Yotsuyanagi *et al*[23] reported that genotype A is more common in patients with AHB in the metropolitan region than in other regions. Sugauchi *et al*[41] found that, in patients with AHB, the proportion with genotype A has increased over time. The present study indicates that the number of patients with AHB in Japan would not have decreased. We found that the proportion of patients with genotype A infection is increasing in the 28 national hospitals in Japan (6.0% in the 1st period, 15.4% in the 2nd, and 39.4% in the 3rd (figure 2)), with the prevalence much higher in the capital than other regions (35.5% vs 19.8% (table 2)).

In this study, there was a time lag in the increase in genotype A infection between the capital region and other regions of Japan (table 2). In the capital region, the prevalence of genotype A started to increase in the late 1990s, and kept increasing through the early 2000s (4.8% in the 1st period, 29.3% in the 2nd, 50.0% in the 3rd, and 56.3% in 2009). In other regions, by contrast, the frequency of genotype A did not change during the late 1990s, and increased significantly in the 2000s (6.5% in the 1st period, 8.5% in the 2nd, 33.1% in the 3rd, and 34.4% in 2009). Thus infiltration of genotype A infection into other regions occurred 5–6 years behind the epidemic in the capital region. This indicates that genotype A infection originated in the capital region and then spread to other areas of Japan.

Some genotypes are classified into several subgenotypes, and they have distinct geographical distributions.[42] Hence, subgenotypes are useful in tracing the route of HBV infection. By phylogenetic analysis (figures 3 and 4), 88.6% of genotype A isolates had the European–American type (A2), and the remaining 11.4% possessed the Asian–African type (A1). Likewise, 76.7% of genotype B isolates had Asian types (B2–B4), and the remaining 23.3% possessed the type endemic to Japan (B1). Of the 157 HBV isolates of genotype A or B, 147 (93.6%) had subgenotypes foreign to Japan. They are thought to have been transmitted from foreign sex workers, and spread among certain populations who share particular sexual behaviours in Japan.[41]

Of note, some HBV isolates of distinct subgenotypes possessed an identical sequence in the preS1/S2/S gene. The isolates of subgenotype A2 were prominent in this regard, and more often had the same sequence than those of other subgenotypes, such as A1, B1 and B2. The high prevalence of subgenotype A2 isolates with an identical sequence would not have been caused by cross-contamination. If cross-contamination had occurred, it would have affected isolates of all subgenotypes, and not influenced subgenotype A2 isolates preferentially. As many as 35% of subgenotype A2 isolates had an identical sequence, and those with the same sequence increased to 56.3% in the recent 2009 survey in Metropolitan Tokyo. Furthermore, some subgenotype A2 isolates in groups I, III and VII clustered locally within short periods, whereas others in groups II and VI were scattered widely over a long period of time. On the basis of these results, it is tempting to speculate that some subgenotype A2 strains would have been transmitted from person to person without undergoing mutations for many years.

In summary, the present study indicates the following. (1) AHB in the 28 national hospitals in Japan has not decreased, because genotype A infections are increasing. (2) Genotype A infections started to increase in the capital region, and then spread to local areas 5–6 years later. (3) Approximately 90% of genotype A in patients with AHB is subgenotype A2. (4) Subgenotype A2 strains with an identical sequence are spreading among younger generations with high sexual activity. (5) On the basis of the results obtained, AHB in Japan is not decreasing, because HBV of subgenotype A2 is prevailing in particular subpopulations at high risk. Finally, in order to prevent further increases in AHB in Japan, universal vaccination of young people deserves consideration.

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Contributors YT, HY and HI designed data collection tools, monitored data collection for the whole study, wrote the statistical analysis plan, cleaned and analysed the data. YT, HY and YM drafted and revised the paper. HY, NM, MN, EM, TK, YW, TM, MS, TH, TS, YM, TK, MT, HK, HO, SH and SA collaborated in data and sample collection.

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Original Article

Anti-hypoalbuminemic effect of branched-chain amino acid granules in patients with liver cirrhosis is independent of dietary energy and protein intake

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Aim: A multicenter prospective intervention study was conducted in 204 patients with uncompensated liver cirrhosis to explore the influence of dietary intake and patient clinical characteristics on improvement of hypoalbuminemia at weeks 12 and 24 of treatment with branched-chain amino acid (BCAA) granules.

Methods: The primary endpoint set in this study was improvement of hypoalbuminemia in patients with liver cirrhosis. The dietary energy and protein intake per day were estimated based on the results of a survey on diet during a 3-day period preceding the start of the study.

Results: As for the primary endpoint, the mean serum albumin level increased significantly at weeks 12 and 24 of BCAA treatment, compared with the baseline level. The mean Child–Pugh score decreased significantly at weeks 12 and 24 of treatment as compared to the mean baseline score. There was a significant increase in the serum albumin level following treatment with BCAA granules regardless of energy intake and of protein intake. The incidence of ascites and edema significantly decreased in the overall patient population both at weeks 12 and 24 of treatment, compared with the baseline incidence. A subgroup analysis conducted in patients stratified according to changes in the serum albumin level at week 12 of treatment as against baseline showed that the incidence of ascites/edema was significantly reduced not only in the increased albumin group but in the unchanged albumin group.

Conclusion: The present data suggest that the anti-hypoalbuminemic effect of BCAA treatment in patients with liver cirrhosis is independent of dietary intake.

Key words: albumin, branched-chain amino acids, food intake, hepatic failure, liver cirrhosis

INTRODUCTION

ALBUMIN IS THE most abundant circulating protein in serum at concentrations as high as 4.2 to 5.1 g/dL. The known physiological functions of albumin include maintenance of colloid osmotic pressure, transport of numerous substances, supply of amino acids, pH buffering, and radical scavenging actions.[1,2]

Serum albumin is the protein synthesized in and secreted by parenchymal cells of the liver into blood and has long been used as an indicator of protein nutrition. In patients with chronic hepatic disorders, especially in liver cirrhosis with concurrent hypoalbuminemia, the serum albumin level is regarded as important not merely as an indicator of protein nutrition but also as factors to estimate hepatic functional reserve and prognosis.[3]

In recent years branched-chain amino acid (BCAA) granules have become available and prescribed for improvement of hypoalbuminemia in patients with

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uncompensated liver cirrhosis who have adequate dietary intake in Japan. It is recommended as grade-A treatment for improvement of hypoalbuminemia in patients with liver cirrhosis in the Japanese guidelines for the treatment of liver cirrhosis published by the Japanese Society of Gastroenterology in 2010.[4] Furthermore, it has been reported in Italy[5] and in Japan[6] that multicenter randomized controlled clinical trials demonstrated not only improvement of hypoalbuminemia but also suppression of the development of serious complications such as aggravation of hepatic failure, that is, prolongation of time to onset of events related to prognosis for survival, in patients with liver cirrhosis receiving BCAA treatment.

Improvement of hypoalbuminemia brought about by treatment with BCAA granules owes primarily to supplementation of deficient BCAA as substrates required for protein synthesis. A recent clarification of the intracellular signal transduction mechanism showed that BCAA activates the mTOR signaling pathways to stimulate initiation of albumin protein translation, thereby enhancing albumin synthesis.[7,8]

Despite the established efficacy of BCAA granules in the treatment of hypoalbuminemia in patients with liver cirrhosis as above, treatment with BCAA granules is not necessarily associated with elevation of the serum albumin level in all patients with liver cirrhosis, and conducting further investigation was considered to determine factors involved in the improvement of hypoalbuminemia brought on by BCAA treatment. We therefore conducted a multicenter prospective intervention study to explore the influence of energy intake and protein intake on the improvement of hypoalbuminemia brought on by BCAA therapy.

METHODS

Study design and protocol

THIS STUDY WAS designed as a multicenter prospective intervention study to clarify the actual state of treatment and dietary intake status in patients with uncompensated liver cirrhosis. Thirty-three medical institutions affiliated with the National Hospital Organization of Japan participated in this study, in which 204 patients with uncompensated liver cirrhosis were enrolled. The study subjects received oral treatment with BCAA granules (Livact Granules, Ajinomoto Pharmaceuticals Co., Ltd, Tokyo) at 4 g (containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine) three times daily after meals.

Patients with uncompensated liver cirrhosis who had hypoalbuminemia were included in the study, except for those who violated any of the following exclusion criteria: (i) a history of treatment with BCAA granules within 24 weeks before enrollment in this study; (ii) suspected concurrent hepatocellular carcinoma; (iii) a history of hepatocellular carcinoma; and (iv) congenital amino acid metabolic abnormality.

The primary endpoint in this study was improvement of hypoalbuminemia at weeks 12 and 24 after the start of BCAA treatment. The effects of dietary intake and patient clinical characteristics were assessed as the secondary endpoints. Baseline patient clinical characteristics included gender, age, height, body weight, etiology of liver cirrhosis, previous medical history, complications, clinical manifestations, and routine laboratory test data. Child–Pugh scoring and grading of encephalopathy and ascites were carried out according to Pugh's modified classification.[9]

The energy intake per day and protein intake per day were estimated based on data obtained in a 3-day diet survey using a disposable camera conducted by Asahi Kasei Life Support Corporation at the start of BCAA treatment.[10]

Written informed consent to participate in this study was obtained from every patient. The protocol for this study was approved by the ethics committee of the National Hospital Organization, and the study was conducted in accordance with the Ethical Guidelines for Clinical Studies.[11]

Rationale for the number of study enrollees

The sample size (n) required for determining the mean of the study population is calculated using the following formula:

$$n = (\text{Standard deviation of the estimated study population})^2 \times (1.96/\text{precision})^2$$

To estimate the mean energy intake with the 95% confidence interval set within 10% of the mean according to a previous study:[12]

$$(478.78)^2 \times (1.96/(1605.2/10))^2 = 34.2 \text{ (35 patients)}$$

(Mean energy intake for 10 patients with liver cirrhosis and standard deviation of the mean: 1605.2 ± 478.78 kcal)

To estimate the mean protein intake with the 95% confidence interval set within 10% of the mean according to the above study:

$$(15.64)^2 \times (1.96/(64.7/10))^2 = 22.4 \text{ (23 patients)}$$

(Mean protein intake for 10 patients with liver cirrhosis and standard deviation of the mean: 64.7 ± 15.64 kcal)

Thus, approximately 35 patients were estimated to be required for estimating the mean energy intake and mean protein intake with the 95% confidence interval set within 10% of the mean. Inasmuch as patients whose pertinent values were below the standard energy intake (25 kcal/kg body weight) and/or below the standard protein intake (1 g/kg body weight) accounted for 25% of the study population in the above study, it was estimated that a total of 140 patients are needed in order to secure at least 35 study subjects for the population. Taking account of possible dropouts during the study period, we finally decided that 200 patients should be enrolled in the study.

Statistical analysis

The analyses were conducted using JMP9.01 and SAS9.2 (both from SAS Institute Inc.). Continuous data such as serum albumin levels over time were expressed as mean ± standard deviation of the mean. Statistical tests used included paired *t*-test, Wilcoxon signed-rank test and McNemar's test, and results were displayed in terms of the *P*-value. The level of significance was assessed as two-sided 5%; in the testing at individual time points, nevertheless, the level of significance was set as two-sided 2.5% by Bonferroni correction, taking multiplicity into account.

RESULTS

Disposition of patients and clinical characteristics

A TOTAL OF 204 patients were enrolled in this study. For the efficacy evaluation, 135 patients were eventually included in the analysis with the exception of the following 69 patients: three patients who were definitely diagnosed as having hepatocellular carcinoma after study enrollment, nine patients who took other amino acid or albumin products during the study period, 19 patients who withdrew from the study prior to week 12 after the start of the study, hence lacking in data other than baseline data, and 38 patients whose serum albumin level had exceeded 3.5 g/dL prior to the study. Table 1 shows clinical characteristics of patients included in the analysis.

Evaluation of efficacy

For the primary endpoint, the mean serum albumin level increased significantly both at week 12 (3.26 ± 0.40) and week 24 (3.31 ± 0.46), compared with the baseline level (3.11 ± 0.35) ($P < 0.0001$ and $P < 0.0001$, respectively, Fig. 1).

The mean Child–Pugh score decreased significantly both at week 12 (7.4 ± 1.4) and week 24 (7.2 ± 1.5) as compared to the baseline mean score (7.8 ± 1.4) ($P = 0.0080$ and $P = 0.0008$, respectively); indicating improvement of hepatic functional reserve (Fig. 2).

Energy intake and protein intake data were assessed according to the following cut-off values (energy intake: 25 kcal/kg or more (33.7 ± 5.7) and less than 25 kcal/kg (19.6 ± 4.0), and protein intake: 1.0 g/kg or more (1.35 ± 0.24) and less than 1.0 g/kg (0.78 ± 0.16)), respectively. For these categories of each parameter, the mean serum albumin level elevated significantly at weeks 12 and 24 as compared to the baseline level (Fig. 3-1,3-2).

A total of 131 patients whose serum albumin measured at both weeks 0 and 12 were classified into the following three groups according to changes in the serum albumin level at week 12 from the baseline: increasing group with changing the serum albumin level by 0.2 g/dL or more (60 patients), no-change group with a change between −0.1 g/dL and 0.1 g/dL (55 patients) and decreasing group with changing the serum albumin level by at least −0.2 g/dL (16 patients). The clinical characteristics among these groups were assessed for any bias, and there were significant differences with respect to ascites, energy intake and protein intake (Table 1). Among them, for energy intake and protein intake, the mean serum albumin level increased as compared to the baseline level for the categories as described above (Fig. 3-1,3-2). As for ascites, the response was assessed in three categories (ascites: none, mild, or moderate), and the mean serum albumin level increased as compared to the baseline level for all these categories (Fig. 4).

To explore the influence of the above factors on the serum albumin level, multiple regression and simple regression analyses were carried out using the absolute change in the serum albumin level at week 24 versus the baseline level as a response variable and the three factors as explanatory variables. None of the three factors proved to have any significant influence.

Of clinical manifestations, the proportion of patients who reported ascites/edema to the whole analysis population was 49.6% at baseline and then significantly

Table 1 Baseline characteristics of the study patients

Changes in the serum albumin level from 0 to 12 weeks	All	Increase ≥0.2	No change ≤0.1 and ≥−0.1	Decrease ≤−0.2	*P*-value
n	135	60	55	16	
Sex					
Male	64 (47.4%)	29 (48.3%)	22 (40.0%)	10 (62.5%)	$P = 0.2644$
Female	71 (52.6%)	31 (51.7%)	33 (60.0%)	6 (37.5%)	
Age (years)	69 (35–89)	67.5 (35–85)	69 (38–89)	70.5 (55–84)	$P = 0.7159$
Height (cm)	155.9 (136–183)	155.8 (136–183)	153.6 (140–177)	158 (142–176)	$P = 0.7610$
Weight (kg)	57.3 (32.0–113.0)	55.3 (32–113)	58 (38–99)	55 (36–89)	$P = 0.6447$
BMI	22.8 (15.7–42.9)	22.7 (15.7–33.7)	23.0 (17.6–42.9)	23.0 (16.0–28.7)	$P = 0.6902$
Cause of hepatic cirrhosis					
HCV	75 (55.6%)	26 (43.3%)	34 (61.8%)	11 (68.8%)	$P = 0.3833$
HBV	6 (4.4%)	3 (5.0%)	3 (5.5%)	0 (0.0%)	
AL	19 (14.1%)	11 (18.3%)	6 (10.9%)	2 (12.5%)	
Other	35 (25.9%)	20 (33.3%)	12 (21.8%)	3 (18.8%)	
Child–Pugh Score					
A	16 (14.3%)	5 (10.9%)	9 (18.8%)	1 (6.7%)	$P = 0.3695$
B	82 (73.2%)	33 (71.7%)	36 (75.0%)	12 (80.0%)	
C	14 (12.5%)	8 (17.4%)	3 (6.3%)	2 (13.3%)	
Diabetes					
None	103 (76.3%)	48 (80.0%)	40 (72.7%)	11 (68.8%)	$P = 0.5268$
Diabetes	32 (23.7%)	12 (20.0%)	15 (27.3%)	5 (31.3%)	
Hepatic encephalopathy					
None	121 (89.6%)	54 (90.0%)	49 (89.1%)	14 (87.5%)	$P = 0.5319$
Grade I	11 (8.1%)	4 (6.7%)	6 (10.9%)	1 (6.3%)	
Grade II	1 (0.7%)	1 (1.7%)	0 (0.0%)	0 (0.0%)	
Unknown	2 (1.5%)	1 (1.7%)	0 (0.0%)	1 (6.3%)	
Ascites					
None	89 (65.9%)	32 (53.3%)	44 (80.0%)	9 (56.3%)	$P = 0.0131$
Mild	23 (17.0%)	11 (18.3%)	7 (12.7%)	5 (31.3%)	
Moderate	16 (11.9%)	13 (21.7%)	1 (1.8%)	2 (12.5%)	
Unknown	7 (5.2%)	4 (6.7%)	3 (5.5%)	0 (0.0%)	
Edema					
None	99 (73.3%)	42 (70.0%)	40 (72.7%)	14 (87.5%)	$P = 0.3696$
Edema	36 (26.7%)	18 (30.0%)	15 (27.3%)	2 (12.5%)	
Serum albumin (g/dL)	3.2 (2.0–3.5)	3.15 (2.0–3.5)	3.2 (2.4–3.5)	3.3 (2.7–3.5)	$P = 0.5449$
Platelet (×10 000/μL)	8.1 (2.2–39.4)	8.3 (2.9–39.4)	7.9 (3.1–21.5)	8.05 (2.2–23.3)	$P = 0.3289$
AST (IU/L)	48 (17–336)	43 (19–336)	50 (17–123)	54 (20–107)	$P = 0.3262$
ALT (IU/L)	32 (7–320)	26.5 (9–320)	36 (10–113)	40 (7–136)	$P = 0.0949$
Total bilirubin (mg/dL)	1.2 (0.3–7.8)	1.22 (0.4–4.5)	1.2 (0.3–7.8)	1.09 (0.5–4.0)	$P = 0.7592$
PT (INR)	1.26 (0.90–2.77)	1.28 (0.90–2.77)	1.25 (0.96–1.71)	1.23 (1.01–1.46)	$P = 0.1984$
BTR	2.62 (1.40–20.70)	3.02 (1.44–6.32)	2.50 (1.40–20.70)	2.65 (2.06–9.12)	$P = 0.4635$
Calorie intake (kcal/kg)	29.3 (8.2–50.1)	27.1 (8.2–43.0)	30.9 (12.7–50.1)	33.3 (16.6–42.7)	$P = 0.0132$
Protein intake (g/kg)	1.16 (0.31–2.05)	1.07 (0.31–1.69)	1.17 (0.53–2.05)	1.39 (0.64–1.92)	$P = 0.0117$

Data were assessed using χ^2 test or Kruskal–Wallis test.
ALT, alannine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BTR, molar ratio of total branched-chain amino acid to tyrosine; PT, prothrombin time.

decreased at week 12 (37.9%) and week 24 (27.0%) ($P = 0.0112$ and $P < 0.0001$, respectively). A subgroup analysis with stratification according to the absolute change in the serum albumin level at week 12 from baseline revealed that the percentage of patients with ascites/edema decreased in the increasing group and the no-change group, whereas in the decreasing group, no such improvement was noted (Fig. 5-1 to 5-4).

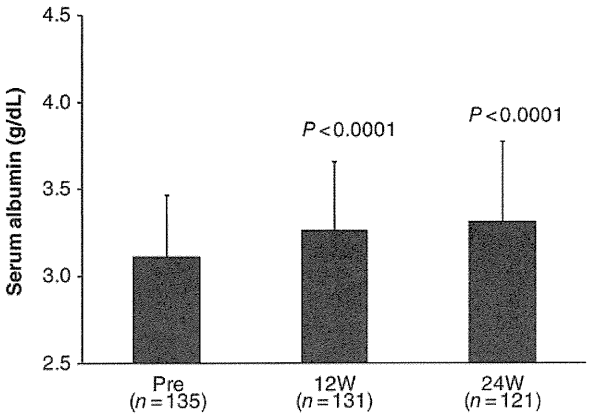

Figure 1 Changes in serum albumin levels (mean, SD). Data were assessed using paired *t*-test (in comparison to the baseline level) and *P*-values are presented.

DISCUSSION

AS REPORTED IN a previous study,[6] a significant elevation in the serum albumin level and a significant improvement in hepatic functional reserve in terms of the Child–Pugh score were observed following treatment with BCAA granules in the present study. At week 12 of study treatment, however, the serum albumin level elevated in 60 patients (45.8%), while it was unchanged in 55 patients (42.0%) and decreased in 16 patients (12.2%). We thus investigated whether these differences in therapeutic response to BCAA treatment are due to patient clinical characteristics. The investigation revealed significant differences among the above

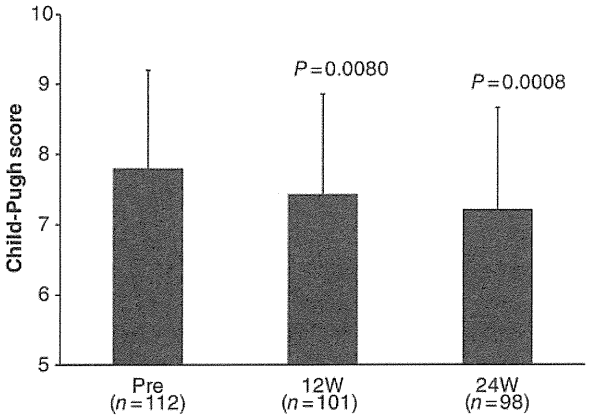

Figure 2 Changes in Child–Pugh score (mean, SD). Data were assessed using Wilcoxon signed ranks test (in comparison to the baseline score) and *P*-values are presented.

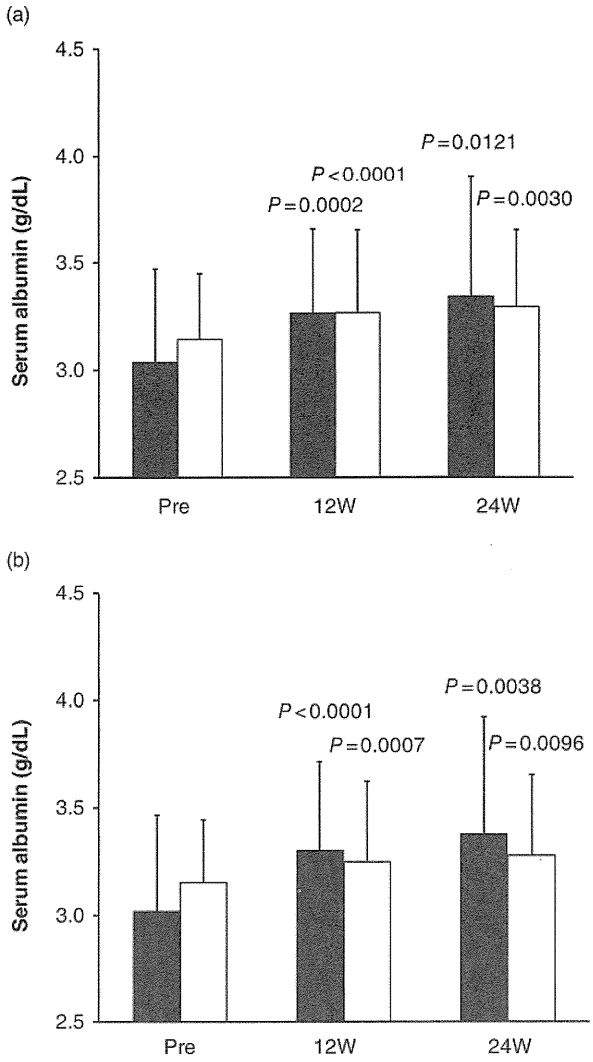

Figure 3 (1) Changes in serum albumin levels by energy intake (mean, SD). Data were assessed using paired *t*-test (in comparison to the baseline level) by energy intake, and *P*-values are presented. The numbers of patients with less than 25 kcal/kg and 25 kcal/kg or more of energy intake were 41 and 90 at baseline, 40 and 87 at week 12, and 37 and 80 at week 24. ■ <25 kcal/kg; □ ≥25 kcal/kg. (2) Changes in serum albumin levels by protein intake (mean, SD). Data were assessed using paired *t*-test (in comparison to the baseline level) by protein intake, and *P*-values are presented. The numbers of patients with less than 1.0 g/kg and 1.0 g/kg or more of protein intake were 41 and 90 at baseline, 39 and 88 at week 12, and 36 and 81 at week 24. ■ <1.0 g/kg; □ ≥1.0 g/kg.

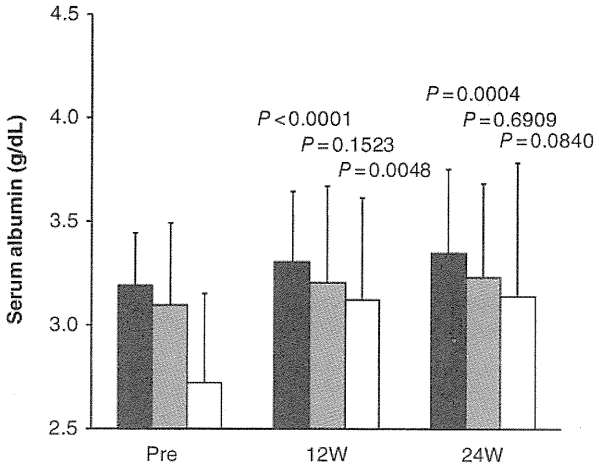

Figure 4 Changes in serum albumin levels by degree of ascites (mean, SD). Data were assessed using paired *t*-test (in comparison to the baseline status) by degree of ascites, and *P*-values are presented. The number of patients with no ascites, mild ascites, and moderate ascites were 89, 23, and 16 at baseline, 85, 23, and 16 at week 12, and 81, 21, and 13 at week 24. ■ none; ▨ mild; □ moderate.

three subgroups with respect to baseline ascites, energy intake and protein intake. Hypoalbuminemia improved irrespective of the degree of ascites. Regarding energy intake and protein intake, their influence was assessed in accordance with the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines[13] using the definition of an adequate energy intake and protein intake of 25 kcal/kg and 1.0 g/kg or more, respectively. In the present study, hypoalbuminemia improved even in patients with liver cirrhosis whose energy intake and/or protein intake was inadequate. We explored the influence in 10 patients (7.4%) whose baseline energy intake was below 1000 kcal, which is considered to be a minimal amount of energy intake. Hypoalbuminemia improved even in these patients with an absolute change in the serum albumin level of 0.47 ± 0.37 at week 12 ($P = 0.0028$) and 0.48 ± 0.74 at week 24 ($P = 0.0904$). The results suggest the possibility that the effect of BCAA treatment may be independent of dietary intake.

The dietary energy intake and protein intake that had been obtained from the 3-day diet survey at the start of BCAA treatment did not necessarily remain practically consistent throughout the study period. In view of this, we checked relevant data on 93 patients who underwent the diet surveys both at the beginning baseline and at week 24 of study treatment. The results showed that the dietary energy intake was either less than ≤25 kcal at both assessment time points or ≥25 kcal or more at both assessment time points in 72% of patients, hence consistent, and the protein intake was either less than ≤1.0 g/kg at both assessment time points or ≥1.0 g/kg or more at both assessment time points in 74% of patients, hence again consistent. It is thus considered reasonable to conclude that the anti-hypoalbuminemic effect of BCAA granules treatment is independent of dietary energy and protein intake. Nevertheless, it is beyond dispute that dietary counseling is important in satisfactorily maintaining the nutritional condition of liver cirrhosis patients, as has been widely advocated.

Taking a BMI of ≤18.5 as a protein-energy malnutrition state in order to discuss the effects of the nutritional condition closely related to dietary intake, there were as few as seven patients (5.3%) with BMI ≤18.5 among 132 patients whose BMI data were available; this percentage was approximate to 5.5% for patients with BMI ≤18.5 among patients enrolled in the Long-Term Survival Study (LOTUS) study. The conclusion that the effect of BCAA granules treatment is independent of dietary energy and protein intake may be ascribed to the fact that the present study population comprised liver cirrhosis patients in Japan where recent patients with protein-energy malnutrition constitute only a small proportion. As for the subgroups in Table 1, the percentage of patients with BMI ≤18.5 was 3.5% for the increasing group, 5.5% for the no-change group and 13.3% for the decreasing group; there was no significant difference among the three subgroups ($P = 0.3242$). The percentage of patients with BMI ≤18.5 tended to be higher in the decreasing group though there were fewer patients (15) in this subgroup; therefore, a relationship between the lack of serum albumin level response to BCAA treatment and the protein-energy malnutrition state cannot be completely ruled out.

The LOTUS study[6] and other studies reported that improvement of symptoms such as ascites/edema, encephalopathy, and jaundice was observed in addition to amelioration of hypoalbuminemia in patients treated with BCAA granules. As the percentage of ascites/edema, encephalopathy, and jaundice (total bilirubin: 2.0 mg/dL or more) was 49.6%, 8.9%, and 23.7% at baseline in the present study, we focused on ascites/edema with the highest incidence. The percentage of patients who reported ascites/edema significantly decreased in association with improvement of the serum albumin level following treatment with BCAA in the increasing group at week 12. This percentage also significantly decreased in the no-change group at week

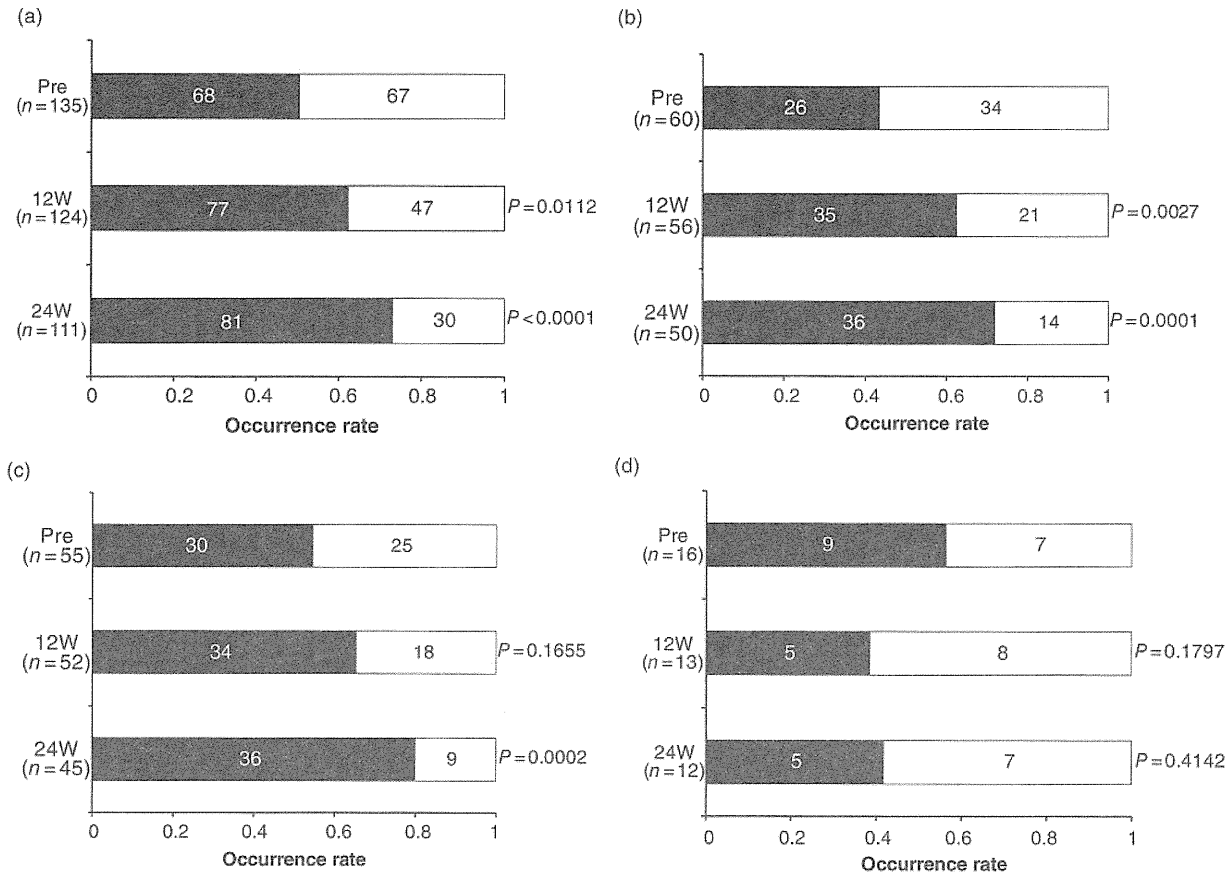

Figure 5 (1) Incidence of ascites/edema. Figures below each horizontal bar represent the number of patients falling into pertinent categories. Data were assessed using McNemar's test (in comparison to the baseline incidence) and *P*-values are presented. ■ none; □ ascites/edema. (2) Incidence of ascites/edema (Patients with increased serum albumin level at week 12). Figures below each horizontal bar represent the number of patients falling into pertinent categories. Data were assessed using McNemar's test (in comparison to the baseline incidence) and *P*-values are presented. ■ none; □ ascites/edema. (3) Incidence of ascites/edema (Patients with unchanged serum albumin level at week 12). Figures below each horizontal bar represent the number of patients falling into pertinent categories. Data were assessed using McNemar's test (in comparison to the baseline incidence) and *P*-values are presented. ■ none; □ ascites/edema. (4) Incidence of ascites/edema (Patients with decreased serum albumin level at week 12). Figures below each horizontal bar represent the number of patients falling into pertinent categories. Data were assessed using McNemar's test (in comparison to the baseline incidence) and *P*-values are presented. ■ none; □ ascites/edema.

12. Although the importance of the serum albumin level in assessing the development of ascites/edema has been reported,[14] the improvement of ascites/edema observed in the above no-change group cannot be explained by the serum albumin level alone since it remained unchanged even at week 24 in this group. The percentage of patients receiving concomitant diuretics for treatment of ascites/edema was 65.0% for the increasing group, 43.6% for the no-change group and 62.5% for the decreasing group, indicating a smaller percentage in the no-change group. This suggests that the use of concomitant diuretics might be unrelated to the improvement of ascites/edema in the no-change group. Watanabe *et al.*[15] reported that the oxidized albumin level increased with the progress of the disease state of hepatic disorders. Sakata *et al.*[16] showed that the oxidized albumin ratio correlated more positively with the extracellular fluid level than the serum albumin level. Therefore, the oxidized albumin ratio in patients with chronic hepatic disorders may be associated with body water retention such as ascites/edema. BCAA treatment reduced the oxidized/reduced albumin level once

elevated in patients with uncompensated liver cirrhosis.[17] It is thus suggested that the improvement of ascites/edema observed in the serum albumin no-change group in this study was brought about by a decreased oxidized albumin ratio following BCAA treatment, and exploration of this hypothesis is currently underway.

In conclusion, hypoalbuminemia significantly improved as well as the Child–Pugh score, an indicator for hepatic functional reserve, in patients receiving BCAA treatment. Improvement of hypoalbuminemia was also noted in patients with liver cirrhosis showing inadequate energy and protein intake. Furthermore, it is important to continue BCAA treatment beyond week 12 because the incidence of ascites/edema decreased at week 24 of treatment even in patients showing no change in the serum albumin level at week 12.

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APPENDIX

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection

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Abstract

Background Nucleotide analogues have recently been approved for the treatment of patients with hepatitis B virus (HBV) infection. However, it is still controversial whether the decrease of HBV-DNA amount induced by treatment with nucleotide analogues can reduce the risk of hepatocellular carcinoma (HCC) development in HBV patients.

Methods A total of 293 HBV patients without HCC who were treated with lamivudine (LAM) were enrolled in a multicenter trial. The incidence of HCC was examined after the start of LAM therapy, and the risk factors for liver carcinogenesis were analyzed. The mean follow-up period was 67.6 ± 27.4 months.

Results On multivariate analysis for HCC development in all patients, age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, cirrhosis, and median HBV-DNA levels of ≥4.0 log copies/ml during LAM treatment were significant risk factors. The cumulative carcinogenesis rate at 5 years was

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3% in patients with chronic hepatitis and 30% in those with cirrhosis. For the chronic hepatitis patients, the log-rank test showed the significant risk factors related to HCC development to be age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, and hepatitis B e antigen negativity, but median HBV-DNA levels of <4.0 log copies/ml (maintained viral response, MVR) did not significantly suppress the development of HCC. In cirrhosis patients, however, the attainment of MVR during LAM treatment was revealed to reduce the risk of HCC development.

Conclusions These results suggest that the incidence of HCC in HBV patients with cirrhosis can be reduced in those with an MVR induced by consecutive LAM treatment.

Keywords Lamivudine · Chronic hepatitis B · Cirrhosis · Hepatocellular carcinoma · HBV-DNA level

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
ADV	Adefovir
ETV	Entecavir
Hbs Ag	Hepatitis B surface antigen
PCR	Polymerase chain reaction
TMA	Transcription-mediated amplification
IVR	Initial viral response
MVR	Maintained viral response
HBe Ag	Hepatitis B e antigen
CT	Computed tomography
MRI	Magnetic resonance imaging
ALT	Alanine aminotransferase

Introduction

More than 350 million people worldwide suffer from chronic infection with hepatitis B virus (HBV) [1–3]. Chronic HBV infection eventually leads to the development of cirrhosis and hepatocellular carcinoma (HCC), and raises the risk of hepatic disease-related death [4–6]. In Japan, up to 15% of HCC patients are diagnosed with HBV-related liver disease [7].

HCC is one of the most common malignancies in Japan and its incidence has been increasing over the past 30 years. Recently, various treatments such as transcatheter arterial embolization/chemoembolization, radio-frequency ablation, and hepatic resection have been reported to yield significant improvements in overall patient survival [8–11]. However, HCC relapse has thus far been observed in a majority of treated patients due to its highly malignant potential. In this regard, successful treatment of chronic HBV infection should prevent the patient's liver from progressing to cirrhosis and reduce the risk of HCC development. In recent years, the treatment of chronic hepatitis has changed greatly with the development of various antiviral therapies with nucleoside/nucleotide analogues such lamivudine (LAM), adefovir (ADV), and entecavir (ETV) [12–15]. LAM has long been used against chronic hepatitis, and many reports have demonstrated that LAM is effective in stabilizing inflammatory activity, suppressing HBV-DNA replication, and improving liver histological findings in chronic hepatitis patients [16, 17] and in HBV-related cirrhosis patients [18]. Furthermore, LAM has been reported to reduce the incidence of HCC in patients with chronic hepatitis B [19]. However, it is still controversial whether or not treatment using nucleotide analogues can reduce the risk of HCC development in HBV-infected patients [20, 21], and the relationship between the effect of HBV suppression and HCC development during LAM treatment has not yet been discussed in detail. Also, the risk factors for HCC development in HBV-infected patients who have been treated with LAM have not been sufficiently evaluated. In this study, we aimed to clarify whether the decrease of HBV-DNA amount induced by LAM therapy could reduce the incidence of HCC in HBV-infected patients.

Patients and methods

Patient selection and study design

This study was conducted at Osaka University Hospital and other institutions participating in the Osaka Liver Forum in Japan. The subjects were 293 consecutive patients with HBV infection who underwent continuous LAM therapy for more than 24 weeks from September 2000 to September 2006. All patients tested positive for hepatitis B surface antigen (HBs Ag) or had detectable levels of HBV DNA in their sera according to findings from a polymerase chain reaction (PCR)-based method or a transcription-mediated amplification (TMA) method. Exclusion criteria were patients with anti-hepatitis C antibody, anti-human immunodeficiency virus antibody, and other liver diseases (alcoholic liver disease, drug-induced liver disease, and autoimmune hepatitis). Also excluded were patients with a history of HCC and those who developed HCC within the first 24 weeks of the follow-up period after the initiation of LAM therapy (because of the possibility that microscopic HCC had been present before the initiation of treatment).

All patients were treated with 100 mg of LAM daily. Of the 293 patients, 129 underwent ADV (10 mg/day) therapy in addition to receiving ongoing LAM treatment. For 43 patients who started ETV administration in lieu of LAM, the observation period was terminated when they started

ETV. LAM resistance was confirmed by virological breakthrough and was defined as an increase in serum HBV-DNA by >1 $\log_{10}$ greater than the nadir [22]. If virological breakthrough developed and alanine aminotransferase (ALT) was elevated over the upper normal limit, the patients received add-on ADV at 10 mg/day.

In this study, all patients were examined for serum HBV-DNA level just before therapy initiation and every 6 months during treatment. The initial viral response (IVR) was defined as HBV-DNA <4.0 log copies/ml in the first 24 weeks of the follow-up period after the initiation of LAM therapy, and the maintained viral response (MVR) was defined as median HBV-DNA levels of less than 4.0 log copies/ml measured every 6 months during therapy.

This study protocol followed the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

HBV testing

HBs Ag, hepatitis B e antigen (HBe Ag) and anti-hepatitis B e antibody (anti-HBe) levels were examined by chemiluminescence immunoassay or enzyme immunoassay. HBV DNA was measured by a PCR-based method (Amplicor HBV monitor; Roche Diagnostics, Tokyo, Japan) or a TMA method (TMA-HPA; Fujirebio, Tokyo, Japan), which have lower detection limits of 2.6 and 3.7 log copies/ml, respectively. The LAM-resistant YMDD mutant virus was examined by a PCR-ELMA method. Serum samples were stored frozen at −80°C.

Diagnosis of HCC and cirrhosis

Ultrasonography was carried out before LAM therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by computed tomography (CT), magnetic resonance imaging (MRI), or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT or MRI. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the patient's consent or the patient was carefully followed until a diagnosis was possible with definite observation by CT, MRI, or hepatic angiography. Cirrhosis was diagnosed by liver biopsy or laparoscopy, and for patients without this information, by clinical data, imaging modalities, and portal hypertension.

Statistical analysis

Quantitative variables were expressed as means ± SD. Quantitative variables at the baseline were compared among two groups, the chronic hepatitis and cirrhosis groups, using the Mann–Whitney *U*-test. Categorical data, such as gender and status of HBe Ag, were compared using Fisher's exact test. The cumulative incidence of HCC was evaluated with a Kaplan–Meiercurve and the differences between groups were analyzed by the log-rank test. For multivariate analysis to investigate factors affecting the cumulative incidence of HCC, Cox's regression analysis was carried out. A value of $p < 0.05$ (two-tailed) was considered to be statistically significant. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of patients

The baseline clinical features of the enrolled patients before LAM administration are shown in Table 1. The mean age of the patients was 48.0 ± 10.7 years, 214 (73%) of the entire group were male, and 163 (56%) tested positive for HBe Ag. Of the 293 patients, 205 (70%) were diagnosed as having chronic hepatitis and 88 (30%) as having cirrhosis. The median HBV-DNA level was 7.0 (range 3.0 to 8.5<) log copies/ml. At baseline, the aspartate aminotransferase (AST) level was 131 ± 151 IU/l, the ALT level was 203 ± 252 IU/l, the total bilirubin level was 1.2 ± 1.6 mg/dl, the albumin (Alb) level was 3.8 ± 0.5 g/dl, and the platelet count was 13.7 ± 5.4 × 10^4/mm^3. The mean follow-up period for all patients was 67.6 ± 27.4 months, with a range of 12–110 months from the start of LAM treatment. There were significant differences between patients with chronic hepatitis and those with liver cirrhosis in age, AST, ALT, total bilirubin, Alb, and platelet counts.

Cumulative incidence of development of HCC

Figure 1a shows the Kaplan–Meier curve of the cumulative HCC incidence for all HBV patients treated with LAM or LAM plus ADV. Of the 293 patients with HBV infection, 32 (10.9%) developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 15% at 7 years.

Figure 1b shows the Kaplan–Meier curve of the cumulative HCC incidence according to initial diagnosis (chronic hepatitis vs. cirrhosis). Eight (4%) of the 205 enrolled chronic hepatitis patients developed HCC and the cumulative carcinogenesis rate was 2% at 3 years, 3% at 5 years, and 5% at 7 years. On the other hand, 24 (27%) of the 88 enrolled cirrhosis patients developed HCC and the cumulative carcinogenesis rate was 15% at 3 years, 30% at 5 years, and 35% at 7 years.

Table 1 Patient characteristics

HBe Ag Hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin

[a] Values are expressed as medians

* $p < 0.05$, ** $p < 0.001$, comparing patients with chronic hepatitis and those with liver cirrhosis using the Mann–Whitney *U*-test for quantitative variables and Fisher's exact test for categorical variables

Factor	All	Chronic hepatitis	Cirrhosis	*p* value
Number of patients	293	205	88	
Age (years)	48.0 ± 10.7	46.3 ± 10.7	51.9 ± 9.8	<0.001**
Sex (male/female)	214/79	147/58	67/21	0.475
HBe Ag (positive	163 (56%)	121 (59%)	42 (48%)	0.068
HBV DNA (log copies/ml)[a]	7.0 (3.0 to 8.5<)	6.8±1.1	6.6 ± 1.1	0.162
AST (IU/l)	131 ± 151	143 ± 162	104 ± 120	0.045*
ALT (IU/l)	203 ± 252	235 ± 269	129 ± 189	<0.001**
Total bilirubin (mg/dl)	1.2 ± 1.6	0.9 ± 0.6	1.8 ± 2.7	<0.001**
Alb (g/dl)	3.8 ± 0.5	3.9 ± 0.4	3.5 ± 0.6	<0.001**
Platelets ($\times 10^4/mm^3$)	13.7 ± 5.4	15.6 ± 9.3	9.3 ± 3.8	<0.001**
Follow-up period (months)	67.6 ± 27.4	68.5 ± 26.5	65.5 ± 29.5	0.393

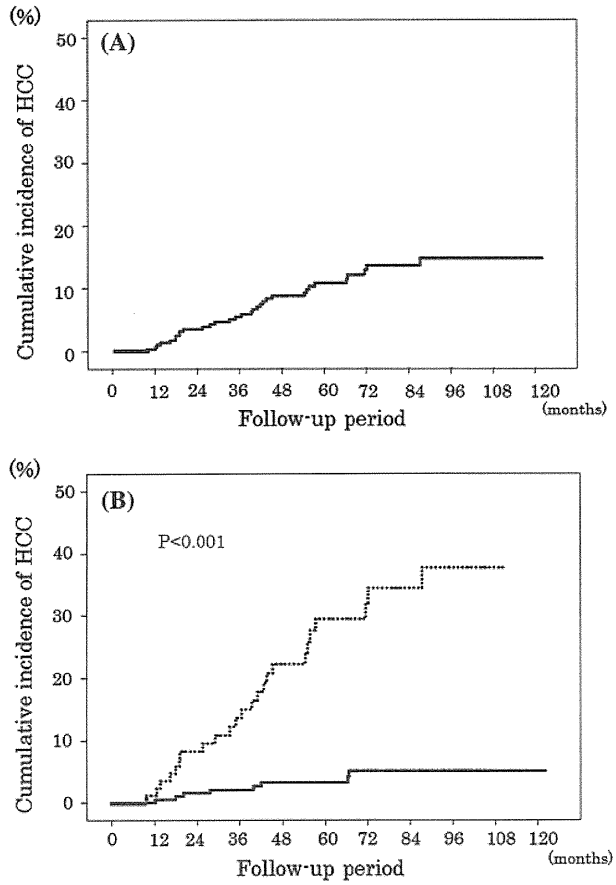

Fig. 1 Cumulative incidence of development of hepatocellular carcinoma (*HCC*) in patients with hepatitis B virus infection treated with lamivudine (LAM). **a** All cases; **b** chronic hepatitis or cirrhosis. *Solid line* Chronic hepatitis, *dotted line* cirrhosis

Risk factors for cumulative incidence of HCC development in all HBV-infected patients

Univariate analysis with the log-rank test was performed for all HBV-infected patients treated with LAM, with the results shown in Table 2. Univariate analysis with the log-rank test showed that the following were significant risk factors for the development of HCC: older age (≥50 years) ($p < 0.001$), cirrhosis ($p < 0.001$), high total bilirubin level (>1.2 g/dl) ($p = 0.004$), low Alb level (<3.8 g/dl) ($p = 0.019$), low platelet count (<14 × $10^4/mm^3$) ($p < 0.001$), and non-MVR ($p = 0.035$).

Stepwise multivariate analyses of four of these variables were performed by Cox's regression analysis for all patients treated with LAM with the results shown in Table 3. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: age ≥50 years [hazard ratio (HR) 3.20, 95% confidence interval [CI] 1.08–9.53, $p = 0.036$], platelet count <14.0 × $10^4/mm^3$ (HR 4.76, 95% CI 0.05–0.96, $p = 0.045$), cirrhosis (HR 4.64, 95% CI 1.75–12.4, $p = 0.002$), and non-MVR (HR 2.70, 95% CI 1.09–6.56, $p = 0.032$).

Cumulative incidence of and risk factors for HCC development in patients with chronic hepatitis and cirrhosis

The results of univariate analysis with the log-rank test for the development of HCC in chronic hepatitis patients treated with LAM are shown in Table 4, and the following were significant risk factors: older age (≥50 years) ($p = 0.002$), HBe Ag negativity ($p = 0.005$), and low platelet count (<14 × $10^4/mm^3$) ($p = 0.004$). Suppression of median HBV-DNA levels to <4.0 log copies/ml by LAM treatment was not associated with the development of HCC in the chronic hepatitis patients. Only non-MVR (median HBV-DNA amount ≥4.0 log copies/ml) was shown to be a significant risk factor for the development of HCC in the cirrhosis patients ($p = 0.029$), while the factors of age, HBe Ag status, and platelet count were not significant in these patients (Table 4).

Table 2 Risk factors for HCC development in all HBV-infected patients by univariate analysis

Factor	95% CI	*p* value
Age (years) (<50/≥50)	2.15–14.5	<0.001
Sex (male/female)	0.33–1.76	0.520
Initial diagnosis (chronic hepatitis/cirrhosis)	3.75–1.176	<0.001
HBe Ag (positive/negative)	0.31–1.29	0.209
HBV DNA (log copies/ml) (<7.0/>7.0)	0.33–1.35	0.262
AST (IU/l) (<40/≥40)	0.33–2.22	0.742
ALT (IU/l) (<40/≥40)	0.17–1.16	0.188
Total bilirubin (mg/dl) (<1.2/>1.2)	1.43–6.72	0.004
Alb (g/dl) (<3.8/≥3.8)	0.19–0.86	0.019
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.02–0.31	<0.001
Emergence of LAM-resistant viruses (positive/negative)	0.51–2.03	0.968
IVR (positive/negative)	0.52–3.25	0.575
MVR (positive/negative)	1.04–5.95	0.035

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *HBe Ag* hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin, *IVR* initial viral response, *MVR* maintained viral response, *LAM* lamivudine

Table 3 Risk factors for HCC development in all HBV-infected patients by multivariate analysis

Factor	Category	Risk ratio	95% CI	*p* value
Age (years)	<50	1	1.08–9.53	0.036
	≥50	3.20		
Initial diagnosis	Chronic hepatitis	1	1.75–12.4	0.002
	Cirrhosis	4.64		
Platelets ($\times 10^4$/mm^8) (<14/≥14)	≥14	1	0.05–0.96	0.045
	<14	4.76		
MVR	Negative	1	1.09–6.56	0.032
	Positive	0.37		

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *MVR* maintained viral response

Cumulative incidence of HCC development according to effectiveness of treatment (MVR vs. non-MVR)

Figure 2a shows the Kaplan–Meier curve of cumulative HCC incidence in all HBV-infected patients treated with LAM according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 5% at 3 years, 13% at 5 years, and 16% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence compared with non-MVR in all HBV-infected patients ($p = 0.035$).

Table 4 Risk factors for HCC development by univariate analysis (chronic hepatitis/cirrhosis)

	95% CI	*p* value
Chronic hepatitis		
Age (years) (<50/≥50)	0.26–8.38	0.002
Sex (male/female)	0.37–6.42	0.556
HBe Ag (positive/negative)	0.01–0.74	0.005
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.11–1.99	0.296
AST (IU/l) (<40/≥40)	0.11–2.64	0.482
ALT (IU/l) (<40/≥40)	0.06–1.41	0.101
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.67–6.67	0.574
Alb (g/dl) (<3.8/≥3.8)	0.13–8.58	0.960
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.01–0.72	0.004
Emergence of LAM-resistant viruses (positive/negative)	0.27–4.28	0.927
IVR (positive/negative)	0.29–8.67	0.590
MVR (positive/negative)	0.51–37.10	0.144
Cirrhosis		
Age (years) (<50/≥50)	0.86–6.17	0.089
Sex (male/female)	0.21–1.82	0.380
HBe Ag (positive/negative)	0.80–4.17	0.149
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.40–2.01	0.795
AST (IU/l) (<40/≥40)	0.27–3.07	0.873
ALT (IU/l) (<40/≥40)	0.13–1.47	0.167
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.82–4.80	0.126
Alb (g/dl) (<3.8/≥3.8)	0.28–1.58	0.354
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.03–1.51	0.084
Emergence of LAM-resistant viruses (positive/negative)	0.44–2.18	0.948
IVR (positive/negative)	0.90–8.32	0.063
MVR (positive/negative)	1.07–0.029	

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *HBe Ag* hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin, *IVR* initial viral response, *MVR* maintained viral response

Figure 2b shows the Kaplan–Meier curve of the cumulative HCC incidence in chronic hepatitis patients according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 0% at 3 years, 0% at 5 years, and 2% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. MVR during LAM did not significantly suppress the cumulative HCC incidence compared with non-MVR in the chronic hepatitis patients ($p = 0.144$).

Figure 2c shows the Kaplan–Meier curve of the cumulative HCC incidence in cirrhosis patients according to the effectiveness of treatment (MVR vs. non-MVR).

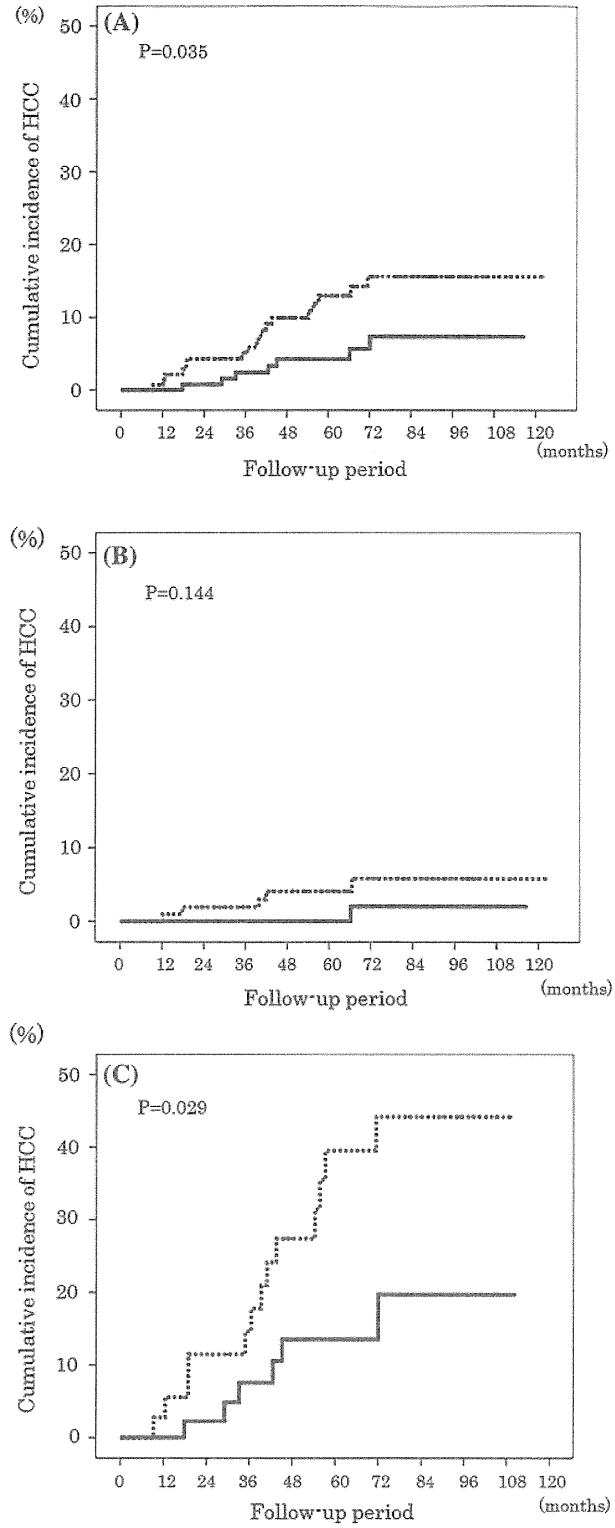

Fig. 2 Cumulative incidence of development of HCC according to the effectiveness of treatment (MVR vs. non-MVR). **a** All cases; **b** chronic hepatitis; **c** cirrhosis. *Solid lines* MVR, *dotted lines* non-MVR. *MVR* Maintained viral response

The cumulative carcinogenesis rate for MVR-positive patients was 8% at 3 years, 14% at 5 years, and 14% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 18% at 3 years, 40% at 5 years, and 44% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence compared with non-MVR in the cirrhosis patients ($p = 0.029$).

Relationship between IVR and MVR

Maintained viral response (MVR) was achieved by 142 (48%) of the 293 patients enrolled in this study. IVR was achieved by 204 (79%) of the 259 patients who were examined for IVR. The relationship between IVR and MVR is shown in Fig. 3; 60% (122/204) of the IVR-positive patients achieved an MVR, while only 16% (9/55) of the IVR-negative patients achieved an MVR ($p < 0.001$). The LAM-resistant YMDD mutant virus was found in 149 (51%) of all patients during follow-up, and in 52% (107/204) of the IVR-positive patients, a finding which was nearly equal to that for the IVR-negative patients (51%, 28/55). Among the IVR-positive patients, the MVR rate was lower in patients with the YMDD mutation, compared with that in those without the YMDD mutation (44%, 47/107 vs. 77%, 75/97, $p = 0.016$), while the MVR rates were low in the IVR-negative patients, irrespective of their YMDD mutation status (with and without the mutation, 15 vs. 18%, respectively). ADV was added to LAM treatment for 73 (68%) of the 107 IVR-positive patients with the YMDD mutation and 20 (36%) of the 55 IVR-negative patients with the YMDD mutation. However, MVR was only achieved at the low rates of 33% (24/73) for the former patients and 20% (4/20) for the latter.

Discussion

Lamivudine treatment has been shown to improve the liver histological findings in patients with HBV-infected liver disease by reducing the HBV load and stabilizing inflammatory activity [16–18]. One report has shown that LAM effectively reduced the incidence of HCC in patients with chronic hepatitis B, but the study only compared LAM-treated patients with non-treated patients in a matched case-controlled study [19]. However, there have been few detailed reports about the relationship between virological response and HCC development in HBV-infected patients during LAM treatment. In the present study, we retrospectively examined the incidence of HCC to clarify the indicators of LAM therapy, including median HBV-DNA levels, for reducing the risk of HCC in HBV-infected patients.

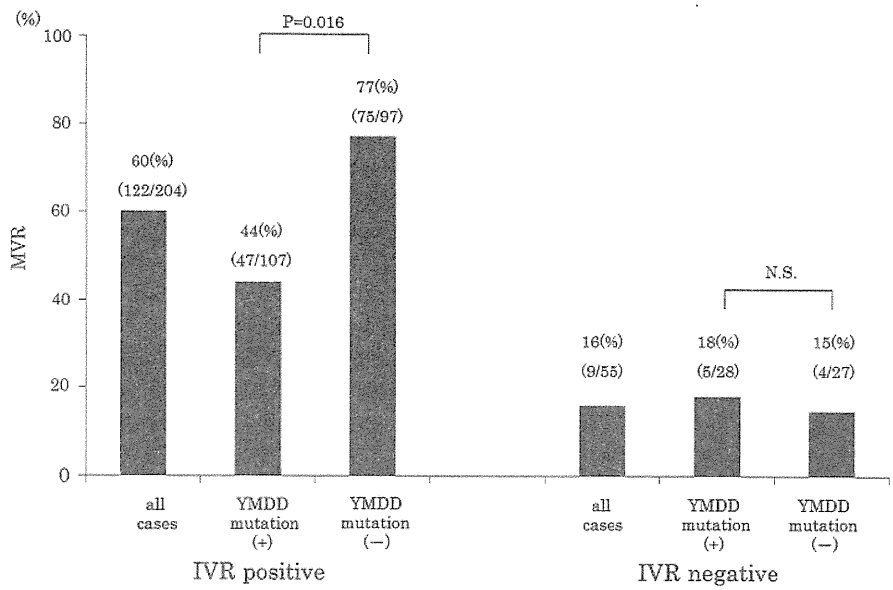

Fig. 3 Relationship between IVR and MVR. *IVR* Initial viral response, *MVR* maintained viral response, *N.S.* not significant

Many investigators have reported that serum HBV DNA levels higher than 4.0–4.5 log copies/ml before HBV treatment serve as a strong risk predictor of HCC [23–25]. Di Marco et al. [26] have reported that the incidence of HCC was higher in patients with serum HBV levels of more than 5.0 log copies/ml, at least once, during LAM therapy than in those in whom serum HBV levels were maintained at 5.0 log copies/ml or less. However, the add-on ADV therapy had not been adopted when the study of Di Marco et al. was reported. When the use of ADV is possible, an evaluation method is needed to measure the antiviral effects of nucleoside/nucleotide analogues against HBV-related liver disease. In the present study, we set the cut-off value for HBV-DNA at 4.0 log copies/ml. The basis of this cut-off value is that a serum HBV DNA level higher than 4.0 log copies/ml before HBV treatment was reported to serve as a strong risk predictor of HCC [23]. MVR, defined as a median HBV-DNA level of less than 4.0 log copies/ml measured every 6 months during therapy, was adopted as an indicator of viral replication, and non-MVR (median HBV-DNA >4.0 log copies/ml) during LAM therapy was shown to be significantly associated with the development of HCC in HBV-infected patients. We also found that a median HBV-DNA level of >4.0 log copies/ml during LAM therapy was a risk factor for HCC development. On the other hand, IVR, defined as HBV-DNA of <4.0 log copies/ml in the first 6 months of the follow-up period after the initiation of therapy, was not associated with the development of HCC in HBV patients in this study. As shown in Fig. 3, 84% of the IVR-negative patients could not achieve an MVR, suggesting that it is crucial to achieve an IVR in order to achieve an MVR. The reason why IVR was not a significant factor for MVR seemed to be the appearance of the YMDD mutation, which reduced the antiviral effect of LAM for HBV in IVR-positive patients. The LAM-resistant YMDD mutant virus was found in 52% of the IVR-positive patients. Although ADV was added to LAM treatment for 73 patients, only 33% of these patients could achieve an MVR. We speculate that the antiviral effect of ADV is not very strong [27] and it takes time to reduce the YMDD mutant virus, which may explain the low MVR rate (33%) in patients with the add-on ADV therapy. The immediate administration of ADV when the LAM-resistant YMDD mutant virus appears can be important [28]. A switch to ETV, which induces resistant virus less frequently, could also raise MVR rates among IVR-positive patients without the YMDD mutant virus.

As the duration of the add-on ADV therapy was included in this study, we compared the cumulative incidence of HCC in patients receiving LAM monotherapy with that in patients who also received the add-on ADV therapy. Sixteen (10%) of the 164 patients who received the LAM monotherapy developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 10% at 5 years, and 15% at 7 years. On the other hand, 16 (12%) of the 129 patients who received LAM plus ADV developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 14% at 7 years. No significant difference was found between these two groups ($p = 0.986$). In addition, we examined the cumulative incidence of HCC development according to the effectiveness of treatment (MVR vs. non-MVR) in patients for whom the observation period was terminated when ADV was added, and the same results were obtained (data not shown).

Older age (≥50 years), cirrhosis, and low platelet count ($<14 \times 10^4/mm^3$) were shown to be significantly associated with the development of HCC in patients with HBV infection. These results were consistent with those of

previous reports [29–31], suggesting that patients of older age with advanced fibrosis should be followed up carefully for longer periods in order to detect early stages of HCC even if LAM therapy does effectively suppress HBV. Of note, in the present study we estimated the cumulative HCC incidence according to the initial diagnosis of chronic hepatitis or cirrhosis. In the chronic hepatitis patients, older age (≥50 years), HBe Ag negativity, and low platelet count ($<14 \times 10^4/mm^3$) were significant risk factors for the development of HCC, but this was not the case in the cirrhosis patients. Because liver biopsies had not been performed, the liver fibrosis stage could not be evaluated with respect to the risk factors for HCC in this study. Instead, the factors of age, HBe Ag status, and platelet count may reflect the degree of liver fibrosis in chronic hepatitis patients. In fact, cirrhotic patients, in comparison with chronic hepatitis patients, were of older age (chronic hepatitis vs. cirrhosis: 46.3 ± 10.7 vs. 51.9 ± 9.8 years, $p < 0.001$), had higher rates of HBe Ag negativity (chronic hepatitis vs. cirrhosis: 39 vs. 51%, $p = 0.065$), and had lower platelet counts (chronic hepatitis vs. cirrhosis: 15.6 ± 4.9 vs. $9.3 \pm 3.8 \times 10^4/mm^3$, $p < 0.001$). This seems to explain why none of these factors were significant risk factors for HCC in cirrhotic patients. On the other hand, in the chronic hepatitis patients, MVR was not a significant factor for HCC development, while MVR was a significant factor for HCC development in the cirrhotic patients. We speculate that HBV suppression induced by LAM therapy could reduce the incidence of HCC in patients infected with HBV, especially those with cirrhosis, who displayed higher malignant potential. Investigation over a longer period is needed to clarify the effect of HBV suppression on the development of HCC in chronic hepatitis patients.

In conclusion, the present study shows that the attainment of an MVR induced by LAM therapy has a significant beneficial effect on the clinical course of HBV-infected patients by decreasing the incidence of HCC. The newer nucleotide analogues, such as ETV and tenofovir, should be able to further reduce the incidence of HCC, given their greater potency.

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Conflict of interest The authors declare that they have no conflict of interest.

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