

# Nucleotide Analogs for Patients with HBV-Related Hepatocellular Carcinoma Increase the Survival Rate through Improved Liver Function

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## Abstract

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**Background and Aim** This study evaluated the outcomes of antiviral therapy with nucleotide analogs for hepatitis B virus infection-related hepatocellular carcinoma.

**Methods** Thirty patients orally received nucleotide analogs and, as a matched control group, 20 patients who were not treated with nucleotide analogs were selected. We compared changes in liver function, HCC recurrence and survival rate between both groups.

**Results** In the nucleotide analog group, serum albumin, AST and ALT were significantly improved compared with baseline values. The Child-Pugh score was significantly decreased in the nucleotide analog group. Furthermore, of the 36 patients curatively treated with the initial treatment, more patients in the nucleotide analog group improved or maintained their Child-Pugh score at the time of recurrent HCC than in the control group ( $p=0.023$ ). The cumulative recurrent-free survival rate of HCC did not significantly differ between the two groups; however, the cumulative survival rates of not only curative-treated patients but also all patients in the nucleotide analog group were significantly higher than those of patients in the control group ( $p=0.047$  and  $p=0.02$ , respectively).

**Conclusion** The results suggest that nucleotide analog treatment increases the survival rate in patients with HCC by contributing to the improvement of remnant liver function.

**Key words:** nucleotide analog, hepatocellular carcinoma, HBV, survival rate

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## Introduction

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Sequelae of chronic infection with hepatitis B virus (HBV) include chronic hepatitis, compensated cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) (1, 2). Several studies have demonstrated that long-standing necro-inflammation and viral replication strongly influence the rate of progression to cirrhosis and development of HCC (3-5). Conceivably, these patients are accompanied by a risk of hepatic decompensation and the development of HCC,

and antiviral therapy such as suppression of HBV replication and necro-inflammation by interferon or nucleotide analogs may reduce the risk of these complications and prolong survival.

HCC is characterized by a high incidence of intrahepatic recurrence, including intrahepatic metastasis and multicentric occurrence, even after curative treatment (6-9); therefore, most patients with recurrent HCC need to be treated repeatedly. Although hepatic resection and radiofrequency ablation (RFA) are recognized as curative treatments, many cirrhotic patients cannot repeatedly undergo curative treatments due

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to the deterioration of liver function after treatment. Furthermore, liver function is the most critical prognostic factor of treatment for HCC (10-12). It is very important to maintain adequate liver function when undergoing therapy and to suppress the recurrence of HCC in order to improve survival; however, HBV-related chronic liver disease gradually progresses to cirrhosis and hepatic decompensation, and leads to a higher probability of contracting HCC. Recently, nucleotide analogs such as lamivudine, adefovir dipivoxil and entecavir have been reported to inhibit the replication of HBV and reduce hepatic necro-inflammation (13-16). Thus, the suppression of HBV in HBV-related chronic hepatitis and cirrhosis prevents the progress of liver failure and the development of HCC (17, 18).

However, few papers have reported the benefit of nucleotide analogs in the outcome after treatment for HCC (19-21). In this study, we investigated the efficacy of nucleotide analogs in patients with HBV-related HCC in terms of liver function, HCC recurrence rate and survival and compared with a nucleotide analog-untreated control.

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## Patients and Methods

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We administered nucleotide analogue to patients who met following conditions: 1) HBV-DNA in serum more than 3.7 LGE/mL, 2) serum ALT more than 40 IU/L, 3) patients wish to have nucleotide analogue treatment. Between January 2002 and December 2006, 62 patients with chronic HBV infection were diagnosed with HCC, of which 30 (48.4%) orally received nucleotide analog daily after HCC treatment because they met all three conditions. Because nine patients were negative (less than 3.7 LGE/mL) for serum HBV-DNA and three patients had normal serum ALT levels (less than 40 IU/L) during observation periods, these twelve patients (19.4%) were not administered nucleotide analogs and were not enrolled in this study. The remaining 20 patients (32.3%) did not want nucleotide analog treatment although they had high serum ALT and were positive for serum HBV-DNA. We set these 20 patients as controls. In the nucleotide analog group, lamivudine (100 mg/day) was administered in 28 patients and entecavir (0.5 mg/day) in 2 patients who started after October 2006. All patients were positive for hepatitis B surface antigen (HBsAg) and HBV-DNA (more than 3.7 LGE/mL) in serum before starting nucleotide analog. These patients continued to take nucleotide analog during the observation period. When the emergence of YMDD mutants in patients who were administered lamivudine was observed, they were additionally administered adefovir dipivoxil or were changed from lamivudine to entecavir. HCC was treated by hepatic resection in 12 patients, radiofrequency ablation (RFA) in 24 patients, percutaneous ethanol injection (PEI) in 5 patients and transcatheter arterial embolization (TAE) in 9 patients. Treatments for HCC were performed at Tottori University Hospital. Biochemical tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time

(PT), albumin, total bilirubin and platelet count, were measured using standard laboratory procedures. HBsAg, HB e antigen (HBeAg), and HB e antibody (HBeAb) were determined by commercially available enzyme immunoassay. HBV-DNA was quantified by transcription-mediated amplification assay (TMA, SRL, Tokyo, Japan) or PCR assay (Amplicor HBV Monitor assay, SRL). We examined the clinical features such as ascites and hepatic encephalopathy by physical findings and laboratory tests every 1-3 months. When HBV-DNA increased during lamivudine treatment, we examined YMDD mutants. All patients were followed using abdominal sonography or computed tomography (CT) every 3 months as well as the measurement of tumor markers, serum alpha-fetoprotein (AFP) and des-gamma-carboxyl prothrombin (DCP) every 1-3 months. When recurrent HCC tumors were identified, HCC of less than 3 cm and fewer than 3 nodules were treated by local ablation (RFA or PEI) and more HCC lesions were treated by TAE or intraarterial chemotherapy.

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## Statistical Analysis

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Data analyses were performed using Stat View version 5.0 (SAS Institute Inc; Cary, NC, USA). Continuous variables are expressed as the mean  $\pm$  SD (standard deviation). For quantitative variables, chi-squared test or Fisher's exact probability test was performed as appropriate. For continuous variables, Student's t-test or the Mann-Whitney U-test was performed. Cumulative survival rates and recurrence-free survival rates were calculated by the Kaplan-Meier method from the date of initial HCC treatment and differences between the two groups were compared by the log-rank test. A p-value of <0.05 was considered significant. We obtained informed consent from each patient. This study was performed in compliance with the declaration of Helsinki.

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## Results

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### **Baseline characteristics**

The baseline characteristics of the nucleotide analog and control groups are summarized in Table 1. There were no significant differences in baseline virological and clinical characteristics between the groups. The duration of administration in the nucleotide analog group was  $28.6 \pm 16.7$  months (range; 5-73 months).

### **Virological markers and the emergence of YMDD mutants**

In the nucleotide analog group, HBV-DNA levels decreased significantly until 21 months compared with baseline values before nucleotide analog administration (baseline:  $5.7 \pm 1.2$  LGE/mL, 3 months:  $3.9 \pm 0.6$ ,  $p < 0.001$ , 6 months:  $3.8 \pm 0.5$ ,  $p < 0.001$ , 9 months:  $4.1 \pm 1.1$ ,  $p < 0.001$ , 12 months:  $4.1 \pm 1.1$ ,  $p < 0.001$ , 15 months:  $3.9 \pm 0.8$ ,  $p <$

**Table 1. Baseline Characteristics of Nucleotide Analog and Control Groups**

	Nucleotide analog group (n=30)	Control group (n=20)	p value
Age (years)	59 ± 70	60 ± 11	NS
Gender (male/female)	24 / 6	15 / 5	NS
HBeAg (+/-)	11 / 19	8 / 12	NS
HBV-DNA (LEG/mL)	5.7 ± 1.2	5.2 ± 1.1	NS
Total bilirubin (mg/dL)	1.5 ± 0.8	1.3 ± 0.6	NS
Albumin (g/dL)	3.3 ± 0.4	3.5 ± 0.4	NS
AST (U/L)	77 ± 44	56 ± 24	NS
ALT (U/L)	78 ± 58	54 ± 29	NS
Prothrombin time (%)	66 ± 14	69 ± 10	NS
Platelet count (× 10 <sup>4</sup> /μL)	9.3 ± 3.2	10.0 ± 4.8	NS
Child-Pugh grade (A/B/C)	19 / 10 / 1	16 / 3 / 1	NS
HCC stage (I:II:III)	14 : 12 : 4	5 : 8 : 7	NS
Initial treatment of HCC (hepatic resection/RFA/PEI/TAE)	5 / 17 / 3 / 5	7 / 7 / 2 / 4	NS
Follow-up periods* (months)	28.6 ± 16.7	36.3 ± 21.6	NS

\*: Mean ± standard deviation

0.001, 18 months:  $3.8 \pm 0.9$ ,  $p < 0.001$ , 21 months:  $3.9 \pm 0.9$ ,  $p = 0.006$ ). Three of 11 patients in the nucleotide analog group who were positive for HBeAg at the start of nucleotide analog administration exhibited seroconversion to HBeAb positively. The emergence of YMDD mutants was observed in 11 (39.3%) of 28 patients who were administered lamivudine. Of these 11 patients, 6 (54.5%) exhibited breakthrough hepatitis. Ten of these 11 patients were additionally administered adefovir depivoxil at a dose of 10 mg/day and one patient was administered entecavir alone at a dose of 1 mg/day. The mean time until the emergence of YMDD mutant was  $19.0 \pm 7.5$  months after lamivudine administration; YIDD and YVDD variants were observed in 4 and 3 patients, respectively. The type of YMDD mutant could not be identified in the remaining 4 patients. The cumulative incidences of developing resistance at 12, 24 and 36 months were 11.2%, 35.9% and 66.4%, respectively.

### Changes in liver function tests

Each parameter of albumin, AST and ALT significantly improved after nucleotide analog administration compared with the baseline, but total bilirubin, platelet count and prothrombin time did not change (Figs. 1-A, C, 2-A, C). The Child-Pugh scores in the nucleotide analog group were significantly decreased compared with the baseline value although those in the control group were significantly increased (Fig. 3).

We compared the alteration of liver function tests between baseline and the time of re-treatment for recurrent HCCs in 37 patients who underwent curative treatment in the initial treatment. These patients consisted of 22 patients in the nucleotide analog group (5 treated by hepatic resection and 17 by RFA) and 14 patients in the control group (7 by hepatic resection and 7 by RFA). Comparing baseline and re-treatment time points with repeated-measures analysis of

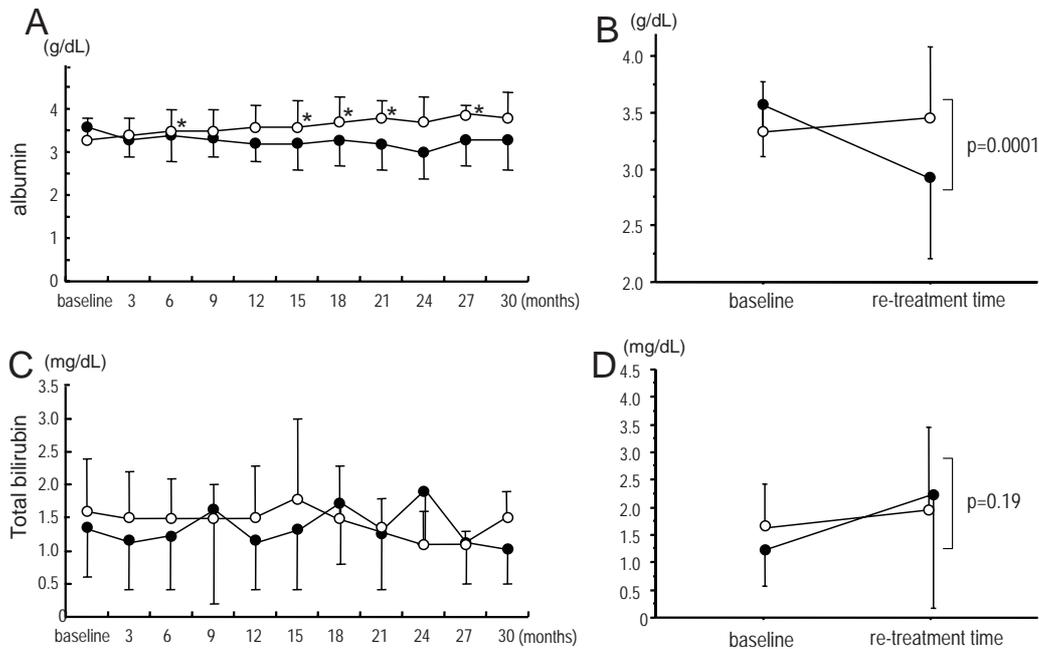
variance, albumin levels increased from  $3.32 \pm 0.46$  g/dL at the baseline to  $3.46 \pm 0.63$  g/dL at the re-treatment time in the nucleotide analog group and those in the control group were  $3.57 \pm 0.45$  g/dL at the baseline and  $2.92 \pm 0.72$  g/dL at the re-treatment time (Fig. 1-B). There was a significant difference between the groups. Similarly, AST, ALT and prothrombin time in the nucleotide analog group were significantly improved compared with the control group (Fig. 1-D, 2-B, D).

We compared the Child-Pugh score at the time of starting nucleotide analog treatment and at the time of re-treatment for recurrent HCC in both groups. In the nucleotide analog group, 8 (47%) of 17 patients had improved at the re-treatment time compared to at the time of starting nucleotide analog, 7 patients (41%) had not changed and 2 (12%) had deteriorated. In the control group, 3 (25%) of 12 patients had improved, 3 (25%) had not changed and 6 (50%) had deteriorated. More patients in the nucleotide analog group improved or maintained their Child-Pugh score than in the control group ( $\chi^2 = 5.148$ ,  $p = 0.0233$ ).

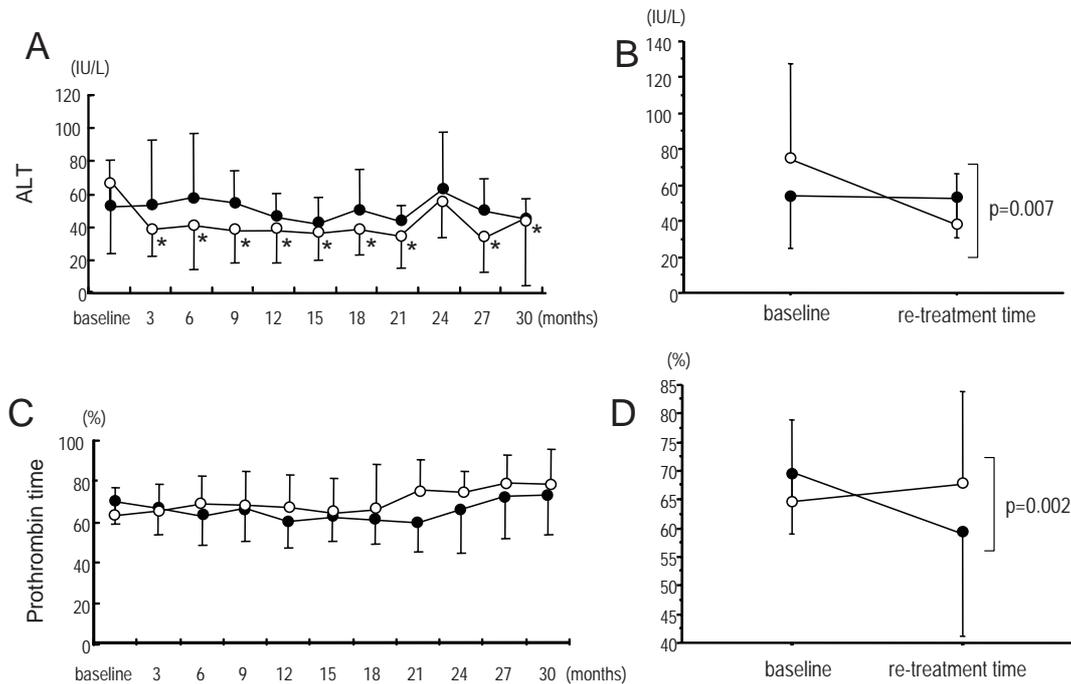
### Comparison of recurrence-free survival rates, cumulative survival rates and cause of death between nucleotide analog and control groups

The mean follow-up period in all patients was  $28.6 \pm 16.7$  months for the nucleotide analog group and  $36.3 \pm 21.6$  for the control group. There was a significant difference in the cumulative survival rate between the two groups ( $p = 0.02$ ) (Fig. 4). As for cause of death, in the control group, 8 (57%) of 14 patients died of HCC progression and 6 (43%) died of progressive liver failure. While, in the nucleotide analog group, 5 (83%) of 6 patients died of HCC progression and only one (17%) died of liver failure after TAE for recurrent HCC.

Next, we compared the recurrence-free survival rate and



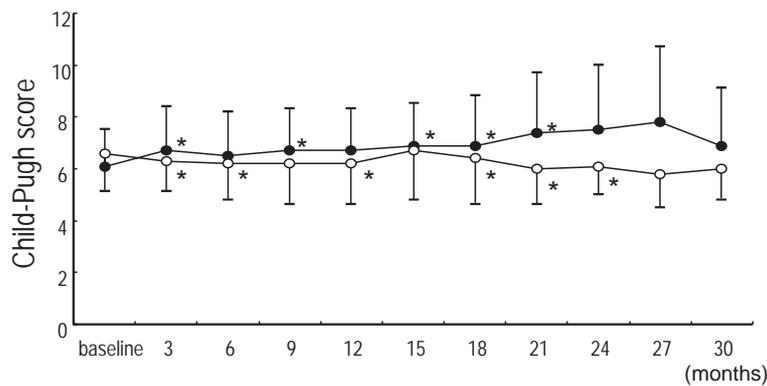
**Figure 1.** Serial changes in (A) serum albumin and (C) total bilirubin in the nucleotide analog group (○) and the control group (●). \*:  $p < 0.05$  vs baseline. Changes from baseline to the re-treatment time between the nucleotide analog group (○) and the control group (●) in curatively treated HCC patients were compared using repeated-measures analysis of variance (B: albumin, D: total bilirubin).



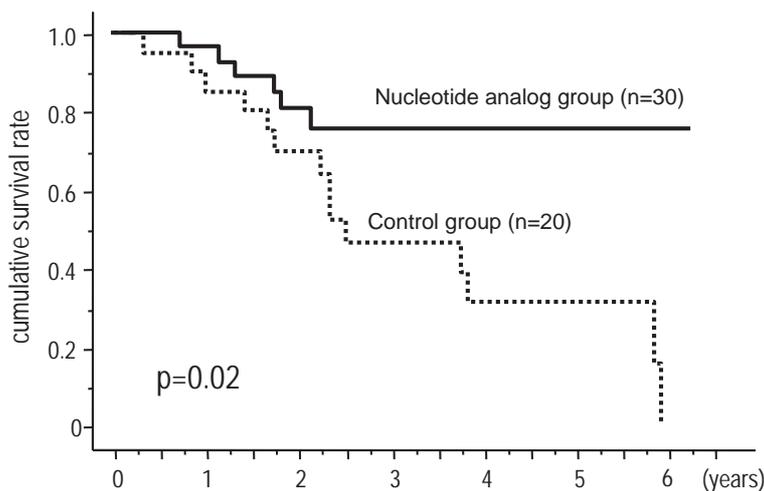
**Figure 2.** Serial changes in (A) alanine aminotransferase (ALT) and (C) prothrombin time in the nucleotide analog group (○) and the control group (●). \*:  $p < 0.05$  vs baseline. Changes from baseline to the re-treatment time between the nucleotide analog group (○) and the control group (●) in curatively treated HCC patients were compared using repeated-measures analysis of variance (B: ALT, D: prothrombin time).

overall survival rate in 36 patients who underwent initial curative treatment. In the nucleotide analog group, 17 (77.3%) of 22 patients and 12 (85.7%) of 14 patients in the

control group had HCC recurrence. The recurrence-free survival rate did not differ between the groups (Fig. 5-A); however, there was a significant difference in the cumulative



**Figure 3.** Serial changes in the Child-Pugh score in the nucleotide analog group (○) and the control group (●). The Child-Pugh score in the nucleotide analog group was significantly decreased compared with the baseline value (baseline:  $6.6 \pm 1.5$ , 3 months:  $6.2 \pm 1.2$ ,  $p=0.023$ , 6 months:  $6.2 \pm 1.4$ ,  $p=0.016$ , 9 months:  $6.2 \pm 1.6$ ,  $p=0.056$ , 12 months:  $6.2 \pm 1.6$ ,  $p=0.02$ , 15 months:  $6.7 \pm 1.9$ ,  $p=0.41$ , 18 months:  $6.4 \pm 1.8$ ,  $p=0.045$ , 21 months:  $6.0 \pm 1.4$ ,  $p=0.007$ , 24 months:  $6.1 \pm 1.1$ ,  $p=0.046$ ) although that in the control group was significantly increased (baseline:  $6.1 \pm 1.4$ , 3 months:  $6.7 \pm 1.7$ ,  $p=0.034$ , 6 months:  $6.5 \pm 1.7$ ,  $p=0.083$ , 9 months:  $6.7 \pm 1.6$ ,  $p=0.036$ , 12 months:  $6.7 \pm 1.6$ ,  $p=0.061$ , 15 months:  $6.9 \pm 1.6$ ,  $p=0.011$ , 18 months:  $6.9 \pm 1.9$ ,  $p=0.044$ , 21 months:  $7.4 \pm 2.3$ ,  $p=0.036$ , 24 months:  $7.5 \pm 2.5$ ,  $p=0.063$ ). \*:  $p < 0.05$  vs baseline



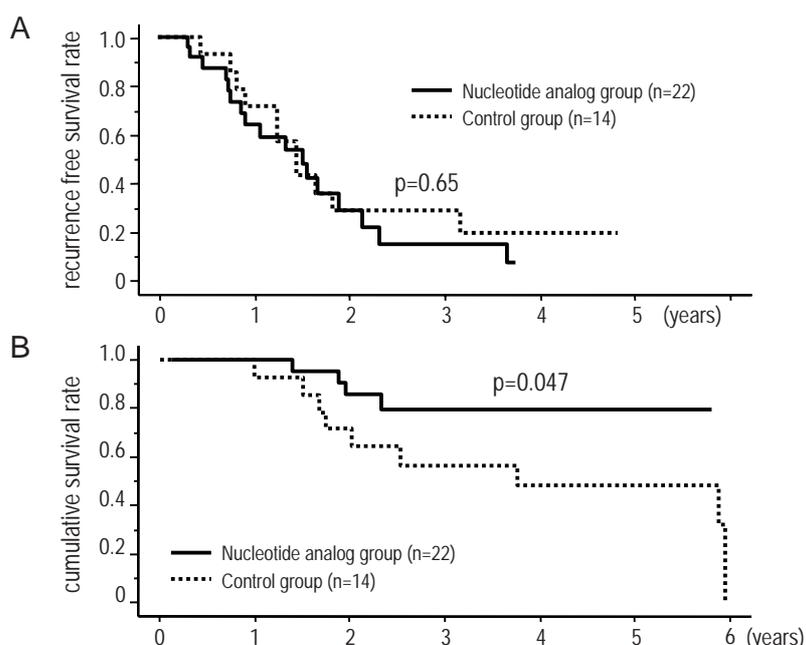
**Figure 4.** Comparison of cumulative survival rates in the nucleotide analog group (n=30) and the control group (n=20).

survival rate between the groups in patients who underwent curative treatment ( $p=0.047$ ) (Fig. 5-B).

## Discussion

This study showed that nucleotide analogs improved the overall survival rate in all HCC patients. In order to evaluate whether nucleotide analogs contribute to preventing the recurrence of HCC after treatment, we selected 36 patients who were successfully treated for HCC by RFA or hepatic resection; however, nucleotide analogs could not reduce the recurrence rate of HCC after curative treatment by RFA or hepatic resection. Indeed, Piao et al (19) and Kuzuya et al (20) have also reported that the cumulative recurrence rates of HCC after initial and curative treatment for HCC did not decrease by the administration of nucleotide analog. There

are two types of HCC recurrence, intrahepatic metastasis and multicentric occurrence. It is difficult to distinguish these using imaging modalities or pathological findings. Continuous treatment with nucleotide analog in patients with chronic hepatitis B or cirrhosis was reported to reduce the risk of hepatocellular carcinoma in a randomized controlled study (17) and a multicenter retrospective study (18). Furthermore, elevated HBV DNA levels were reported to be strong risk predictors of HCC recurrence (21) and the HBV DNA level in the nucleotide analog group was markedly decreased; therefore, it should be possible for the administration of nucleotide analogs to reduce the incidence of multicentric recurrence. In this study, however, because HCC recurrence generally occurred within three years after treatment, it was considered to be mainly derived from intrahepatic metastases. To confirm the efficacy of nucleotide ana-



**Figure 5.** A: Comparison of recurrence-free survival rates in the nucleotide analog group (n=22) and the control group (n=14) in curatively treated HCC patients. B: Comparison of cumulative survival rates in the nucleotide analog group (n=22) and the control group (n=14) in curatively treated HCC patients.

logs against the recurrence of HCC, further studies with a larger number of patients and longer follow-up period are needed.

The suppression of HBV replication by nucleotide analogs delays progression to cirrhosis in patients with chronic hepatitis B or deterioration of the Child-Pugh grade in cirrhotic patients (14, 15, 17, 18). In the present study, serum ALT and AST levels decreased and serum albumin increased, involving reduction of the serum HBV-DNA level compared with before the administration of nucleotide analogs. The alterations of serum ALT, AST, albumin and prothrombin time in the nucleotide analogs group significantly improved compared with the control group when we evaluated changes from baseline to re-treatment time for recurrent HCCs in the two groups. Furthermore, Child-Pugh scores at the time of HCC recurrence in the nucleotide analog group were significantly improved compared with the control group. These results indicated that remnant liver functions in the nucleotide analog group were maintained better than those in the control group. Remnant liver function is an important factor in selecting further treatment for HCC recurrence and is a prognostic factor for the survival rate (22). Therefore, patients treated by nucleotide analogs

have several re-treatment options for HCC recurrence, resulting in improving the cumulative survival rate.

The most serious problem in patients treated with lamivudine is breakthrough hepatitis by the emergence of YMDD mutants, resulting in fatal liver failure (23). In the present study, YMDD mutants were found in 39.3% of lamivudine-treated patients and 54.5% of these patients exhibited breakthrough hepatitis; however, none resulted in fatal liver failure because adefovir dipivoxil or entecavir were administered immediately. The appearance rate of YMDD mutants in this study was similar to previous reports (16). Recently, we chose entecavir as the first choice in place of lamivudine because there are fewer mutants for entecavir than for lamivudine (16).

In conclusion, treatment with nucleotide analogs for HBV-related HCC improves remnant liver function and provides the changes necessary for receiving effective treatment options for recurrent HCC, resulting in prolonged survival, however, there are limitations in interpreting the results because this study is a retrospective study and not a randomized one. Our findings should be confirmed by prospective and randomized studies with larger numbers of patients and a longer follow-up period.

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