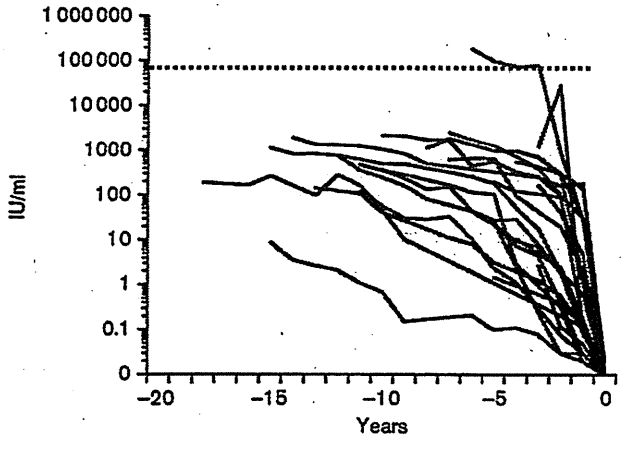
Serial changes in hepatitis B surface antigen, alanine aminotransferase level, and platelets before and after hepatitis B surface antigen seroclearance

The levels of HBsAg, ALT, and platelets in the patients with HBsAg seroclearance were evaluated annually (Figs 1, 2a and b). The average follow-up period after HBsAg seroclearance was 6.5 ± 5.7 years. HBsAg reappeared in three patients at 8, 10, and 11 years after HBsAg seroclearance. Two patients showed HBsAg seroclearance again within 2 and 3 years of the reappearance of HBsAg, but one patient could not be followed up after the reappearance of HBsAg. All 25 patients were negative for HBeAg and HBV DNA and had normal ALT levels. In addition, ALT levels did not fluctuate in these patients after HBsAg seroclearance. Platelets in the patients with HBsAg seroclearance did not show any difference between entry (180 000 ± 44 000/μl) and the end (179 000 ± 55 000/μl) of the follow-up period (paired *t*-test), although three of eight patients with less than 150 000/μl of platelets at HBsAg seroclearance showed an increase in platelets after HBsAg seroclearance.

Factors associated with the future seroclearance of hepatitis B surface antigen

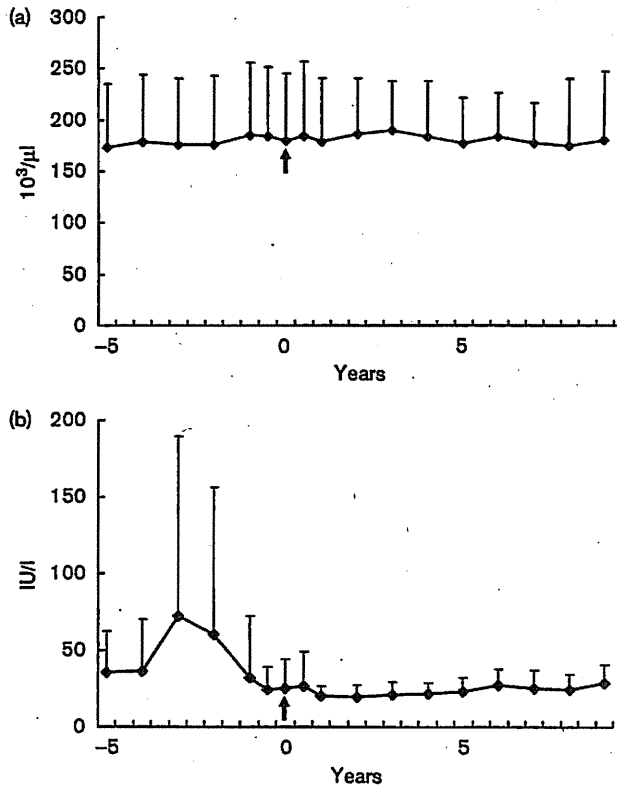
Next, we used the Cox proportional hazards model to investigate the factors associated with the future seroclearance of HBsAg (Table 2). Univariate analysis revealed that age [compared with younger patients: odds ratio (OR) = 1.06, 95% confidence interval (CI): 1.03–1.10], HBeAg negativity (compared with HBeAg positivity: OR = 7.88, 95% CI: 2.34–26.6), HBV DNA level (compared with patients with a low HBV DNA level: OR = 0.58, 95% CI: 0.46–0.75), and HBsAg titer (compared with patients with a low HBsAg level: OR = 0.39, 95% CI: 0.29–0.53) at baseline were predictive factors for HBsAg seroclearance. Multivariate analysis revealed that HBsAg titer (compared with patients with a low HBsAg level: OR = 0.45, 95% CI: 0.29–0.70) at baseline was a predictive factor for HBsAg seroclearance. Thus, these analyses revealed that a low HBsAg level was the most important factor associated with the future seroclearance of HBsAg. We performed the multivariate analysis again, changing the threshold of HBsAg from 1.0 to 5.0 log IU/ml in 1.0 log increments. We determined the threshold when the value of probability was the smallest. As a result, the threshold of HBsAg levels was determined to be 3.0 log IU/ml. The hazard ratio (95% CI) and the *P*-value were 5.32 (1.77–15.9) and 0.003, respectively. When the HBV carriers were divided into two groups, over 1000 IU/ml of HBsAg or not, HBsAg seroclearance occurred in HBV carriers with less than 1000 IU/ml of HBsAg at a higher rate and with a significant difference (log-rank test, $P < 0.01$; Fig. 3).

Fig. 1



Serial changes in hepatitis B surface antigen (HBsAg) levels in patients with HBsAg seroclearance. The average level of HBsAg at entry among all the patients was 16 994 IU/ml (dotted line), although the levels of most patients with HBsAg seroclearance were below the average. Twenty-five patients with HBsAg seroclearance showed a decline in the HBsAg level several years before HBsAg seroclearance.

Fig. 2



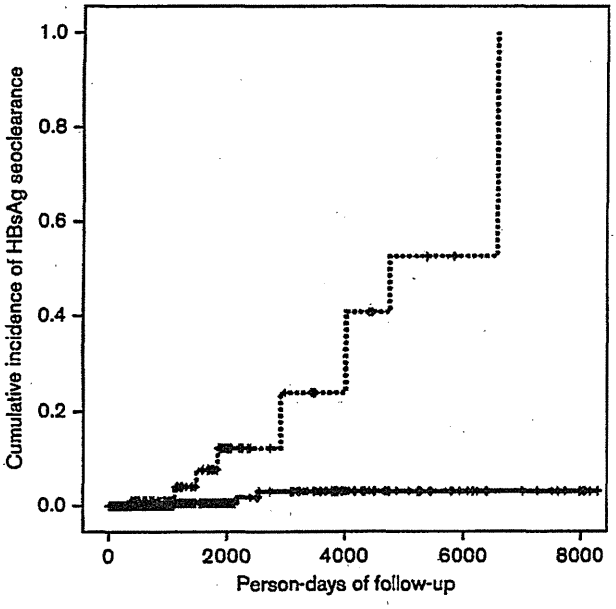
Serial changes in (a) the number of platelets and (b) alanine aminotransferase (ALT) levels before and after hepatitis B surface antigen (HBsAg) seroclearance. Platelets showed no change before and after HBsAg seroclearance. ALT levels fluctuated before HBsAg seroclearance, but did not fluctuate afterward. The arrows indicate the year of HBsAg seroclearance.

Table 2 Cox regression analysis for the predictive factors for hepatitis B surface antigen seroclearance

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age	1.06 (1.03–1.10)	0.001	1.03 (0.98–1.07)	NS
Sex *male*	2.35 (0.97–5.68)	NS		
HBeAg negative	7.88 (2.34–26.6)	0.001	2.62 (0.62–11.0)	NS
HBV-DNA	0.58 (0.46–0.75)	<0.001	0.94 (0.66–1.35)	NS
ALT	1.00 (1.00–1.00)	NS		
Platelet	1.00 (0.99–1.00)	NS		
Genotype A	1.92 (0.92–4.00)	NS		
Past use of interferon	1.47 (0.34–6.27)	NS		
HBsAg (log)	0.39 (0.29–0.53)	<0.001	0.45 (0.29–0.70)	<0.001

ALT, alanine aminotransferase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NS, not significant.

Fig. 3



Cumulative occurrence of seroclearance of hepatitis B surface antigen (HBsAg) based on the HBsAg levels over 1000 IU/ml of HBsAg or not by the Kaplan–Meier method. A significant difference was observed by the log-rank test ($P<0.01$). The dotted line indicates the group with a low HBsAg level.

Discussion

HBsAg is the fundamental diagnostic marker of HBV infection. HBsAg is a component of the Dane particle, which contains the viral genome, and of subviral particles. But the mechanisms that regulate the production of HBsAg, particularly the subviral particles, are largely unclear [13]. HBsAg seroclearance is a clinical goal for HBV carriers, because, after HBsAg seroclearance, clinical outcomes of HBV carriers are favorable and the incidence of liver failure and HCC in patients with HBsAg seroclearance is much lower than that in HBsAg-positive patients [2,3,14,15]. Individuals who become HBsAg negative can be considered to have resolved CHB. If we can predict the seroclearance of HBsAg among HBV carriers, this can help physicians manage CHB patients.

Spontaneous HBsAg seroclearance has been well documented and predictive factors for the seroclearance of HBsAg were also clarified. Liu *et al.* [4] reported that the level of HBV DNA was an important factor and Kim *et al.* [16] reported that old age and a normal ALT level were factors associated with HBsAg seroclearance. Tai *et al.* [7] reported that male sex, HBeAg negativity, older age, low maximal ALT level, and hepatic steatosis were factors associated with HBsAg seroclearance and that the estimated HBsAg seroclearance rates increased with age and reached a plateau after the age of 50 years. Our study clarified that the level of HBsAg, not the HBV DNA level, is a predictive factor for the clearance of HBsAg. In the previous reports [17,18] and ours [10], the level of HBV DNA showed a good correlation with the level of HBsAg, but there were quite a few outliers. In fact, nine (36.0%) of 25 patients with HBsAg seroclearance showed a high HBV DNA level (over 5.0 log copies/ml) at baseline. In contrast, only three (12.0%) of 25 patients showed a high HBsAg level (over 4.0 $\log_{10}$ IU/ml) at baseline. As far as HBsAg seroclearance is concerned, the HBsAg level is the most reliable predicting factor for it, and future analysis for the outliers between HBsAg and HBV DNA levels might provide a clue toward clarification of the mechanism of HBsAg seroclearance. In this study, the age at HBsAg clearance varied from 27 to 67 years and was scattered and showed no particular trend. This difference was attributed to the difference in the method of HBsAg quantification. Our study involved quantification of the HBsAg level using an assay with higher sensitivity (the cutoff level was 0.03 IU/ml) than traditional and qualitative analysis of HBsAg (the cutoff level was almost 1.0 IU/ml). In addition, most studies had not evaluated the quantitative HBsAg level as a prognostic factor for HBV carriers. In any case, to evaluate the HBsAg seroclearance precisely, HBsAg should be evaluated using a quantitative method.

Nine patients with HBsAg seroclearance showed ALT elevation within 5 years before HBsAg seroclearance. Five of nine patients showed a high HBV DNA level during ALT elevation, which might indicate a severe immune reaction for HBV. These results suggested that there exist two types of progress reaching to HBsAg seroclearance: one with a flare in the ALT level as a severe immune reaction for HBV and the other without it. We should clarify the difference between these two types in the future.

IFN therapy has antiviral and immunomodulatory effects and has been used in the treatment of CHB. In meta-analysis, IFN therapy could induce HBsAg seroclearance at the end of follow-up for at least 3 years [19,20]. In our

study, IFN therapy was not related to HBsAg seroclearance. This difference might be attributable to the difference in the HBV genotype, the small number of patients with IFN treatment, or the past use of IFN.

The average number of platelets in the patients with HBsAg seroclearance did not change after HBsAg seroclearance. In contrast, three of eight patients with less than 150 000/μl of platelets showed an increase in platelets, which was also reported in a previous study [21]. We have reported that the number of platelets is one of the most important factors predicting the prognosis of HBV carriers [22,23]. We do not know the reason for the difference between those with and without an increase in platelets after HBsAg seroclearance; therefore, we should clarify this in the future.

In conclusion, the predictive factor for the seroclearance of HBsAg was a lower level of HBsAg. Therefore, measurement of HBsAg level is one of the most effective means to follow up HBV carriers accurately.

Acknowledgements

Conflicts of Interest

The authors thank our staffs for their help. We have no conflict of interest disclosure.

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Longitudinal changes of the laboratory data of chronic hepatitis C patients with sustained virological response on long-term follow-up

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SUMMARY. There is no study that follows up longitudinal changes in laboratory data of patients with C-viral chronic liver disease (C-CLD) who achieved sustained virological esponse (SVR) with interferon treatment in a long-term study. We investigated the laboratory data in a long-term retrospective cohort study of 581 patients with C-CLD who underwent liver biopsy between January 1986 and December 2005. 467 were treated with interferon and 207 of these patients achieved SVR with follow-up periods of 8.36 ± 5.13 years. Alanine aminotransferase (ALT) levels, albumin levels, platelet counts, and the aspartate aminotransferase (AST)-to-platelet ratio index (APRI) values were serially examined during the follow-up period. None of the 207 patients with SVR exhibited hepatitis C virus (HCV) RNA positivity more than 6 months after the end of IFN treatment. Platelet counts and albumin levels increased only in those with eradication of HCV. APRI values decreased more in patients with SVR than in those with nonsustained virological responses (non-SVR). Patients who achieved SVR and had fibrosis stage 0–1 and 2–4 at enrolment had platelet counts that longitudinally increased by 2.81 ± 3.95 and 5.49 ± 4.53 × $10^3/\mu$L during the 10-year follow-up period, respectively. Albumin levels continuously increased during the first 2 years by 0.15 ± 0.31 and 0.33 ± 0.37 in fibrosis stage 0–1 and 2–4, respectively and then plateaued. ALT levels decreased rapidly one year after the start of treatment by 110.3 ± 140.0 and 100.5 ± 123.4 in fibrosis 0–1 and 2–4, respectively. HCV RNA negativity persisted in all patients with SVR, and laboratory data including APRI longitudinally improved during the long-term follow-up period.

Keywords: aspartate aminotransferase-to-platelet ratio index, chronic hepatitis C, cohort studies, interferon, platelet counts, sustained virological response.

INTRODUCTION

There are approximately 130–170 million hepatitis C virus (HCV) carriers worldwide, estimated to comprise 3–4% of the population [1,2]. In Japan, the number of HCV carriers was estimated at approximately 1.7 million [2]. HCV infection is persistent in nearly 70% of patients after acute infection, and these patients progress to chronic hepatitis. Thus, chronic inflammation leads to liver cirrhosis and hepatocellular carcinoma (HCC) [3]. Once the infection becomes chronic, the spontaneous viral clearance rate is very low indeed [4], and there was no therapy to eradicate the virus before 1986. Beginning in 1986, interferon-α (IFN-α) was used for the first time to treat patients with chronic HCV infection, and this antiviral therapy enabled eradication of the virus [5]. At present, administration of pegylated IFN with ribavirin is the most common treatment for chronic hepatitis C. Patients with chronic hepatitis C have a high risk of HCC, but sustained virological response (SVR) after IFN treatment induced not only long-lasting normal liver function but also improvement of liver fibrosis and significant reduction in the rate of HCC development [5–7].

There is no study that follows up longitudinally continuous changes in laboratory data of patients with chronic hepatitis C who achieved SVR with IFN treatment in a long-term study. We investigated the laboratory data of such patients in a long-term retrospective cohort study.

Abbreviations: ANOVA, analysis of variance; APRI, AST-to-platelet ratio index; C-CLD, C-viral chronic liver disease; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN-α, interferon-α; SVR, sustained virological response.

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PATIENTS AND METHODS

Patients

Of 853 patients with C-viral chronic liver disease (C-CLD) who underwent liver biopsy at the Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University Hospital between January 1986 and December 2005, 581 patients were enrolled in this study after excluding 272 patients for the reasons shown in Fig. 1. The follow-up period started from the date of liver biopsy in patients who did not receive IFN therapy and from the date when IFN therapy was initiated in those who did receive IFN therapy. The mean duration between the date of liver biopsy and the start of IFN treatment was 5.48 ± 16.18 months.The average age of the 581 patients was 50.52 ± 12.46 years (50.32 ± 12.26 years for males and 50.80 ± 12.77 years for females, $P = 0.485$). Among 581 patients, 467 were treated with IFN, and 207 of these patients achieved SVR (Fig. 1). The average follow-up periods were 8.36 ± 5.13 years among all patients, 8.54 ± 4.91 years among untreated patients, 8.31 ± 5.19 years among treated patients, 8.40 ± 5.31 years among nonsustained response (non-SVR) patients, and 8.20 ± 5.04 years in those with SVR.

Laboratory and imaging examination

Liver biopsy specimens were examined by two independent liver pathology specialists (F.I. and O.Y.) who were blinded to the clinical etiology according to the criteria of Desmet *et al.* [8], with fibrosis staging defined as F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), or F4 (cirrhosis) and the activity of inflammation being defined as A0 (no inflammation), A1 (mild inflammation), A2 (moderate inflammation), or A3 (severe inflammation).

All patients were positive by second- or third-generation HCV antibody tests. Early patients were diagnosed later with sera stored at −20 °C. Because the methods of quantifying HCV RNA levels varied during the follow-up period, we categorized patients into high titre and low titre groups. Patients who had HCV RNA of ≥100 KIU, ≥100 kc, ≥1.0 Mequiv., or $\geq 1 \times 10^4$ 50/μL or who had HCV core antigen levels of ≥30 pg/mL were classified into the high titer group, and the remaining patients were classified into the low titer group [9–12]. The serotype or genotype of HCV RNA was also examined. Laboratory data were examined every 1–3 months. To detect HCC, abdominal ultrasonography was performed every 3–6 months, and if there was a possibility of HCC development, further evaluation was performed such as computed tomography, magnetic resonance imaging, hepatic angiography, or ultrasonography-guided tumour biopsy.

SVR was defined as HCV RNA negativity, determined using the Amplicor quality test or TaqMan method, for more than 6 months after the end of IFN treatment, and any other response was considered a non-SVR. In the early patients treated with IFN, SVR was confirmed later using the Amplicor quality test on stored serum.

We utilized the laboratory data from at least 1 year before death in patients who died and before HCC detection in those who developed HCC during follow-up; we used the laboratory data until just before the start of IFN retreatment in those who were treated with IFN at the last follow-up point.

Statistical analysis

The Wilcoxon signed-rank tests and the analysis of variance (ANOVA) were used to analyze the data. Ratios were examined using Pearson's chi-square test. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using the Dr. SPSS 2 statistical software package (SPSS Japan Inc., Tokyo, Japan).

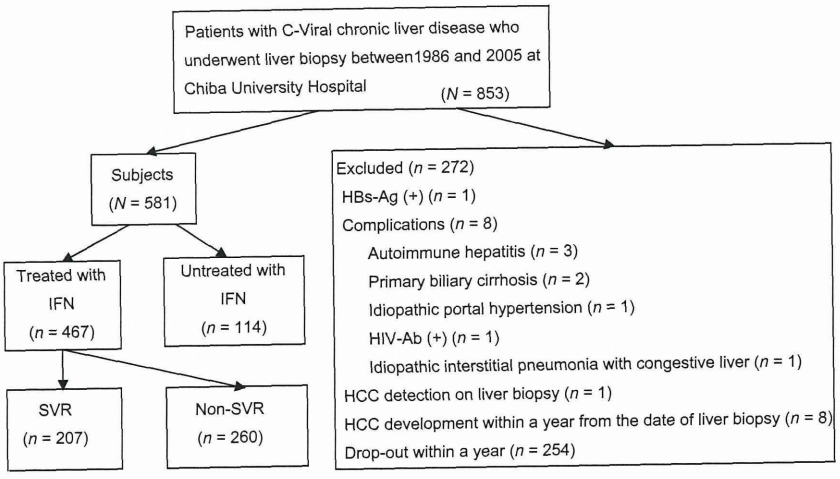

Fig. 1 Flow chart of patients analyzed. IFN, interferon; SVR, sustained virological response; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; HCC, hepatocellular carcinoma.

RESULTS

Patient characteristics

Patient characteristics are shown in Table 1. Among 467 patients who received IFN therapy, 100 were treated with IFN-α (38 with SVR), 8 were treated with a sequential combination of IFN-α and IFN-β (3 with SVR), 105 were treated with rIFNα-2a (24 with SVR), 80 were treated with rIFNα-2b (40 with SVR), 9 were treated with IFN-β followed by rIFNα-2b (5 with SVR), 28 were treated with rIFNα-2b and ribavirin (15 with SVR), 17 were treated with IFN-α con1 (12 with SVR), 1 was treated with IFN-α con1 and ribavirin (1 with SVR), 44 were treated with IFN-β (23 with SVR), 26 were treated with pegIFNα-2a (17 with SVR) and 49 were treated with pegIFNα-2b and ribavirin (29 with SVR).

Abnormal ALT level in SVR patients during follow-up

Fourteen (6.76%) of 207 patients with SVR had abnormal ALT levels (≥40 IU/L) during the follow-up period, and they were all male. The highest ALT value in each patient was 116, 62, 51, 51, 48, 46, 44, 43, 42, 42, 42, 41, 41, and 40 IU/L. Abdominal ultrasonography showed that 9 of the 14 patients had fatty liver, of which 1 patient had an alcohol consumption of >20 g/day and 2 patients had body mass index values of >25 kg/m^2. Among the remaining 5 of the 14 patients, 1 had high alcohol consumption and 4 had abnormal ALT levels of unknown cause.

No reappearance of HCV RNA in patients with SVR

None of the 207 patients with SVR exhibited HCV RNA positivity more than 6 months after the end of treatment. The duration between the date of confirmation of SVR and the date of last examination of HCV RNA was 7.5 ± 5.3 years (0–21.3 years). HCV RNA was examined using a TaqMan method in 119 of these patients, and the duration between the date of confirmation of SVR and the date of last examination of HCV RNA was 8.2 ± 5.5 years (0.5–21.3 years).

ALT levels, platelet counts, albumin levels, and AST-to-platelet ratio index values at initiation and end of follow-up

ALT levels significantly decreased in every group at the conclusion of follow-up compared to those before the initiation of follow-up (Fig. 2a). The decreased value in each group was −46.23 ± 125.17 IU/L in untreated patients, −58.02 ± 83.31 in patients with non-SVR, and −112.75 ± 125.17 in those with SVR.

Platelet counts decreased significantly in untreated patients ($P < 0.001$) and patients with non-SVR

Table 1 Patient characteristics at enrolment

	Untreated	IFN treated	Non-SVR	SVR
Patients, *n*	114	467	260	207
Age (years)*	54.1 ± 11.5	49.7 ± 12.6	51.7 ± 11.9	47.5 ± 13.1
Sex (male/female), *n*	53/61 (46.5%/53.5%)	290/177 (62.1%/37.9%)	154/106 (59.2%/40.8%)	136/71 (65.7%/34.3%)
Fibrosis stage: F0/F1/F2/F3/F4	2/55/24/12/21 (1.8%/48.2%/21.1%/10.5%/18.4%)	15/225/105/73/49 (3.2%/48.2%/22.5%/15.6%/10.5%)	5/119/51/51/34 (1.9%/45.8%/19.6%/19.6%/13.1%)	10/106/54/22/15 (4.8%/51.2%/26.1%/10.6%/7.2%)
ALT level (IU/L)*	101.9 ± 120.0	126.7 ± 105.6	121.9 ± 85.9	132.9 ± 126.0
Platelet count (×10^9/L)*	16.0 ± 6.8	17.0 ± 6.1	16.6 ± 6.2	17.6 ± 6.0
Albumin level (g/dL)*	4.1 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.3 ± 0.3
APRI*	1.76 ± 1.72	1.78 ± 1.55	1.92 ± 1.63	1.62 ± 1.42
HCV load: high/low, *n*	23/81 (22.1%/77.9%)	142/305 (31.8%/68.2%)	44/203 (17.8%/82.8%)	98/102 (49.0%/51.0%)
HCV serotype: 1/2, *n*	78/23 (77.2%/22.8%)	324/127 (71.8%/28.2%)	211/42 (83.4%/16.6%)	113/85 (57.1%/42.9%)
Serotype1 and high viral load/others, *n*	62/35 (63.9%/36.1%)	227/208 (52.2%/47.8%)	172/70 (71.1%/28.9%)	55/138 (28.5%/71.5%)

IFN, interferon; SVR, sustained virological response; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; HCV, hepatitis C virus.
*Data are shown as mean ± SD.

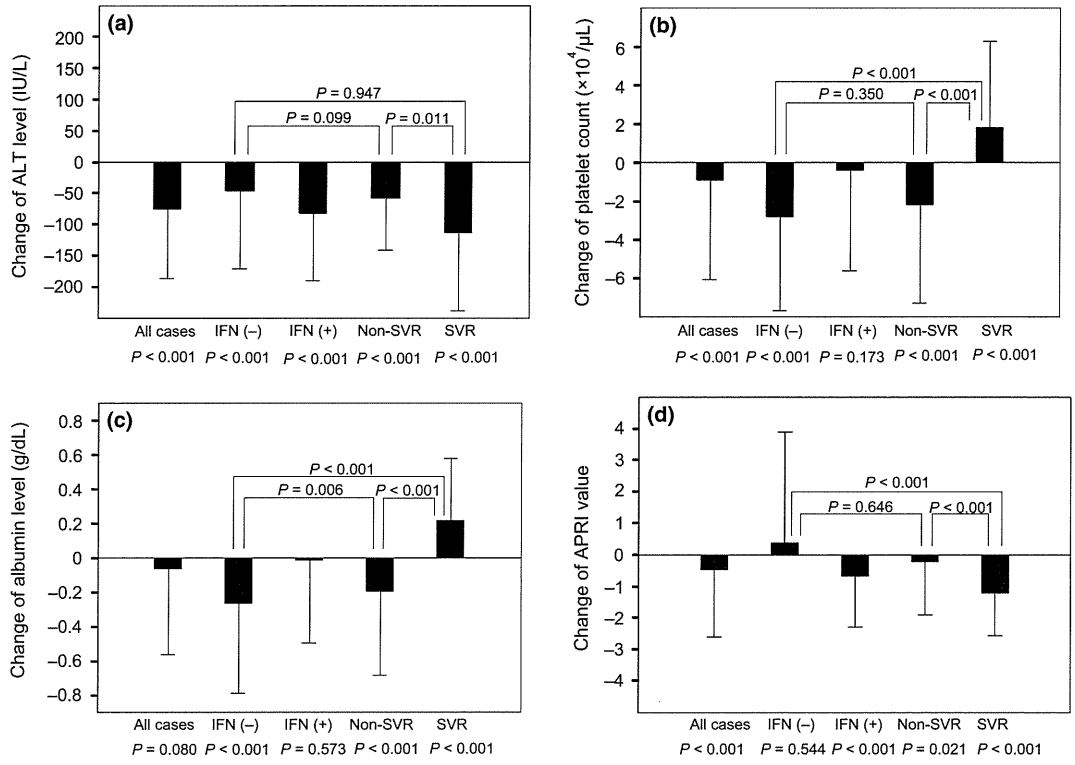

Fig. 2 Changes in (a) ALT levels, (b) platelet counts, (c) albumin levels and (d) APRI values according to IFN treatment. *P* values represent comparisons between the values at the start of observation and those at the end of observation by using the Wilcoxon signed-rank test. Changes in values were compared using the ANOVA followed by *post hoc* tests for multiple comparisons (Tukey's test). ALT, alanine aminotransferase; IFN, interferon; SVR, sustained virological response.

($P < 0.001$) but increased in those with SVR ($P < 0.001$) (Fig. 2b). The degree of change in each group was -2.83 ± 4.84 in untreated patients, -2.21 ± 5.07 in those with non-SVR, and $+1.82 \pm 4.41$ in those with SVR, indicating that platelet count increased only in those with eradication of HCV. There was a significant difference in values between untreated and SVR patients ($P < 0.001$), and between patients with non-SVR and those with SVR ($P < 0.001$) but not between untreated and non-SVR patients ($P = 0.350$), suggesting that platelet counts decreased in non-SVR patients at the same pace as observed in untreated patients and only increased in patients with SVR.

Albumin levels decreased significantly in untreated patients ($P < 0.001$) and in those with non-SVR ($P < 0.001$) but increased significantly in those with SVR ($P < 0.001$) (Fig. 2c). The degree of change in albumin levels in each group was -0.26 ± 0.53 in untreated patients, -0.19 ± 0.49 in those with non-SVR, and $+0.21 \pm 0.37$ in those with SVR, indicating that albumin levels increased only in those with eradication of HCV. There was a significant difference in values between untreated and non-SVR patients ($P = 0.006$), suggesting that albumin levels decreased in untreated patients at a greater rate than in non-SVR patients.

AST-to-platelet ratio index (APRI) has been reported to be useful for evaluating liver fibrosis [13], and high APRI values could indicate progression of liver fibrosis. In our study, APRI values tended to increase in untreated patients, and decrease significantly in IFN-treated patients, especially in those with SVR and even in those with non-SVR (Fig. 2d). The decrease in the APRI value in patients with SVR was greater than that in those with non-SVR (-1.208 ± 1.335 *vs* -0.297 ± 1.399, $P < 0.001$).

Serial changes in ALT levels, platelet counts, albumin levels, and APRI values in patients with SVR

Next, the serial changes in ALT levels, platelet counts, albumin levels, and APRI values were analyzed in the 207 patients with SVR. Histological examination before IFN treatment showed F0–F1 fibrosis in 116 (F0: 10, F1: 106) and F2–F4 fibrosis in 91 (F2: 54, F3: 22, F4: 15) of these patients. Laboratory data were obtained for 193 patients after 6 months (F0–F1: 110, F2–F4: 83), for 194 patients after 1 year (F0–1: 111, F2–4: 83), for 190 patients after 2 years (F0–F1: 105, F2–F4: 85), for 165 patients after 4 years (F0–F1: 95, F2–F4: 70), for 115 patients after 7 years (F0–F1: 68, F2–F4: 47), and for 77 patients after 10 years (F0–F1: 52, F2–F4: 25).

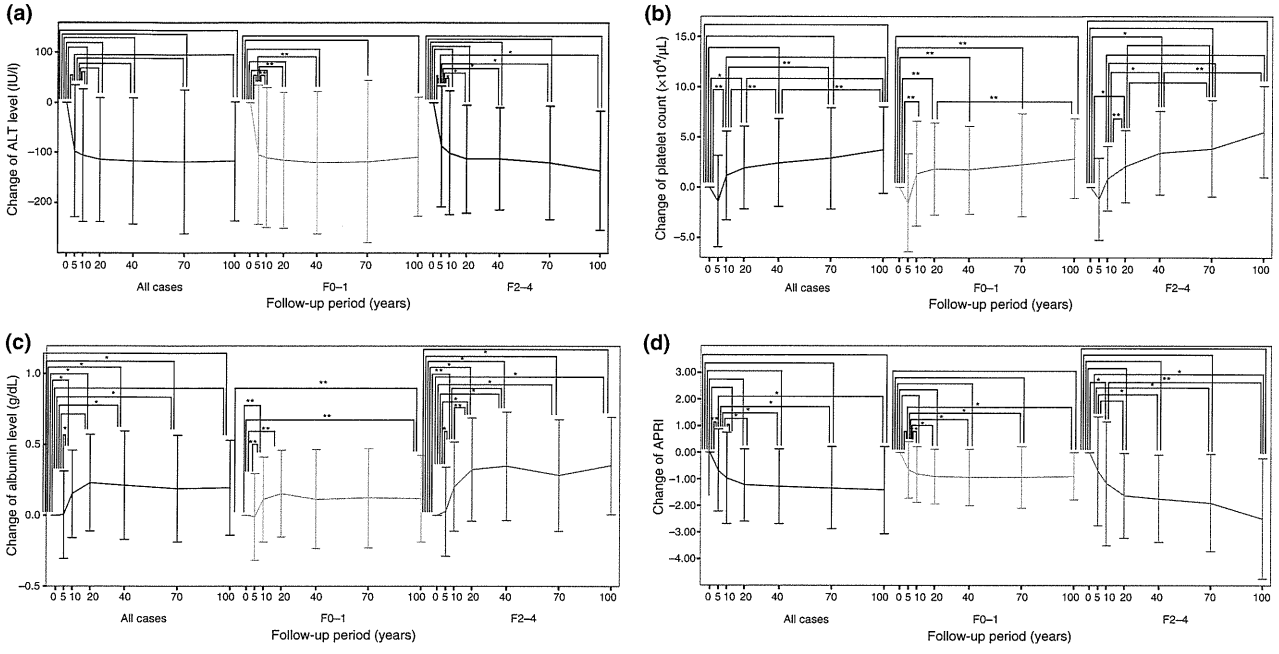

Fig. 3 Serial changes in (a) ALT levels, (b) platelet counts, (c) albumin levels and (d) APRI values in patients with SVR. Changes in values were compared using the one-way repeated-measures ANOVA. No asterisk, $P < 0.001$; $^{*}P < 0.01$; $^{**}P < 0.05$. ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index.

ALT levels decreased rapidly 6 months after the initiation of treatment and gradually over the next 6 months by 110.3 ± 140.0 and 100.5 ± 123.4 in F0–F1 and F2–F4, respectively. After that, ALT levels nearly plateaued in F0–F1 patients but continued to decrease gradually in F2–F4 patients (Fig. 3a).

Platelet counts continued to increase significantly for 10 years after IFN treatment, especially in F2–F4 patients. The mean platelet count was 14.57 ± 4.57 before IFN treatment, and the increase in this value was +0.84 ± 3.22 at 1 year, +2.03 ± 3.58 at 2 years, +3.38 ± 4.20 at 4 years, and +5.49 ± 4.53 at 10 years after the start of IFN treatment (Fig. 3b). The mean platelet count was 18.85 ± 5.26 at 10 years after the start of IFN treatment in F2–F4 patients. In F0–F1 patients, platelet counts markedly increased 1 year after IFN therapy and gradually increased thereafter with smaller changes compared to F2–F4 patients (+1.34 ± 5.18 at 1 year, +2.81 ± 3.95 at 10 years after the start of IFN treatment, respectively). The mean platelet count was 19.89 ± 5.98 before IFN treatment and 22.12 ± 5.79 at 10 years after the start of IFN treatment in F0–F1 patients.

Albumin levels increased only during the first 2 years in F0–F1 and F2–F4 patients (+0.15 ± 0.31 and +0.33 ± 0.37, respectively). The mean albumin level was 4.35 ± 0.27 and 4.18 ± 0.37 before IFN treatment, and 4.48 ± 0.24 and 4.49 ± 0.33 at 2 years after the start of IFN treatment in F0–F1 and F2–F4 patients, respectively. Albumin levels did not increase beyond 2 years of follow-up (Fig. 3c).

APRI values significantly decreased for the first 2 years in F0–F1 patients (−0.93 ± 1.03 in the 2 years after the start of IFN treatment compared to the baseline value of 1.19 ± 1.01 before IFN treatment), whereas in F2–F4 patients, APRI values tended to decrease continuously throughout the follow-up period (−1.64 ± 1.59 in the 2-year period after the start of IFN treatment, −2.50 ± 2.26 in the 10-year period after the start of IFN treatment compared to the baseline value of 2.15 ± 1.67 before IFN treatment) (Fig. 3d).

DISCUSSION

We showed favourable outcomes regarding laboratory data of chronic hepatitis C patients who achieved SVR. During a long-term follow-up period of a mean 7.5 years (0–21.3 years), reappearance of HCV RNA in serum was detected in none of the 207 patients with SVR. Previous reports on long-term follow up studies of patients who achieved SVR have shown that the rate of relapse of HCV RNA is relatively low but not rare [14–24]. In contrast, reports that HCV RNA was not detected in serum for long periods are few. Tsuda *et al.* reported that HCV RNA was not detected in serum in 38 patients over a 4.4–12.0-year (median 6.8 years) observation period [25]. Adamek *et al.* reported the nonreappearance of HCV RNA in the sera of 78

patients for 0.5–5.3 years (median, 1.8 years) [26]; George *et al.*, in 150 patients for 1.0–7.8 years (median, 5.1 years) [27]; Formann *et al.*, in 187 patients for 1.0–14.3 years (mean, 2.4 years) [28]; and Maylin *et al.*, in 344 patients for 0.5–18 years (mean, 3.3 years) [29]. In all of these reports excluding that of Maylin *et al.*, the patient numbers were smaller and the observation periods were shorter than those in our study. The study by Maylin *et al.* had larger numbers than our study, but the observation period in our study was more than twofold larger. In addition, in our study, HCV RNA was examined in 119 of 207 patients by using the TaqMan PCR method, one of the most specific and sensitive tests, 0.5–21.3 years (8.2 ± 5.5 years) after the confirmation of SVR, and HCV RNA was not detected in any of these patients. No report mentioned the use of TaqMan methods except our study.

McHutchinson *et al.* reported that 7 (2%) of 400 patients with SVR had detectable HCV RNA in liver biopsy specimens, and 2 patients had reappearance of serum HCV RNA 12 months after therapy [30]. HCV might replicate in extrahepatic tissues such as bone marrow and lymphocytes, as the antisense strand of HCV RNA was detected in these organs [31–34]. In our study, some patients with SVR exhibited elevated ALT levels without any definite cause, and there is a possibility of an effect of residual HCV in the liver.

ALT levels decreased in all patients during the follow-up period. Platelet counts and albumin levels decreased in untreated patients and non-SVR patients, and they increased only in patients with SVR. These results may indicate that the progression of liver fibrosis is different between untreated or non-SVR patients and SVR patients. Toccaceli *et al.* reported that histological improvement was achieved in 44% of 112 SVR patients after 36–76 months of follow-up [35], and Shiratori *et al.* reported that improvement of liver fibrosis was obtained in 59% of 183 SVR patients after a mean follow-up period of 3.7 years [36]. However, Shindo *et al.* reported that the fibrotic stage did not significantly improve in non-SVR patients after a follow-up period of 2 years [37]. Our investigation was based on the laboratory data in serum, and our data were in accordance with these reports, indicating that the improvement of hepatic fibrosis could be surmised by transition of common serum data.

Serial changes in ALT levels, platelet counts, and albumin levels in patients with SVR according to the hepatic fibrosis stage at enrolment showed that ALT levels decreased for only 12 months after initiation of IFN treatment and almost plateaued for 10 years. Platelet counts continued to increase significantly for 10 years, especially in F2–F4 patients. Therefore, serial changes in platelet counts may be useful to estimate the long-term improvement of liver fibrosis after achievement of SVR. In contrast to platelet counts, albumin levels increased significantly only for the first 2 years. Previous studies reported that ALT levels normalized in most patients after SVR [18], but there are few reports analyzing consecutive serum data. George *et al.* followed 150 patients with SVR, and 136 of these patients (91%) were followed over 3 years [27]. They found no differences in ALT levels, albumin levels, and platelet counts at the last follow-up point compared to those at 6 months after the end of IFN treatment. However, our data revealed gradual increases in platelet counts after IFN treatment, especially in patients with advanced fibrosis. There is no study that follows up longitudinal changes in laboratory data of patients with C-CLD who achieved SVR with interferon treatment in a long-term study except our study.

Liver biopsy is the most reliable examination to confirm the fibrotic stage of the liver, but it has some risk of complications, sampling variability, procedural discomfort, and added cost. Recently, noninvasive methods have been developed to evaluate liver fibrosis by ultrasonography, such as Fibroscan [38] or real-time tissue elastography [39], but not all institutions can perform these examinations because they lack the necessary equipment. Hyaluronic acid is superior in evaluating liver fibrosis independently, but this examination is not performed at every institution. Wai *et al.* reported that APRI was useful to distinguish between F0–F1 and F2–F4 fibrosis by AUROC 0.80, and a cut-off value of 1.5 showed that a positive predictive value of 88% was obtained for the diagnosis of F2–F4 fibrosis [13]. There are some reports that improvement in liver fibrosis for a few years after SVR was determined by liver biopsy as previously explained. However, there is no report showing that liver fibrosis improved for 10 long years continuously in SVR patients by any method, and we proved that by using platelet counts and APRI, the consequential indices of fibrosis.

In conclusion, we showed a favourable clinical course in patients who achieved SVR. HCV RNA remained negative in all patients, and laboratory data including APRI showed longitudinal improved during the long-term follow-up period.

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D. Maruoka planned and conducted this project, analyzed the data, and wrote this paper. F. Imazeki helped plan the project, analyze data, and write the paper and performed physical examinations of the patients. M. Arai, T. Kanda, and K. Fujiwara helped collect data and performed physical examinations of the patients. O. Yokosuka helped plan the project and performed physical examinations of the patients.

DISCLOSURE

None of the authors have any conflicts of interest or financial disclosures to declare.

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Research Paper

Efficacy of Lamivudine or Entecavir on Acute Exacerbation of Chronic Hepatitis B

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Abstract

Background/Aims: Spontaneous acute exacerbation of chronic hepatitis B virus (HBV) infection occasionally occurs in its natural history, sometimes leading rapidly to fatal hepatic failure. We compared the effects of lamivudine (LAM) with those of entecavir (ETV) treatments in acute exacerbation of chronic hepatitis B with 500 IU/L or higher alanine aminotransferase (ALT) levels.

Methods: Thirty-four patients with acute exacerbation were consecutively treated with LAM /ETV. Their clinical improvements were compared.

Results: Among LAM-treated and ETV-treated patients, none showed a reduction of <1 log IU/mL in HBV DNA after 1 or 3 months of treatment. Initial virological response, defined as a reduction of 4 log IU/mL in HBV DNA at 6 months, with LAM and ETV, respectively, was 83.3% and 100%. One LAM patient developed hepatic encephalopathy, but all patients in both groups survived. Twelve months after treatment, 41.6% of 24 LAM group patients switched to another drug or added adefovir to their treatment due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change drugs.

Conclusions: ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should carefully start to treat these patients as soon as possible.

Key words: acute exacerbation, ALT, entecavir, HBV, lamivudine

INTRODUCTION

Chronic hepatitis B infection is associated with the development of hepatocellular carcinoma [1]. Infection with hepatitis B virus (HBV) also leads to wide a spectrum of liver injury, including acute, self-limited infection, fulminant hepatitis, and chronic hepatitis with progression to cirrhosis and liver failure, as well as to an asymptomatic chronic carrier state [2, 3].

Reactivation of hepatitis B is a well-characterized syndrome marked by the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactivated or resolved HBV infection [4]. Reac-

tivation is often spontaneous, but can also be triggered by cancer chemotherapy and immune suppression. Spontaneous acute exacerbation of chronic hepatitis B infection is seen with a cumulative probability of 15-37% after 4 years of follow-up [5]. Prognosis is generally poor in HBV carriers with spontaneous acute exacerbation together with high alanine aminotransferase (ALT) levels, jaundice, and liver failure [4, 6, 7]. This condition has been defined as acute-on-chronic liver failure according to a recent Asia-Pacific consensus recommendation [8]. Acute exacerbation occasionally leads to a critical scenario, meaning that clinicians need to treat this condition immediately.

Lamivudine (LAM) is a reverse-transcriptase inhibitor of viral DNA polymerase with an excellent profile of safety and tolerability, causing inhibition of viral replication, and it is approved for antiviral treatment of hepatitis B patients [9, 10]. LAM suppresses serum HBV DNA values in up to 98% of patients within a median period of 4 weeks, leading to aminotransferase normalization, increased hepatitis B e antigen (HBeAg) seroconversion rate, and improvement of histological parameters [11, 12]. A study from Taiwan showed that LAM had a survival benefit and was effective for patients with baseline bilirubin levels below 20 mg/dL [7].

Entecavir (ETV), a deoxyguanosine analogue, is a potent and selective inhibitor of HBV replication; its *in vitro* potency is 100- to 1,000-fold greater than that of LAM, and it has a selectivity index (concentration of drug reducing the viable cell number by 50% [CC_{50}]/concentration of drug reducing viral replication by 50% [EC_{50}]) of ~8,000 [13, 14]. At present, the Japanese national health insurance system approves ETV as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is a nucleoside analogue (NUC) belonging to a new subgroup, cyclopentane [15], and it has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing ALT, although NUCs do not eradicate the virus. ETV develops less resistance than LAM.

We undertook a retrospective study to compare the efficacy of LAM with that of ETV in the reduction of HBV DNA levels and associated improvement in disease severity and biochemical recovery in patients with acute exacerbation together with higher ALT levels due to HBV reactivation.

MATERIALS AND METHODS

Patients

A retrospective analysis of LAM/ETV-treated chronic hepatitis B patients at Chiba University Hospital and Numazu City Hospital, Japan, between May 2003 and December 2009 was performed. The inclusion criteria were: acute exacerbation of chronic hepatitis B characterized by an elevation of ALT level ≥ 500 IU/L along with HBV DNA ≥ 4.5 log IU/mL presenting in a patient with diagnosed chronic liver disease. The exclusion criteria were: acute hepatitis B, superinfection with other viruses (hepatitis E, A, D, or C), other causes of chronic liver failure [16, 17], coexistent hepatocellular carcinoma, portal thrombosis, coexistent renal impairment, pregnancy, coinfection with human immunodeficiency virus (HIV), or patients who had received a previous course of NUC treatment. This retrospective study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of Chiba University, Graduate School of Medicine [18].

Baseline assessment of patients

Retrospectively collected data included patient demographics, clinical findings, all laboratory variables including virological tests and abdominal ultrasound. HBsAg, HBeAg, anti-HBe antibody and immunoglobulin M (IgM) anti-HBc antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [19]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [20]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan).

Definitions

Primary antiviral treatment failure was defined as a reduction of < 1 log IU/mL in HBV DNA after 3 months of therapy. Initial virological response (IVR) was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

Follow-up

Clinical assessment and routine investigations were done every 15 days or every month for at least 6 months. HBV DNA measurements were repeated monthly.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7 and StatView 5 (SAS Institute Inc, Cary, NC). Continuous variables were expressed as mean ± standard deviation and were compared by two-factor analysis of variance (ANOVA) and two-way repeated measures ANOVA. Categorical variables were compared by Chi-square

test. Baseline was taken as the date when the first dose of LAM/ETV was administered. Statistical significance was considered at a *P*-value < 0.05.

RESULTS

Patients

Between May 2003 and December 2009, 34 patients with spontaneous acute exacerbation of chronic hepatitis B, with ALT levels ≥ 500 IU/mL and treated with LAM or ETV, were consecutively enrolled and retrospectively analyzed. 24 (70.5%) were treated with LAM at 100 mg daily and 10 (29.4%) were treated with ETV at 0.5 mg daily. All patients were followed for at least 6 months. Mean follow-up in the LAM and ETV groups was 55.5 ± 25.4 and 16.5 ± 9.9 months, respectively.

Baseline characteristics

Baseline characteristics in the two patient groups were similar **(Table 1)**. Median age was 37 (21-73) years and 79.4% were men. One patient of the LAM group developed hepatic encephalopathy, but recovered. All patients in both groups survived. At admission, the serological profile showed HBsAg positivity in all 34 (100%); 22 (64.7%) were HBeAg positive. The median HBV DNA level was 7.4 log IU/mL in the LAM group and 7.9 log IU/mL in the ETV group **(Table 1)**.

Table 1 Demographic, Clinical, and Laboratory Variables of Patients at Entry.

Parameters	Total Patients (N=34)	LAM (N=24)	ETV (N=10)	*P*-value
Age (years)	37 (21-73)	37 (21-73)	39 (24-67)	NS
Male (%)	27 (79.4)	18 (75)	9 (90)	NS
Cirrhosis (+/-)	2/32	2/22	0/10	NS
ALT (IU/L)	986 (523-2,450)	995 (523-2,450)	1,046 (523-2,140)	NS
T. Bil (mg/dL)	2.0 (0.8-22.0)	2.4 (0.8-20.6)	1.6 (1.9-22.0)	NS
PT (%)	83 (24-121)	81.5 (24-119)	83.6 (35-121)	NS
HBeAg (+/-)	22/12	18/6	4/6	NS
HBV DNA (log IU/mL)	7.6 (4.8-8.7)	7.4 (5.2-8.7)	7.9 (4.8-8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in HBV DNA of total patients

LAM significantly reduced HBV DNA levels from baseline 7.24 log IU/mL to 3.27 log IU/mL at 1 month ($P < 0.001$), to 2.21 log IU/mL at 3 months ($P < 0.001$), and to 1.53 log IU/mL at 6 months ($P < 0.001$). ETV also significantly reduced HBV DNA levels from baseline 7.56 log IU/mL to 3.12 log IU/mL at 1 month ($P < 0.001$), to 2.14 log IU/mL at 3 months ($P < 0.001$), and to 1.77 log IU/mL at 6 months ($P < 0.001$). There were no differences in HBV DNA levels from baseline to 6 months between the two groups. None with primary antiviral treatment failure was identified in either group. There were no significant differences in IVR between the two groups **(Figure 1)**.

Reduction in ALT levels of total patients

LAM significantly reduced ALT levels from baseline 1,130 IU/mL to 102 ($P < 0.001$) at 1 month, to 28.6 ($P < 0.001$) at 3 months, and to 23.1 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,210 IU/mL to 117 ($P < 0.001$) at 1 month, to 25 ($P < 0.001$) at 3 months, and to 24.4 ($P < 0.001$) at 6 months. There were no differences in ALT levels from baseline to 6 months between the two groups **(Figure 2)**.

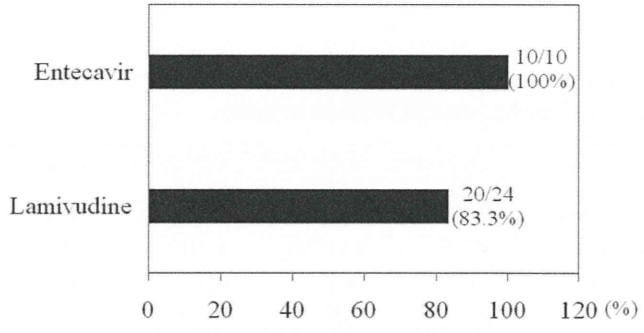

Figure 1 Initial virological response (IVR). IVR was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

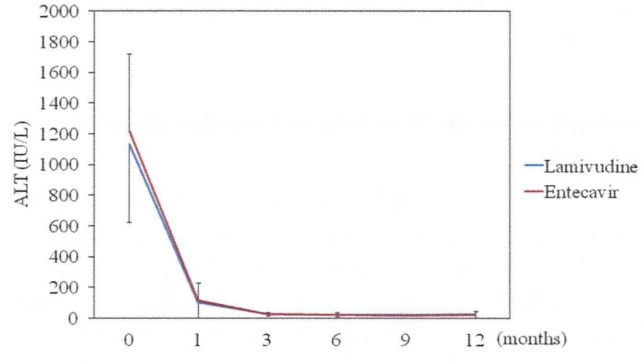

Figure 2 Efficacy of lamivudine and entecavir for ALT levels. Lamivudine (N=24) vs. entecavir (N=10); data are shown as mean ± SD.

Reduction in HBV DNA of HBeAg-positive patients

It has been demonstrated that the levels of HBV DNA in the HBeAg-positive phase were generally higher than those in the ant-HBe-positive phase [19, 22]. HBeAg positivity is also associated with HBV viremia and increased ALT levels in HIV/HBV co-infected patients [23]. Next, we compared the response to LAM or ETV in 18 or 4 HBeAg-positive patients, respectively **(Table 2)**. LAM significantly reduced HBV DNA levels from baseline 7.52 log IU/mL to 3.35 log IU/mL ($P < 0.001$) at 1 month, to 2.38 log IU/mL ($P < 0.001$) at 3 months, and to 1.55 log IU/mL ($P < 0.001$) at 6 months. ETV also significantly reduced HBV DNA levels from baseline 8.42 log IU/mL to 3.87 log IU/mL ($P < 0.001$) at 1 month, to 2.90 log IU/mL ($P < 0.001$) at 3 months, and to 2.22 log IU/mL ($P < 0.001$) at 6 months. There were no differences in HBV DNA levels from baseline to 6 months between the two groups. Primary antiviral treatment failure was not observed in either group. Four patients in the LAM group did not achieve IVR.

Table 2 Demographic, Clinical, and Laboratory Variables of HBeAg-positive Patients at Entry.

Parameters	Total Patients (N=22)	LAM (N=18)	ETV (N=4)	*P*-value
Age (years)	34.5 (21-51)	36.5 (21-51)	30 (24-33)	NS
Male (%)	18 (81.8)	14 (77.7)	4 (100)	NS
Cirrhosis (+/-)	1/21	1/17	0/4	NS
ALT (IU/L)	1,030 (523-2,450)	1,990 (523-2,450)	1,363 (980-1,620)	NS
T. Bil (mg/dL)	1.75 (0.8-20.6)	2.0 (0.8-20.6)	1.5 (1.0-18.7)	NS
PT (%)	77 (24-119)	73.6 (24-119)	95.0 (44.1-113)	NS
HBeAg (+)	22	18	4	
HBV DNA (log IU/mL)	7.6 (5.5- 8.8)	7.6 (5.5- 8.7)	8.6 (7.6- 8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in ALT levels of HBeAg-positive patients

LAM significantly reduced ALT levels from baseline 1,150 IU/mL to 84 ($P < 0.001$) at 1 month, to 27.5 ($P < 0.001$) at 3 months, and to 22.0 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,460 IU/mL to 230 ($P = 0.0038$) at 1 month, to 22.2 ($P = 0.0016$) at 3 months, and to 24.0 ($P = 0.0016$) at 6 months. At 1 month after treatment, the ALT levels of the LAM groups were lower than those of the ETV group ($P < 0.0001$) **(Figure 3)**. During follow-up periods, 10 and 1 sero-converters of HBeAg to anti-HBe antibody phase were seen in 18 LAM-treated and in 4 ETV-treated patients, respectively.

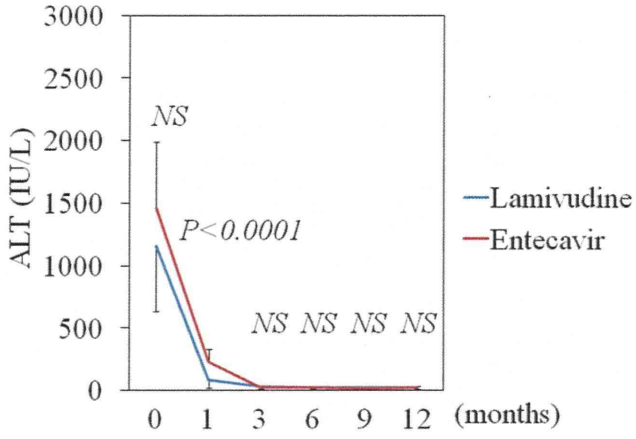

Figure 3 Efficacy of lamivudine and entecavir for ALT levels in HBeAg-positive patients. Lamivudine (N=18) vs. entecavir (N=4); data are shown as mean ± SD.

Safety

No patient stopped taking medications. Twelve months after treatment, 10 of 24 patients (41.6%) in the LAM group switched from LAM to ETV (n=4) or added adefovir (n=6) due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change their medication.

DISCUSSION

The present study compared the use of NUCs, LAM and ETV, for the treatment of acute exacerbation of chronic hepatitis B. The results clearly showed significant benefits of a rapid reduction of HBV DNA levels, compared with untreated patients in a previous report [4].

It was reported that ETV treatment is associated with increased short-term mortality in patients with severe acute exacerbation of chronic hepatitis B, but that it achieves better virological response in the long run [24]. We used LAM or ETV for patients with acute exacerbation of chronic hepatitis B presenting with ALT ≥ 500 IU/L in the present study. The effects of LAM on HBV DNA levels were the same as those of ETV (Figure 1). But the effects of LAM on ALT levels after 1 month were stronger than those of ETV in HBeAg-positive patients **(Figure 3)**. In spite of the limited number of these patients, the effects were possibly related to immunomodulating activities of LAM [25]. The patients' prognoses were more favorable than in the previous report [4]. This might have

depended on the fact that, in the present study, treatment was begun as soon as possible, and some patients may have had a milder grade of acute exacerbation of chronic hepatitis B than those in the previous report [4]. We believe that patients with acute exacerbation of chronic hepatitis B need to be subjected to treatment as promptly as possible.

The major routes of HBV infection in our country have been mother-to-child transmission and blood transfusion. However, cases with HBV transmitted through sexual contact are increasing, especially among HIV-1-seropositive patients [26]. One should bear in mind that knowledge about interactions between ETV and anti-HIV nucleoside analogues is limited [27]. Because long-term use of LAM induces LAM-resistant mutants [28], we can only use LAM for short-term treatment of patients with acute exacerbation of chronic hepatitis B. On the other hand, the present study also revealed that patients receiving ETV did not need to change drugs.

Recently, there have been several reports that reactivation of HBV is a fatal complication following systemic chemotherapy or other immunosuppressive therapy including rituximab and steroid therapies mainly in HBsAg-positive and -negative lymphoma patients. It is important to enable early diagnosis of HBV reactivation as well as initiation of antiviral therapy [29, 30].

In conclusion, ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should start to treat these patients with NUCs as soon as possible.

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ABBREVIATIONS

ETV: Entecavir; HIV: Human immunodeficiency virus; IVR: Initial virological response; LAM: Lamivudine; NUC: Nucleoside analogue.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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