk in Chronic Hepatitis B Patients without Detectable Lamivudine Resistance

Jeong-Hoon Lee, Yuri Cho, Dong Hyeon Lee, Minjong Lee, Jeong-ju Yoo, Won-mook Choi, Young Youn Cho, Yun Bin Lee, Su Jong Yu, Jung-Hwan Yoon, Hyo-Suk Lee, Yoon Jun Kim

Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea

The efficacy of entecavir (ETV) treatment in chronic hepatitis B (CHB) patients who were exposed to lamivudine (LAM) but had no detectable LAM resistance (LAM-R) is not well evaluated. In this study, we aimed to evaluate whether the probability of developing genotypic resistance to ETV in LAM-exposed patients with or without LAM-R is comparable to that in antiviral-naive patients. This retrospective cohort study included 500 consecutive patients with CHB who started ETV monotherapy at a single tertiary hospital in Korea. The patients were divided into three groups: nucleos(t)ide analogue (NA)-naive patients (group 1, $n = 142$), patients who were previously exposed to LAM and had no currently or previously detected LAM-R (group 2, $n = 233$), and patients with LAM-R when starting ETV (group 3, $n = 125$). The overall median ETV treatment duration was 48.7 months. The probabilities of virologic breakthrough were significantly increased not only in group 3 (hazard ratio [HR] = 14.4, $P < 0.001$) but also in group 2 (HR = 5.0, $P < 0.001$) compared to group 1. Genotypic ETV resistance (ETV-R) developed more frequently in group 2 (HR = 13.0, $P = 0.013$) as well as group 3 (HR = 43.9, $P < 0.001$) than in group 1: the probabilities of developing ETV-R in groups 1, 2, and 3 were <1.0%, 8.0%, and 28.2%, respectively, at month 48. The results of this study indicate that ETV-R occurred more frequently in LAM-exposed patients, even though they had no detectable LAM-R, than in NA-naive patients. Therefore, LAM-exposed CHB patients, regardless of the presence or absence of LAM-R, should be monitored more cautiously for the development of ETV-R during ETV monotherapy.

Entecavir (ETV) is an orally administered guanosine analogue that has been approved for treatment of chronic hepatitis B (CHB). In antiviral-naive CHB patients, ETV has shown excellent antiviral efficacy with remarkably low probabilities of genotype resistance (1.2%) and virologic breakthrough (0.8%) for up to 5 years of treatment (1). In contrast to antiviral-naive patients, the rates of genotypic resistance to ETV are much higher in patients with lamivudine (LAM) resistance (LAM-R) (i.e., 51% 5-year cumulative probability) (1). Consequently, ETV is now recommended as one of the first-line therapeutic regimens for antiviral-naive patients with CHB but not for patients who had developed LAM-R (2–4).

The emergence of LAM-R variants has been relatively frequent even in antiviral-naive patients with CHB: approximately 20% of patients treated with LAM develop LAM-R at 1 year and 70% to 80% at 5 years of treatment (5–7). Although some selected cases that have succeeded in achieving treatment endpoints (i.e., hepatitis B e antigen [HBeAg] seroconversion and/or maintenance of complete virologic suppression) were able to discontinue LAM without developing LAM-R (8, 9), the rates of durable response after cessation of LAM have been low (10, 11). Unfortunately, the retreatment strategies for virologic relapse in those cases are still indefinite. Since LAM was the first approved nucleoside analogue for the treatment of hepatitis B virus (HBV) infection and had been widely used as a first-line therapy for CHB before the introduction of more-potent nucleos(t)ide analogues (NAs), including ETV (2), there are already a number of CHB patients who have been receiving LAM or who have experienced prior LAM treatment. Although ETV may not be recommended to those who developed LAM-R variants, the applicability of ETV in those patients who were exposed to LAM without previously or currently detected LAM-R variants remains unclear. In this study, therefore, we aimed to compare the risk of developing virologic breakthrough and genotypic resistance to ETV (ETV-R) in patients who were previously exposed to LAM without previous or current detectable LAM-R to the risk in either patients with LAM-R or NA-naive patients.

MATERIALS AND METHODS

Patients. A retrospective longitudinal cohort study was performed in consecutive patients treated with ETV monotherapy for CHB between 1 January 2007 and 5 November 2010 at a single tertiary hospital (Seoul National University Hospital, Seoul, Republic of Korea). Among the patients, LAM-R was defined as a virologic breakthrough associated with genotypic resistance to LAM (i.e., rtL180M, rtL180V, rtM204I, rtM204V, and rtM204S). Virologic breakthrough was defined as at least a 1 $\log_{10}$ increase in serum HBV DNA (IU/ml) compared to the on-treatment nadir (12). "Genotypic ETV-R" refers to the detection of HBV variants with amino acid substitutions that conferred attenuated susceptibility to ETV (i.e., rtT184G, rtT184S, rtT184A, rtT184I, rtT184L, rtS202G, rtS202I, and rtM250V) by a direct-sequencing method (13, 14). Patients with the following conditions were excluded from the study: coinfection with hepatitis C, hepatitis D, or human immunodeficiency virus; previous treat-

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Address correspondence to Yoon Jun Kim, yoonjun@snu.ac.kr.
J.-H.L. and Y.C. contributed equally to this article.

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TABLE 1 Baseline characteristics[a]

Variable	Value(s)			
	Group 1 ($n = 142$)	Group 2 ($n = 233$)	Group 3 ($n = 125$)	P
Mean age, yr	51.6 ± 12.1	54.3 ± 10.8	52.6 ± 10.8	0.060
Male sex, *n* (%)	92 (64.8)	145 (62.2)	85 (68.0)	0.280
Liver cirrhosis, *n* (%)	73 (51.4)	115 (49.4)	54 (43.2)	0.377
HBeAg positive, *n* (%)	54 (38.0)	83 (35.6)	62 (49.6)	0.063
Median HBV DNA, $\log_{10}$ IU/ml (range)	6.67 (2.07–9.81)	5.39 (3.70–9.81)	6.03 (4.32–9.81)	0.578
ALT > 2× ULN, *n* (%)	119 (83.8)	202 (86.7)	100 (80.0)	0.702
Median prior LAM treatment duration, mos (range)	NA	23.5 (2.0–93.5)	38.3 (6.0–121.1)	<0.001
Median ETV treatment duration, mos (range)	45.2 (21.9–80.7)	54.7 (19.1–84.3)	41.7 (10.4–83.1)	0.165

[a] Mean age data are given as means ± standard deviations. Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal; NA, not applicable.

ment for HBV with alpha interferon and NAs other than LAM, before and during ETV therapy; history of prior LAM-R without evidence of LAM-R at baseline; liver transplantation before and during the rescue therapy; a glomerular filtration rate of <50 ml/min, estimated by the Cockcroft-Gault equation; prior or current malignancy, including hepatocellular carcinoma (HCC); and concomitant serious medical illness such as hematological disease and heart failure. Subjects who did not undergo the clinical and laboratory assessments described below were also excluded. The patients were grouped into three groups: NA-naive patients (group 1), patients who experienced LAM with no currently or previously detected LAM-R (group 2), and patients who had LAM-R at baseline (group 3).

Follow-up and endpoints. All patients were followed every 2 to 3 months with routine biochemical liver function tests and assessment of hepatitis B e antigen (HBeAg) and antibody (anti-HBe Ab) and serum HBV DNA levels. Compliance with treatment was assessed by interview during every visit. Serum HBV DNA levels were quantified at baseline and at each follow-up visit, with a low detection limit of approximately 20 IU/ml (15). HBV DNA was obtained from serum samples, and the HBV polymerase gene was amplified using nested PCR. A BigDye Terminator version 3.1 ready-reaction cycle sequencing kit (Applied Biosystems, Foster City, CA) was used with an ABI Prism 3730 genetic analyzer (Perkin-Elmer, Foster City, CA) to perform the cycle sequencing reaction. Genotypic variants resulting in LAM-R and ETV-R were determined by direct-sequencing analysis of serum samples obtained either when virologic breakthrough occurred during ETV treatment or at the time of starting ETV treatment in LAM-exposed patients (groups 2 and 3).

The primary endpoints of this study were the emergence of genotypic ETV-R variants and virologic breakthrough. The secondary endpoints included the following: (i) biochemical response (normalization of serum alanine aminotransferase [ALT] level) and (ii) complete virologic suppression (undetectable serum hepatitis B virus [HBV] DNA by real-time PCR). The upper limit of normal ALT was defined as 30 IU/liter for men and 19 IU/liter for women (16).

The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital.

Statistical analysis. Survival analysis was performed using the Kaplan-Meier method, the life table method, the Cox regression model, or Firth-based penalized logistic regression analysis to estimate and compare the times to the emergence of genotypic resistance, biochemical response, complete virologic suppression, and virologic breakthrough. Univariate and multivariate analyses were performed with Firth-based penalized logistic regression analysis. Variables with $P < 0.05$ in univariate analysis or those with clinical implications were added to the multivariate logistic regression model to identify independent risk factors after adjusting for other variables. In multivariate Firth-based penalized logistic regression analysis, a stepwise method was used to select variables to be maintained in the final model; the conditional probabilities for stepwise entry and stepwise removal of a factor were 0.05 and 0.20, respectively. Statistical analysis was performed with SPSS version 17.0 (SPSS Institute, Inc., Chicago, IL), STATA version 10.0 (STATA Corp., College Station, TX), and SAS version 9.2 (SAS Institute Inc., Cary, NC). $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics. A total of 500 patients started ETV therapy during the study period. Their mean age was 53.1 ± 11.2 years; 332 patients (66.4%) were male. The median overall ETV treatment duration was 48.7 months (range, 10.4 to 84.3 months). A total of 142 patients were included in group 1 (NA-naive patients), 233 in group 2 (LAM-exposed patients without prior or current LAM-R), and 125 in group 3 (patients with current LAM-R at baseline). Table 1 shows the baseline characteristics of these three groups. A total of 115 patients (49.4%) in group 2 had discontinued LAM without LAM-R after achieving treatment endpoints (HBeAg seroconversion and/or maintenance of complete virologic suppression). Seventy-six patients who experienced virologic breakthrough without LAM-R during prior LAM treatment in group 2 were treated with 1.0 mg/day of ETV and the remaining 157 patients in group 2 with 0.5 mg/day of ETV. All the patients in group 3 were treated with 1.0 mg/day of ETV.

The median overall LAM treatment duration of group 3 (38.3 months; range, 6.0 to 121.1 months) was longer than that of group 2 (23.5 months; range, 2.0 to 93.5 months) ($P < 0.001$). Sixty-two patients (49.6%) had experienced virologic breakthrough during prior LAM treatment in group 3, which was significantly more than in group 2 (76 patients; 32.6%) ($P < 0.001$).

Nineteen patients (8.2%) in group 2 and 9 patients (7.2%) in group 3 showed primary nonresponse at 3 months of LAM treatment, and there was no significant difference between the groups. A total of 126 patients (54.1%) in group 2 showed partial response at 6 months of LAM treatment, while 45 patients (36%) in group 3 showed partial response at 6 months of LAM treatment ($P = 0.006$). A total of 104 patients (44.6%) in group 2 achieved complete virologic suppression during prior LAM treatment, and the median duration of continuing LAM after achieving complete virologic suppression was 18.3 months (range, 3.0 to 76.8 months). In group 3, only 19 patients (15.2%) had achieved complete virologic suppression during prior LAM treatment and the median duration of treatment was 12.1 months (range, 4.0 to 26.8 months).

The median treatment-free duration (i.e., interval between cessation of LAM and initiation of ETV) of group 2 was 6.0 months (range, 0 to 54 months) and that of group 3 was 5.4 months (range, 0 to 25.2 months) ($P = 0.543$).

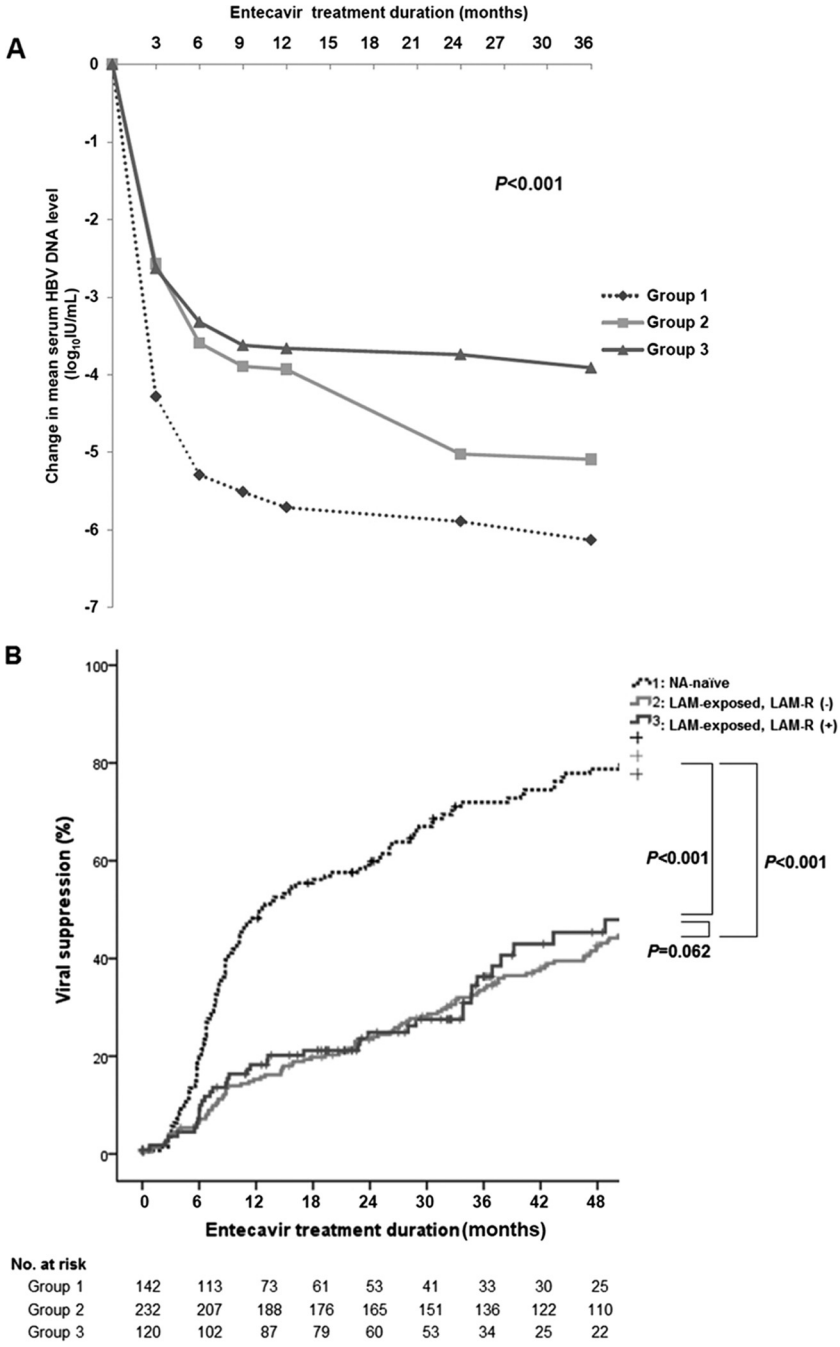

FIG 1 Efficacy with ETV therapy. (A) Changes in mean log values of the serum HBV DNA levels from baseline during ETV therapy. The decrease in HBV DNA was significantly less prominent in group 3 than in either group 1 or 2 at all the time points (all $P < 0.001$). An independent sample *t* test was used for the statistical analysis at each time point. (B) Cumulative incidence of complete virologic suppression (undetectable serum HBV DNA). Analysis was done by the Kaplan-Meier analysis method ($P < 0.001$ by log rank test). Group 3 showed significantly lower probability of complete virologic suppression than either group 1 or group 2.

Among the included patients, 35 patients were evaluated for HBV genotype and all of them had genotype C HBV. Actually, almost all of the patients with CHB in Korea (96 to 100%) have been known to have genotype C virus (17, 18).

Biochemical, virologic, and serological responses. During the ETV treatment period, except for six patients with an initially normal ALT level, the overall rate of biochemical response was 94.9% (355 of 494). The cumulative probabilities for biochemical responses at month 36 were 95% in group 1, 97% in group 2, and 94% in group 3. There was no significant difference among the three groups ($P = 0.830$).

Figure 1A and Table 2 show the mean changes in the HBV DNA level at each time point. The decrease in HBV DNA was significantly less prominent in group 3 than in either group 1 or 2

TABLE 2 Treatment responses during entecavir therapy

Outcome[a]	Group 1	Group 2	Group 3
Reduction of HBV DNA (log_{10} IU/ml), mean ± SD			
Mo 3	−4.28 ± 1.82	−2.57 ± 2.54	−2.63 ± 2.12
Mo 6	−5.29 ± 1.61	−3.59 ± 2.96	−3.32 ± 2.18
Mo 12	−5.71 ± 1.40	−3.93 ± 3.24	−3.66 ± 2.49
Mo 24	−5.89 ± 1.52	−5.02 ± 2.72	−3.74 ± 2.47
Mo 36	−6.13 ± 1.59	−5.09 ± 2.80	−3.91 ± 2.66
Complete virologic suppression, cumulative incidence[b] (no. of patients at risk)			
Mo 12	48% (73)	15% (188)	18% (87)
Mo 24	60% (53)	24% (165)	25% (60)
Mo 36	72% (33)	34% (136)	36% (34)
Virologic breakthrough, cumulative incidence[b] (no. of patients at risk)			
Mo 12	0% (136)	0% (222)	3% (111)
Mo 24	1% (129)	1% (209)	7% (98)
Mo 36	1% (101)	3% (179)	15% (80)
Mo 48	3% (59)	10% (42)	32% (52)
Genotypic resistance to ETV, cumulative incidence[b] (no. of patients at risk)			
Mo 12	0% (73)	0% (56)	3% (58)
Mo 24	0% (53)	1% (40)	11% (44)
Mo 36	0% (33)	2% (27)	16% (31)
Mo 48	0% (25)	8% (18)	28% (16)

[a] Abbreviations: HBV, hepatitis B virus; SD, standard deviation; HBeAg, hepatitis B e antigen.
[b] Data are given as cumulative incidence as a percentage of the number of patients at risk.

at all the time points (all $P < 0.001$). At month 36, group 1 showed significantly more profound HBV DNA suppression (−6.31 ± 1.59 log_{10} IU/ml) than group 2 (−5.09 ± 2.80 log_{10} IU/ml, $P < 0.001$) as well as group 3 (−3.91 ± 2.66 log_{10} IU/ml, $P < 0.001$) (Fig. 1A).

During the treatment period, complete virologic suppression with undetectable serum HBV DNA was achieved in 365 patients (73.0%). The cumulative probabilities of complete virologic suppression at month 36 were 72% in group 1, 34% in group 2, and 36% in group 3 (Table 2). There was no statistically significant difference between groups 2 and 3 (hazard ratio [HR], 0.530; 95% confidence interval [CI], 0.271 to 1.039; $P = 0.062$). Group 3 showed a significantly lower probability of complete virologic suppression than either group 1 (HR, 0.077; 95% CI, 0.039 to 0.151; $P < 0.001$) or group 2 (HR, 0.144; 95% CI, 0.086 to 0.241; $P < 0.001$) (Fig. 1B).

Among the 199 patients positive for HBeAg at the time of initiating ETV therapy, 105 patients (52.8%) achieved HBeAg seroconversion. The cumulative probabilities of the serological responses at month 36 were 86% in group 1, 83% in group 2, and 73% in group 3. There was no significant difference among the three groups ($P = 0.111$).

Virologic breakthrough and ETV-resistant genotypic variants. Virologic breakthrough occurred in 84 patients (16.8%) during the treatment period. The cumulative probabilities of virologic breakthrough at months 36 and 48, respectively, were as follows: 1% and 3% in group 1; 3% and 10% in group 2; and 15% and 32% in group 3 (Table 2). Both LAM-exposed groups (group 2 [HR, 5.007; 95% CI, 1.916 to 13.083; $P < 0.001$] and group 3 [HR, 14.368; 95% CI, 5.470 to 37.739; $P < 0.001$]) showed significantly more frequent virologic breakthrough than the NA-naive group (group 1). Group 3 showed significantly more frequent virologic breakthrough than group 2 (HR, 2.870; 95% CI, 1.719 to 4.789; $P < 0.001$) (Fig. 2A).

Genotypic ETV-R was documented in 50 (10%) patients during the treatment period, and all ETV-R variants were accompanied by LAM-R. Univariate and subsequent multivariate analyses showed that exposure to LAM was an independent predictor of genotypic resistance to ETV. Compared to group 1, both group 2 (HR, 13.039; 95% CI, 1.721 to 98.777; $P = 0.013$) and group 3 (HR, 43.885; 95% CI, 5.871 to 328.021; $P < 0.001$) showed a significantly higher risk of developing ETV-R, after adjustment for HBeAg status (Table 3). ETV-R was not found in group 1. The cumulative probabilities of an ETV-R variant at months 6, 12, 24, 36, and 48 were, respectively, as follows: <1%, <1%, 1%, 2%, and 8% in group 2 and 2%, 3%, 11%, 16%, and 28% in group 3 (Fig. 2B). Table 2 summarizes the efficacy and breakthrough data for each group.

Predictors for the development of genotypic resistance in group 2. Since no ETV-R variant occurred in group 1 (NA-naive patients) and international guidelines no longer recommend ETV monotherapy for patients in group 3 (patients who had LAM-R) (2, 3), we tried to determine the pre- and on-treatment predictors for the emergence of an ETV-R variant in group 2 patients (i.e., those who had experienced LAM but who had no prior or current LAM-R variant).

Among the 233 patients included in group 2, 19 patients developed an ETV-R variant and the shortest time to developing a variant was 33.3 months (range, 33.8 to 48 months). Thirteen of them had rtS202G variants, 5 had rtT184I, and 1 had both rtS202G and rtT184I variants. With ETV therapy, 215 patients (92.3%) included in group 2 showed primary responses, defined as a ≥1 log_{10} decrease in serum HBV DNA (IU/ml) within 3 months of antiviral therapy (16), and 188 patients (81.0%) achieved complete virologic suppression within 12 months. Univariate and multivariate analysis showed that complete virologic suppression within 12 months of ETV treatment was a sole independent predictor of developing an ETV-R variant (HR, 0.019; 95% CI, 0.004 to 0.087; $P < 0.001$) (Fig. 3); the other factors, including HBeAg status, baseline serum HBV DNA levels, and duration of prior LAM treatment, or interruption of NA, were not (Table 4). There was significant interaction between complete virologic suppression during prior LAM treatment and complete virologic suppression within 12 months of ETV treatment ($P < 0.001$).

Response to prior LAM treatment and ETV-R during ETV treatment. We performed subgroup analysis of patients who were exposed to prior LAM treatment (groups 2 and 3). Among the patients who had experienced virologic breakthrough during previous LAM treatment, ETV-R was less frequent in group 2 (16 of 76, 21.0%) than in group 3 (26 of 62, 43.5%) at month 48 ($P = 0.002$).

Among patients who had achieved complete virologic suppression for more than 1 year with prior LAM treatment, ETV-R occurred less frequently in group 2 (2 of 104, 1.9%) than in group 3 (2 of 19, 10.5%) at month 48 ($P < 0.001$).

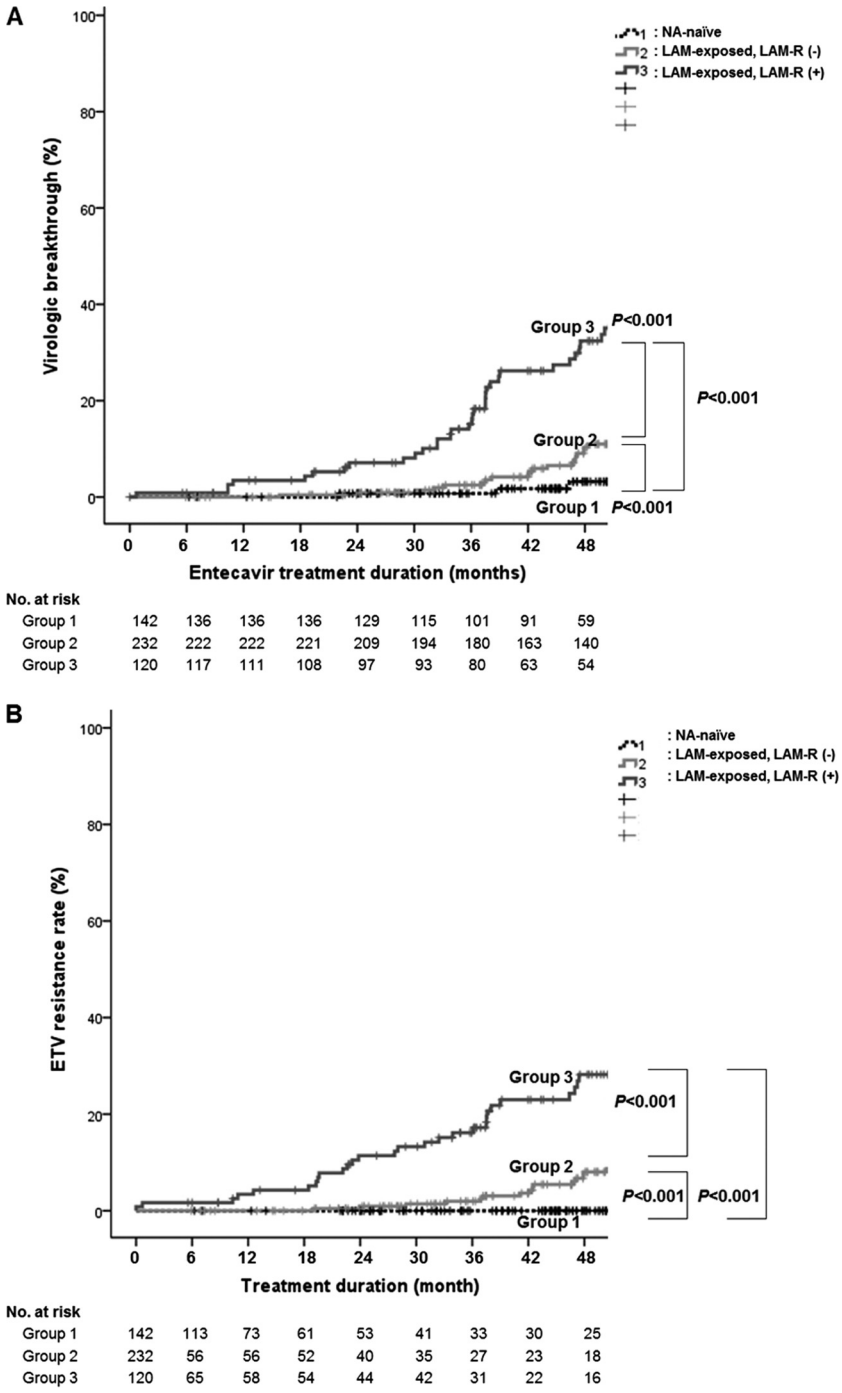

FIG 2 Breakthrough with ETV therapy. (A) Cumulative incidence of virologic breakthrough during ETV treatment using Kaplan-Meier curve (all $P < 0.001$ by log rank test). LAM-exposed groups (both group 2 and group 3) showed significantly more frequent virologic breakthrough than the NA-naive group (group 1). Group 3 showed significantly more frequent virologic breakthrough than group 2. (B) Cumulative incidence of emergence of ETV-R with ETV therapy determined using Kaplan-Meier curve (all $P < 0.001$ by log rank test). Compared to group 1, both group 2 and group 3 showed a significantly higher risk of developing ETV-R.

DISCUSSION

Antiviral efficacy of ETV in LAM-exposed patients without LAM-R had not yet been well evaluated. Therefore, there are no current guidelines regarding the use of antiviral agents in LAM-exposed patients who had no detectable LAM-R and who were frequently treated as antiviral-naive patients in clinical practice. Although a previous study showed that the cumulative probability of achieving virologic response during ETV therapy was slightly decreased in LAM-experienced patients without LAM-R compared to LAM-naive patients (19), there were no data comparing the virologic breakthrough and genotypic resistance characteristics of LAM-experienced patients and NA-naive patients.

TABLE 3 The independent risk factors for genotypic resistance to entecavir[a]

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Treatment group				
1	1.000 (reference)		1.000 (reference)	
2	12.519 (1.657–94.567)	0.014	13.039 (1.721–98.777)	0.013
3	45.500 (6.241–346.474)	<0.001	43.885 (5.871–328.021)	<0.001
HBV DNA, $\log_{10}$ IU/ml	1.203 (0.946–1.531)	0.132		
Presence of LC	0.665 (0.324–1.363)	0.265		
HBeAg positivity	2.625 (1.266–5.443)	0.009	2.545 (1.361–4.760)	0.003

[a] Abbreviations: HR, hazard ratio; CI, confidence interval, HBV, hepatitis B virus; LC, liver cirrhosis; HBeAg, hepatitis B e antigen.

This was the first study to examine the risk of developing ETV-R during ETV therapy in patients who were exposed to prior LAM treatment without detectable LAM-R (group 2) compared to either NA-naive patients (group 1) or patients with LAM-R (group 3). The results clearly demonstrated that ETV-R was significantly more frequent in LAM-exposed patients without prior or current LAM-R than in NA-naive patients but was slightly less frequent in patients having current LAM-R. LAM-exposed patients (both group 2 and group 3) showed a significantly lower reduction of the serum HBV DNA level and a higher risk of virologic breakthrough than LAM-naive patients. This study also indicated that developing ETV-R in LAM-exposed patients was significantly related to failure to achieve complete virologic suppression within 12 months of ETV treatment, which in turn was significantly related to achieving complete virologic suppression during prior LAM treatment.

In this study, ETV showed excellent antiviral efficacy in antiviral-naive patients (group 1) without developing any virologic breakthrough or a genotypic resistance variant for up to 84.3 months of median treatment duration, which is consistent with the findings of previous studies (20–22). In contrast, in LAM-experienced patients with current LAM-R (group 3), the probabilities of developing virologic breakthrough and ETV-R at month 48 were as high as 32% and 28%, respectively, in spite of the higher dose of ETV (1.0 mg/day); this is also comparable to the results of previous reports (23, 24). Surprisingly, LAM-exposed patients without prior or current LAM-R (group 2) also revealed relatively high probabilities of developing virologic breakthrough and ETV-R at month 48 (10% and 8%, respectively) during long-term ETV treatment. As shown in subgroup analysis, even among the patients without LAM-R who had complete virologic suppression for more than 1 year with LAM treatment, 1.9% of patients developed ETV-R after 2 years of ETV treatment. Thus, sustained complete virologic suppression with prior LAM treatment failed to ensure developing no ETV-R.

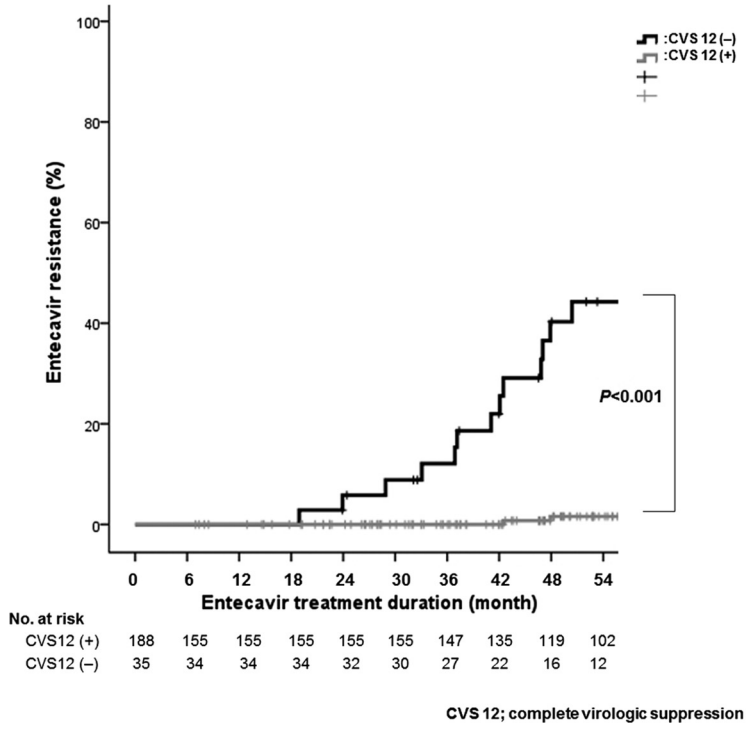

FIG 3 Impact of complete virologic suppression within 12 months (CVS 12) during ETV treatment on the development of ETV-R in group 2 patients. Patients with CVS 12 had a significantly lower probability of developing ETV-R ($P < 0.001$ by log rank test).

TABLE 4 The independent risk factors for genotypic resistance to entecavir in group 2

Variable[a]	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Initial HBV DNA, $\log_{10}$ IU/ml	1.281 (1.045–1.569)	0.017	0.981 (0.729–1.319)	0.897
Presence of LC	0.409 (0.151–1.104)	0.078		
HBeAg positivity	3.794 (1.449–9.931)	0.007	1.390 (0.384–5.029)	0.616
Duration of prior LAM treatment	0.991 (0.967–1.016)	0.485		
Duration of NA interruption	0.998 (0.993–1.004)	0.533		
Complete virologic suppression within 12 mos of ETV treatment	0.018 (0.005–0.068)	<0.001	0.019 (0.004–0.087)	<0.001

[a] Abbreviations: HBV, hepatitis B virus; LC, liver cirrhosis; HBeAg, hepatitis B e antigen; LAM, lamivudine; NA, nucleos(t)ide analogue; ETV, entecavir.

HBV may exist in the form of quasispecies in CHB patients (25), and an antiviral-resistant strain(s) sometimes cannot be detected in time due to the limitation of test sensitivity, especially when its proportion is less than 20% in the pool of viral quasispecies (13, 26). The sensitivity of direct sequencing is reported as 43.2% to 66.7% (27–29). Thus, theoretically, there might have been a small number of LAM-resistant strains, although tests failed to detect them at the time of initiating ETV treatment. Once LAM-resistant variants have been developed, they do not disappear but are archived and retained in the virus population (30). During ETV treatment, those inferior LAM-resistant strains would readily become predominant strains by positive selection by ETV, since they are less susceptible to ETV (31, 32). According to the "two-hit" theory, this positive selection of LAM-resistant strains by ETV acts as the first hit, and the second hit of the additional variant in these selected strains could easily occur to establish ETV-R (13). Surprisingly, ETV-R occurred even in a patient who was exposed to LAM for only 2 months. ETV-R developed in patients as long as 6 months after LAM cessation and occurred during more than 33.3 months of ETV treatment in group 2. These findings collectively indicated that a short duration of LAM treatment might be enough to select LAM-resistant strains which could survive even after discontinuation of LAM to affect the long-term efficacy of subsequent antiviral therapy.

This report provides another important clinical implication that prior antiviral treatment with low-potency drugs (i.e., LAM) might significantly affect the risk of developing strains resistant to the next antiviral treatment with ETV, a highly potent drug, even when there was no evidence of preexisting genotypic resistance to prior drugs. In this study, moreover, some patients who stopped low-potency NA treatment even after reaching treatment endpoints (HBeAg seroconversion and/or maintenance of complete virologic suppression) experienced resistance to subsequent ETV, which has great implications. This finding again highlights the importance of using not low-potency NAs but high-potency NAs (e.g., ETV and tenofovir disoproxil fumarate [TDF]) for the initial treatment of CHB. A previous Japanese study reported that LAM-to-ETV switching therapy may be feasible, since there was neither virologic breakthrough nor ETV-R during 2 years of ETV treatment and since LAM-to-ETV switching therapy was significantly superior to continuing LAM therapy in terms of virologic breakthrough (33). Compared to our study, that study had a shorter follow-up duration (median, 20 months versus 48.8 months), and approximately two-thirds of the ETV-R variants were detected after 2 years of ETV therapy in group 2 of our study. Therefore, the conclusion of the Japanese study should not be translated into an expectation of excellent long-term efficacy of switching therapy from low-potency drugs to ETV. Close monitoring of the serum DNA level and the genotypic ETV-R variant may be required during ETV treatment in LAM-experienced patients, even in those who never developed LAM-R. In addition, monotherapy with TDF, which is not cross-resistant to LAM, or combination therapy with nucleoside and nucleotide analogues (e.g., adefovir plus LAM, TDF plus LAM, TDF plus ETV, and TDF plus emtricitabine), rather than ETV monotherapy, could be useful for previously or currently LAM-exposed patients, especially those who failed to achieve complete virologic suppression either during prior LAM treatment or at 12 months of ETV treatment, since those regimens may suppress LAM-R variants more effectively. Our results suggest that the addition of a more potent drug that does not show cross-resistance (i.e., adding TDF to LAM or telbivudine or adding ETV to adefovir) may be more beneficial than switching therapy for those patients who had suboptimal treatment response with a low-genetic-barrier drug (e.g., LAM, adefovir, or telbivudine). It should also be questioned whether ETV could be one of the drugs of choice for LAM-exposed patients as well as for NA-naive patients, since only 2 months of prior exposure to LAM triggered LAM-resistant strains in our study. Considering that a number of patients have been exposed to LAM, further prospective studies on proper treatment strategy in LAM-exposed patients may be required to establish treatment guidelines of CHB. In particular, regarding safety and cost of therapy, further study is warranted to evaluate whether monotherapy with TDF, which shares no cross-resistance with LAM, might be a good treatment option in patients exposed to LAM.

In conclusion, the results of this study indicate that prior exposure to LAM treatment, even though the patients did not exhibit prior or current LAM-R, is significantly related to a high risk of the emergence of ETV-R during long-term ETV treatment. More attention should be paid to those LAM-experienced patients who are currently treated with ETV regardless of prior or current LAM-R, and it could be judicious to treat the high-risk patients, who were previously treated with LAM but failed to achieve complete virologic suppression, with combination therapy or with more-potent regimens rather than ETV monotherapy. In addition, the importance of therapy with highly potent antivirals (i.e., ETV and TDF) from the first-line therapy of CHB patients cannot be overemphasized.

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