Two cases of development of entecavir resistance during entecavir treatment for nucleoside-naïve chronic hepatitis B

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Abstract

Background Entecavir (ETV) is a potent nucleoside analogue against hepatitis B virus (HBV), and emergence of drug resistance is rare in nucleoside-naïve patients

because development of ETV resistance (ETVr) requires at least three amino acid substitutions in HBV reverse transcriptase. We observed two cases of genotypic ETVr with viral rebound and biochemical breakthrough during

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ETV treatment of nucleoside-naïve patients with chronic hepatitis B (CHB).

Results *Case 1:* A 44-year-old HBeAg-positive man received ETV 0.1 mg/day for 52 weeks and 0.5 mg/day for 96 weeks consecutively. HBV DNA was 10.0 log₁₀ copies/ml at baseline, declined to a nadir of 3.1 at week 100, and rebounded to 4.5 at week 124 and 6.7 at week 148. Alanine aminotransferase (ALT) level increased to 112 IU/l at week 148. Switching to a lamivudine (LVD)/adefovir-dipivoxil combination was effective in decreasing HBV DNA. *Case 2:* A 47-year-old HBeAg-positive man received ETV 0.5 mg/day for 188 weeks. HBV DNA was 8.2 log₁₀ copies/ml at baseline, declined to a nadir of 2.9 at week 124, and then rebounded to 4.7 at week 148 and 6.4 at week 160. ALT level increased to 72 IU/l at week 172. The ETVr-related substitution (S202G), along with LVD-resistance-related substitutions (L180M and M204V), was detected by sequence analysis at week 124 in both case 1 and case 2.

Conclusions ETVr emerged in two Japanese nucleoside-naïve CHB patients after prolonged therapy and incomplete suppression and in one patient after <0.5 mg of dosing. ETV patients with detectable HBV DNA or breakthrough after extended therapy should be evaluated for compliance to therapy and potential emergence of resistance.

Keywords Entecavir · HBV · Chronic hepatitis B · Drug resistance · Nucleoside-naïve

Introduction

Hepatitis B virus (HBV) infection is a serious health problem because of its high prevalence, estimated to be infecting more than 350 million people worldwide, and its potential for inducing chronic hepatitis, cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) [1, 2]. It has been demonstrated that the most potent risk factor for development of cirrhosis or HCC is serum HBV DNA level [3, 4], and it seems that suppressing serum HBV load is essential for improving the prognosis of HBV carriers. Treatment of chronic hepatitis B (CHB) has evolved markedly with the introduction of nucleoside-analogue antivirals, that is, lamivudine (LVD), adefovir-dipivoxil (ADV), entecavir (ETV), and telbivudine, to clinical practice. LVD, the first approved nucleoside analogue against HBV, was shown to be effective in suppressing HBV DNA replication, improving transaminase levels, improving liver histology, inducing hepatitis B e antigen (HBeAg) seroconversion, and suppressing hepatic insufficiency and hepatocarcinogenesis in CHB and compensated cirrhosis [5, 6]. However, the effectiveness of LVD is limited because of frequent development of drug resistance

followed by a hepatitis flare and, occasionally, hepatic failure [7, 8].

ETV, a novel anti-HBV nucleoside analogue, has more than 1,500 times greater potency than LVD in vitro [9]. In clinical trials, ETV administration demonstrated potent anti-HBV activity with a marked decline in serum HBV DNA level and a significant improvement in liver histology than LVD in nucleoside-naïve HBeAg-positive and -negative patients [10, 11]. In addition, emergence of ETV resistance (ETVr) or viral rebound was shown in these studies to be rare. From these results, recent treatment guidelines have recommended ETV as the first-line nucleoside analogue for nucleoside-naïve CHB patients, including those with cirrhosis [12, 13].

It has been reported that the development of ETVr in nucleoside-naïve patients is very rare, even after 4 years of therapy. Recently, however, rare cases of ETVr, which developed in nucleoside-naïve patients in clinical studies, have been reported [14–16]. We also observed two patients who developed ETVr-associated HBV reverse transcriptase (RT) substitutions, followed by *virologic rebound*, defined as an elevation in serum HBV DNA of more than 1 log₁₀ copy/ml from nadir, and biochemical breakthrough in long-term ETV treatment of nucleoside-naïve CHB patients. In this article, we report these two cases in detail.

Case report

Case 1

A 44-year-old Japanese male CHB patient was positive for hepatitis B surface antigen (HBsAg), HBeAg, serum HBV DNA, and had HBV genotype C, had elevated alanine aminotransferase (ALT) levels, and had no history of nucleoside analogue treatment. The patient had a history of acute appendicitis at age 30, ureteral stone at age 35, and hyperlipidemia at age 43. He had a habit of drinking alcohol (700 ml) daily but did not smoke. At age 27, he was diagnosed for the first time by health screening as an asymptomatic HBV carrier in the immune-tolerant phase, defined by HBsAg positivity and normal liver enzymes, and he was followed up regularly elsewhere with blood tests for liver enzymes. He was found to have ALT elevation. He was referred to our hospital at age 44 and was diagnosed with CHB. Serum HBV DNA level determined by Roche AmplicorTM Monitor PCR assay (lower limit of detection is 2.6 log₁₀ copies/ml = 400 copies/ml; Roche Diagnostics K.K., Tokyo, Japan) [17] was 10.0 log₁₀ copies/ml and serum ALT level was 199 IU/l. Histologic diagnosis by percutaneous liver biopsy at baseline revealed chronic hepatitis with mild fibrosis and mild activity (CH F1/A1, according to the New Inuyama Classification) [18].

Table 1 Baseline characteristics

	Normal range	Unit	Case 1	Case 2
Age	–	–	44 years	47 years
Gender	–	–	Male	Male
T. Bil	0.2–1.0	mg/dl	0.8	0.5
AST	10–40	IU/l	113	48
ALT	5–40	IU/l	199	74
ALP	115–359	IU/l	268	216
BUN	6–20	mg/dl	9.5	15.9
CREA	0.61–1.04	mg/dl	0.95	0.83
ALB	4.0–5.0	g/dl	4.2	4.3
WBC	3500–8500	/ μ l	6,800	5,650
Hb	13.5–17.0	g/dl	15.7	14.8
PLT	13.1–36.2	10^4 / μ l	18.9	14.5
Prothrombin time	10–13	second	10.8	11.2
INR	–	–	1.0	0.9
HBsAg (CLIA)	0–0.05	IU/ml	>100 (positive)	>100 (positive)
anti-HBs (CLIA)	0–10	IU/ml	0 (negative)	0 (negative)
HBeAg (CLIA)	0–1		120 (positive)	190 (positive)
anti-HBe (CLIA)	0–50	%	<35 (negative)	0 (negative)
HBV DNA (PCR)	<2.6	log ₁₀ copies/ml	10.0	8.2
HBV genotype			Genotype C	Genotype C
YMDD (sequencing)			YMDD+	YMDD+
			YVDD–	YVDD–
			YIDD–	YIDD–
Liver histology ^a			CH F1/A1	CH F2/A2

^a Diagnosed according to New Inuyama classification. T. Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkalinephosphatase, BUN: blood urea nitrogen, CREA: serum creatinine, ALB: serum albumin, WBC: white blood cell count, Hb: hemoglobin, PLT: platelet count, INR: international normalized ratio, HBsAg: hepatitis B surface antigen, CLIA: chemiluminescent immunoassay, anti-HBs: antibody to hepatitis B surface antigen, HBeAg: hepatitis B e antigen, anti-HBe: antibody to hepatitis B e antigen, HBV: hepatitis B virus, PCR: polymerase chain reaction, YMDD: tyrosine-methionine-aspartate-aspartate motif, YVDD: tyrosine-valine-aspartate-aspartate motif, YIDD: tyrosine-isoleucine-aspartate-aspartate motif, CH F1/A1: chronic hepatitis with mild fibrosis and mild activity, CH F2/A2: chronic hepatitis with moderate fibrosis and moderate activity

Other baseline characteristics are shown in Table 1. He was enrolled in a phase II clinical trial of ETV and was randomized into 0.1- and 0.5-mg dosage groups. The trial was conducted in Japan in compliance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and Articles/Notifications of the Ministry of Health, Labor and Welfare (H. Kobashi et al., J Gastroenterol Hepatol, in press). He was assigned into the 0.1-mg dosage group and administered ETV at daily dose of 0.1 mg for an initial 52 weeks. Subsequently, he was administered ETV continuously at a daily dose of 0.5 mg for the following 96 weeks. The serum HBV DNA level, which was 10.0 log₁₀ copies/ml at baseline, declined to a nadir of 3.1 log₁₀ copies/ml at week 88 of ETV treatment. Thereafter, HBV DNA level increased from 4.5 log₁₀ copies/ml at week 124 to 6.3 log₁₀ copies/ml at week 140 and 6.7 log₁₀ copies/ml at week 148. ALT levels increased

from 28 IU/l at week 144 to 112 IU/l at week 148. The patient discontinued ETV therapy at week 148, and then received a combination therapy of 100 mg of LVD and 10 mg of ADV per day. Afterwards, HBV DNA level dropped to below 2.6 log₁₀ copies/ml and ALT level was normalized after 28 weeks of LVD/ADV dosing (Fig. 1).

HBV DNA sequence analysis was performed using PCR-amplified HBV DNA from preserved serum samples at baseline and at every 24 weeks via HBV DNA polymerase sequence assay (developed at SRL, Inc., Tokyo, Japan). Although sequence analysis of the baseline isolate revealed no substitution in the RT domain of the HBV DNA polymerase gene, analysis of the isolates collected over time revealed the M204I substitution at week 100 and the L180M, S202G, and M204V substitutions at weeks 124 and 144, respectively (Table 2). In addition, a polymorphic residue N238 was found as mixed N238 N/H at week 100 and thereafter. The

Fig. 1 Clinical course of case 1, a 44-year-old man with nucleoside-naïve CHB. ETV treatment reduced ALT levels to below the upper normal limit at week 12 and reduced HBV DNA load to a nadir of 3.1 log₁₀ copies/ml at week 88. However, HBV DNA re-elevated to 4.5 log₁₀ copies/ml at week 124 (virologic breakthrough) and 6.3 log₁₀ copies/ml at week 140, as well as ALT level re-elevated at week 148 (biochemical breakthrough). Sequence analysis of the

HBV DNA polymerase gene using serum sample obtained at weeks 124 and 144 revealed the emergence of L180M, M204V (related to LVD resistance), and S202G (related to ETVr) substitutions. SNP-PCR assay revealed that LVDr M204V and ETVr S202G substitutions were detected first at week 124 (98%) and increased at week 148 (>99%). Switching from ETV to LVD/ADV combination treatment at week 148 was successful in reducing HBV DNA load and ALT again

Table 2 Population sequence analysis of isolates from case 1 on ETV therapy

Week	Reverse transcriptase position				
	180	202	204	223	238
0	L	S	M	S/A	N
24	L	S	M	S/A	N
100	L	S	M/I	S/A	N/H
124	M	G	V	S	N/H
144	M	G	V	S	N/H

polymorphic residue S223, which was mixed as S/A at baseline, was found to be only S at weeks 124 and 144.

In addition, preserved serum samples from this patient at baseline and at every 24 weeks were analyzed by an ultrasensitive, single-nucleotide-polymorphism (SNP)-PCR assay, using a method similar to Punia et al. [19] for identification of resistance substitutions, as well as analyzing the sequence of individual clones to determine the genetic linkage of substitutions. SNP-PCR analysis was performed for the two LVD-resistance (LVDr) substitutions, M204V (codon GTG) and M204I (codons ATA and ATT), and the ETVr substitution S202G. Both wild-type and positive control plasmids containing the correct sequence were used at various concentrations to establish the background level as well as the level of detection for each substitution. For clonal analysis, the amplified RT

gene from the patient's HBV was cloned into plasmids, as well as 22 to 24 individual clones were selected and sequenced, to determine the genetic linkage of the different substitutions observed.

SNP-PCR analysis for ultrasensitive detection of the resistance substitutions revealed that the LVDr M204V(GTG) and ETVr S202G(GGT) substitutions were not detected (<0.1%) at baseline, week 24, or week 100. The M204I substitution (codon ATA) was detected at low levels at week 24 (0.4%), increased levels at week 100 (6.6%), and was present but at reduced levels at weeks 124 and 148 (0.4% at both time points). The LVDr M204V and ETVr S202G substitutions were detected first at week 124 (98%) and increased levels at week 148 (>99%). The levels of M204I(ATA) were lower at weeks 124 and 144, likely as a result of the dominant M204V/S202G virus (Table 3). Samples at weeks 48 and 76 could not be analyzed conclusively because of low yields of HBV DNA from serum samples.

Clonal analysis revealed that position 223 was a mixture of S and A residues at baseline, the LVDr substitutions L180M and M204V, as well as the ETVr substitution S202G, all emerged simultaneously and were linked in the same virus isolate clones at week 124, isolates that also contained S at position 223. These substitutions did not appear to arise from the LVDr isolates with M204I because the M204I substitution emerged in an isolate with substitution S223A.

Table 3 SNP-PCR analysis of case 1 isolates

Week	M204V		S202G		M204I (ATA)		M204I (ATT)	
	Mut/WT	Ave (%)	Mut/WT	Ave (%)	Mut/WT	Ave (%)	Mut/WT	Ave (%)
0	1/5,424	0.018	1/15,453	0.0065	1/4,199	0.024	1/37,940	0.0026
24	1/5,655	0.018	1/19,000	0.0052	<u>1/243</u>	<u>0.410</u>	1/46,518	0.0021
100	1/3,846	0.026	1/16,038	0.0062	<u>1/14</u>	<u>6.569</u>	1/50,456	0.0020
124	<u>48/1</u>	<u>97.973</u>	<u>59/1</u>	<u>98.327</u>	<u>1/265</u>	<u>0.377</u>	1/12,879	0.0078
144	<u>706/1</u>	<u>99.859</u>	<u>1,250/1</u>	<u>99.920</u>	<u>1/237</u>	<u>0.421</u>	1/10,573	0.0095

Cells with bold and underlined font are considered positive ($>1/1000$ or $>0.1\%$ mutant/wild-type)

Mut/WT, mutant/wild type, mean ($N = 3$)

Ave %, average % in total HBV DNA

Case 2

A 47-year-old Japanese male CHB patient was positive for HBsAg, HBeAg, serum HBV DNA, and had HBV genotype C, had elevated ALT levels, and had no history of nucleoside analogue treatment. At age 33, he was diagnosed for the first time as an asymptomatic HBV carrier in the immune-tolerant phase because of positive HBsAg and normal liver enzymes. At age 44, he was found to have ALT elevation, referred to our hospital, and diagnosed with CHB. Histologic diagnosis by percutaneous liver biopsy revealed chronic hepatitis with moderate fibrosis and moderate activity (CH F2/A2 according to the New Inuyama Classification). He was treated with ursodeoxycholic acid at a daily dose of 600 mg orally and glycyrrhizin preparation (stronger Neo-Minophagen CTM) 40 ml i.v. thrice per week for 3 months. However, liver enzymes did not normalize. Interferon- $\alpha 2b$ administration, three mega units i.m. thrice per week, was started at age 45 and continued for 24 weeks. Although HBV DNA level was reduced transiently to below $3.7 \log_{10}$ copies/ml at the end of therapy, it rose 9 months after cessation of interferon therapy to $8.2 \log_{10}$ copies/ml and ALT level increased to 483 IU/l. At age 47, the patient was started on ETV treatment as the subject enrolled in the ETV clinical trial (ETV-053) in Japan at a daily oral dose of 0.5 mg and continued for 188 weeks. A liver biopsy performed 1 month before starting the ETV treatment showed chronic hepatitis with moderate fibrosis and moderate activity (CH F2/A2, according to the New Inuyama Classification). The baseline serum HBV DNA level was $8.2 \log_{10}$ copies/ml, ALT level was 74 IU/l, and other baseline characteristics were as shown in Table 1. The serum HBV DNA level declined to $3.2 \log_{10}$ copies/ml and ALT level decreased to below the upper limit of normal at week 32. Liver histology improved to mild-to-moderate fibrosis and mild activity (CH F1-2/A1) at week 48 and chronic hepatitis with mild-to-moderate fibrosis and mild activity (CH F1/

A1) at week 148. HBV DNA level was suppressed to a nadir of $2.9 \log_{10}$ (794) copies/ml at week 124 and rose again to $4.7 \log_{10}$ copies/ml at week 148, $5.4 \log_{10}$ copies/ml at week 152, and $6.4 \log_{10}$ copies/ml at week 160 and $7.0 \log_{10}$ copies/ml at week 164. ALT level rose to 79 IU/l at week 172 and remained between 40 and 50 IU/l thereafter. ETV at 0.5 mg/day was continued until this time (Fig. 2).

HBV DNA sequence analysis revealed no resistance substitutions in the patient's baseline virus. However, the LVDr-related substitutions L180M and M204V, as well as ETVr-related substitution S202G, were detected at week 124, as a mixed population with wild type, and at week 148, as a pure population (Table 4). In addition, the patient displayed evidence of several polymorphic substitutions at baseline, indicating a mixed quasi-species, which became enriched for those with the resistant virus over time.

SNP-PCR analysis was used to determine the first appearance of the resistance substitutions, using the same method as for case 1. There was no antiviral resistance detected at baseline ($<0.1\%$). The M204V (0.65%) and S202G substitutions were detected first at week 24 but not again until week 124. At weeks 124 and 148, the resistant isolate had become enriched to 43% (M204V) and 98% (M204V), respectively (Table 5).

Clonal analysis was performed to determine the genetic linkage of the various substitutions observed, using the same method as for case 1. The amplified RT gene from the patient's virus was cloned into plasmids, and 24 to 27 individual clones were selected and sequenced. From the clonal analysis, it can be seen that there are three positions that contain mixtures at baseline; position 55 is a mixture of H and R residues, position 221 is a mixture of Y and F residues, and position 269 is a mixture of I and L residues. The substitutions L180M and M204V, as well as the ETVr-related substitution S202G, all emerge simultaneously and in an isolate with H at position 55, Y at position 221, and I at position 269.

Fig. 2 Clinical course of case 2, a 47-year-old man with nucleoside-naïve CHB. ETV treatment reduced ALT level to below the upper normal limit at week 30 and reduced serum HBV DNA level to a nadir of 2.9 log₁₀ copies/ml at week 124. However, HBV DNA level re-elevated to 4.7 log₁₀ copies/ml (virologic breakthrough) at week 148 and 7.0 log₁₀ copies/ml at week 168, as well as ALT level re-

elevated to 79 IU/l at week 172. Sequence analysis of the HBV DNA polymerase gene using serum sample obtained at weeks 124 and 148 revealed the emergence of L180M, M204V (related to LVD resistance), and S202G (related to ETVr) substitutions. SNP-PCR assay revealed that the resistant isolate was enriched to 43 (M204V) and 98% (M204V), respectively

Table 4 Population sequence analysis of isolates from case 2 on ETV therapy

Week	RT position								
	55	76	180	191	195	202	204	221	269
0	H/R	S	L	V	F	S	M	Y/F	I/L
24	H/R	S	L	V	F	S	M	Y/F	I/L
52	H/R	S	L	V	F	S	M	Y/F	I/L
100	H	S	L	V	F	S	M	Y/F	I/L
124	H	S/T	L/M	V/I	F/S	S/G	M/V	Y	I/L
148	H	S	M	V	F	G	V	Y	I

Table 5 SNP-PCR analysis of case 2 isolates

Week	S202G ^a	M204V (GTG, %)	M204I (ATA, %)	M204I (ATT, %)
0	Negative	0.016	0.020	0.0065
24	Positive	0.65	0.029	0.018
52	Negative	0.021	0.020	0.018
100	Negative	0.020	0.021	0.010
124	Positive	43	0.33	0.010
148	Positive	98	2.9	0.016

^a S202G PCR was non-quantitative. A positive indicates 4-fold, 5085-fold, and 10475-fold the wild-type background for weeks 24, 124, and 148, respectively. The baseline isolate gave 1.1-fold the wild-type background

Discussion

The most important limitation of long-term nucleoside analogue treatment for CHB is the emergence of drug-resistant mutant HBV followed by viral breakthrough and hepatitis flare [12]. The most common mutation associated with LVDr involves substitution of methionine in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV DNA polymerase gene RT domain with valine or isoleucine (M204V/I), with or without a leucine-to-methionine substitution in an upstream region (rtL180M) [20]. It was reported that LVDr was detected at a rate of 14 to 32% after 1 year and 60 to 70% after 5 years of LVD treatment [12]. The substitutions conferring resistance to ADV are asparagine to threonine (N236T) and alanine to valine or threonine (A181V/T) [21], and the cumulative probability of ADV resistance with elevation of HBV DNA level has been reported to be 20% at 5 years in HBeAg-negative patients [22] and as high as 42% in HBeAg-positive patients [23].

In the case of ETV, it has been reported that resistance to the drug requires at least one of three substitutions in HBV RT, that is, rtT184, rtS202, and rtM250, as well as LVDr-related substitutions rtL180M and M204V [24]. Phenotypic analyses of samples associated with virologic breakthrough confirmed that ETV susceptibility correlates

with the spectrum of these additional substitutions conferring genotypic resistance and the increased level of circulating HBV DNA [25].

There is a high genetic barrier to resistance to ETV in nucleoside-naïve patients and <1% experience virologic breakthrough with ETVr through 4 years of therapy [15]. However, in LVD-refractory patients, the barrier to resistance is lower because the suppression of HBV replication is not as great and these patients mostly harbor virus with two of the three substitutions required for high-level ETVr [26]. This results in virologic breakthrough with ETVr in LVD-refractory patients at 1% in the first year but increasing to 39.5% after 4 years of therapy [15].

In this article, we report two cases with confirmed genotypic resistance to ETV, virologic rebound, and biochemical breakthrough during long-term ETV treatment for nucleoside-naïve CHB patients. In the first case, the patient received a lower dose of ETV (0.1 mg daily for 52 weeks) than is currently recommended in product labeling. It was shown that LVD–ADV combination therapy was apparently effective for the ETV-resistant strain, presumably because there is no cross-resistance between ETV and ADV [26, 27].

SNP-PCR analysis for resistance substitutions revealed that the LVD_r M204V(GTG) and the ETV_r S202G(GGT) substitutions were negative at baseline and emerged simultaneously at week 124 in both patients. The three resistance substitutions L180M, M204V, and S202G appeared to be genetically linked and did not arise in a stepwise manner in nucleoside-naïve patients, as has been described previously.

ETV displays several properties for consideration as the first-line nucleoside analogue because of its potent antiviral activity and a lower frequency of drug resistance than LVD, ADV, or telbivudine [13]. Although ETV is effective in LVD-refractory patients, the potency is reduced somewhat and the barrier to resistance is diminished by the presence of rtM204I/V and rtL180M substitutions. The fact that ETV_r may develop in nucleoside-naïve patients, even if the chance is small, is noteworthy. In case 1, the patient received a lower dose of ETV (0.1 mg daily), which may be a possible contributing factor to resistance. The common features of our two cases were: HBeAg-positivity, male, high viral load, slow decrease of HBV DNA, and persistently detectable HBV DNA by PCR (>2.6 log₁₀ copies/ml) during the treatment course; however, these characteristics were also present in some other patients who did not develop ETV_r. Patient compliance with prescribed therapy also should be assessed in such situations. It is believed that some subpopulations of HBV that proliferate very actively and are not completely suppressed by ETV may have a chance of being selected for the resistance substitutions required for ETV virologic failure. Accordingly, such cases

with persistent HBV DNA after extended ETV treatment should be evaluated for emergence of drug-resistance substitutions with close monitoring of HBV DNA level, even in nucleoside-naïve patients.

The rate at which resistant mutants are selected is related to pretreatment serum HBV DNA level, rapidity of viral suppression, duration of treatment, and prior exposure to nucleoside analogue therapies [12]. For the management of the emergence of drug resistance in nucleoside analogue treatment of CHB and cirrhosis, prediction and early detection of drug-resistant HBV by close monitoring of serum viral load and genotypic resistance are necessary. Keeffe et al. [28] reported the “road-map concept,” that is, on-treatment monitoring strategy, for selection of nucleoside analogues by early prediction of efficacy and resistance using assessment of viral responses at weeks 12 and 24. Although this “concept” seems imperfect because the probability of emergence of resistance to particular nucleoside analogue is not taken into account, a similar strategy for management of drug resistance by close monitoring of viral load and confirming genotypic resistance with consideration of the property of each nucleoside analogue should be established for antiviral treatment of CHB using nucleoside analogues.

Conclusions

We reported two cases of emergence of genotypic resistance to ETV accompanied by virologic breakthrough in nucleoside-naïve CHB patients. One patient was treated with a lower than recommended dose of ETV. Although development of ETV_r-related gene mutations is rare in nucleoside-naïve patients, the patients with a slow decline of HBV DNA or persistent HBV DNA (>2.6 log₁₀ copies/ml) after ETV administration should be evaluated carefully for the potential emergence of ETV_r.

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