Underlying Liver Diseases

All HCC patients had underlying chronic liver diseases, such as CH or LC. We compared the underlying liver diseases among those aged 50 years or younger and those aged older than 50 years by HBV genotype (fig. 4). In 13 patients with HBV/Ba, only 1 (20.0%) of the 5 patients aged 50 years or younger had LC, while 7 (87.5%) of the 8 patients aged older than 50 years had LC ($p < 0.05$). However, in patients with HBV/Bj or HBV/C, the ratios of underlying liver diseases were approximately identical even when compared by age.

Discussion

The clinical and virologic features of patients with chronic HBV infection are specific according to their HBV genotypes [4, 15]. However, to date, there has been no report on the relationship between the HBV genotypes of Ba, Bj and C, and the clinical characteristics of HCC patients. We therefore analyzed the relationship between the clinical characteristics of Japanese HCC patients identified throughout Japan, and their HBV genotypes, including the HBV subtypes of Ba and Bj. In this study, we demonstrated that HBV/Ba (4.4%), HBV/Bj (7.4%) and HBV/C (86.5%) were found in Japanese HCC patients, and that there were distinct clinical differences among the three HBV genotypes, in geographic distribution, age distribution, and the frequency of positive HBeAg.

Of the Japanese patients with chronic HBV infection, including asymptomatic carriers, CH, LC and HCC, 1.7% were HBV/A, 12.2% HBV/B, 84.7% HBV/C, 0.4% HBV/D, and the others 1.0%, as reported previously [7]. In this study, we collected 296 serum samples from patients with HCC throughout Japan. In addition, we recently developed a new method for detecting HBV/Ba and HBV/Bj with restriction fragment length polymorphism [11]. Thus, we showed that 1.0% was HBV/A, 4.4% HBV/Ba, 7.4% HBV/Bj, 86.5% HBV/C, and mixed genotype 0.7% in Japanese HCC patients. This prevalence in HCC patients is almost identical to that in all patients with chronic HBV infection [7]. In addition, the geographic distribution of HBV/B and HBV/C in HCC patients is also identical to that in all patients. However, when we analyzed the HBV subtypes of HBV/Ba and HBV/Bj in patients with HBV/B, a high proportion of patients with HBV/Bj is found in the highly endemic HBV/B area, the Tohoku district and Okinawa, while the prevalence of HBV/Ba is approximately identical between the highly endemic HBV/C area, the other areas of Japan, and the highly endemic HBV/B area. Thus, HBV/Bj is specifically distributed in the Tohoku district and Okinawa.

As reported previously, HBV/Ba is ubiquitous in all Asian countries including Japan, although HBV/Bj is specific to Japan and is not found in other countries [11]. In Okinawa, it is reported that a high proportion of patients with chronic HBV infection have HBV/B and a good prognosis compared with patients with HBV/C [16, 17]. In contrast, in Taiwan, close to Japan, a higher proportion of patients aged 50 years or younger with HBV/B have HCC and CH [15]. The underlying liver diseases in those who developed HCC were compared among each HBV genotype group. In the HBV/Ba group, up to 75% of the patients aged 50 years or younger had CH as the underlying liver disease, compared with patients aged over 50 years. On the other hand, in the group with HBV/Bj or HBV/C, more than 60% of the patients had LC regardless of their age. The mean age of the patients with HBV/Ba in Japan is more than 10 years younger than those with HBV/Bj. So, more younger patients with HBV/Ba tend to have CH than the other patients. However, the molecular mechanism is unclear why patients with HBV/Ba develop HCC at a younger age and often have CH.

It is unclear why Japanese patients with HBV/B have a good prognosis while Taiwanese patients with HBV/B often have more advanced liver diseases, such as HCC. The frequency of patients positive for HBeAg in the HBV/Ba and HBV/C groups was higher than in the HBV/Bj group. So, the viral activity of HBV may be higher in patients with HBV/Ba or HBV/C than those with HBV/Bj. Thus, these differences in subtypes of HBV/Ba and Bj could be one of the reasons why the discrepancy in prognosis exists between Japanese and Taiwanese patients with HCC.

The differences in DNA sequences between HBV/Ba and HBV/Bj can be characterized in the core gene [10]. It has been reported that HBV/Ba, not HBV/Bj, recombines with HBV/C in the core gene. The product of the core gene is reported to be a cytotoxic T-cell epitope [18], suggesting that patients with HBV/Ba and HBV/C may be exposed to severe immune responses for destroying hepatocytes compared with those with HBV/Bj. In addition, patients with HBV/Ba more often have core promoter mutations at nucleotide 1762/1764 than those with HBV/Bj [11], which is associated with more advanced liver diseases [6, 19]. Taken together, these facts may indicate a poor prognosis in patients with HBV/Ba compared to those with HBV/Bj.

In the patients with HBV/C, the mean ALP and γ-GTP levels were higher than those with the other genotypes. In this study, there may exist some bias of regarding the tumor size of HCC between patients with HBV/C and the other patients. It is considered that more patients with a rather large size of HCC were found in the patients with HBV/C, resulting in elevation in ALT and γ-GTP levels.

To investigate the hepatocarcinogenesis and risk factors of HCC, it is important to study the differences in host, environmental and viral factors. The various genetic alterations, such as mutations of cancer-associated genes or loss of some chromosomes, are found in the HCC cells [20]. However, the genetic polymorphism varies among populations [21]. The differences in host genomes are still unknown between Japanese and other Asian populations. The association of environmental factors, such as air, water and food contaminated with some chemical agents, and HCC is still unclear, although aflatoxin affects the mutation of p53 in HCC [22]. However, with respect to the viral factors, a survey of the distribution of HBV genotypes or subtypes will be important clues for solving these problems.

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Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients

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Abstract

A retrospective survey of Japanese patients histologically diagnosed with chronic hepatitis B was conducted to determine the effectiveness of lamivudine in preventing hepatocellular carcinoma (HCC). Of the 2795 patients who satisfied criteria for analysis after treatment from any of 30 medical institutions, 657 had received lamivudine and the remaining 2138 had not. A Cox regression model with liver biopsy as the starting point revealed seven factors related to HCC: lamivudine therapy, gender, family clustering of hepatitis B, age at liver biopsy, hepatic fibrosis stage, serum albumin level, and platelet count. In a matched case-controlled study, 377 patients in a lamivudine-treated group and 377 matched patients in a non-treated group were selected based on their propensity scores. The mean follow-up period was 2.7 years in the lamivudine group and 5.3 years in the control group. In the lamivudine group, HCC occurred in four patients (1.1%) with an annual incidence rate of 0.4%/(patient/year), whereas in the control group HCC occurred in 50 patients (13.3%) for a rate of 2.5%/(patient/year). A comparison of the cumulative HCC incidence between the two groups by the Kaplan–Meier method showed a significantly lower incidence of HCC in the lamivudine group ($p < 0.001$). These findings suggest that lamivudine effectively reduces the incidence of HCC in patients with chronic hepatitis B.

Keywords: Chronic hepatitis B; Hepatocellular carcinoma; Anti-viral treatment; Lamivudine

1. Introduction

An estimated 350 million people worldwide are chronically infected with the hepatitis B virus (HBV), most in southeast Asia [1,2]. In this region, infection occurs during infancy, including that through mother–child transmission. Infected persons with HBV are initially asymptomatic, and

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active hepatitis emerges years later. In most patients, seroconversion from hepatitis Be antigen (HBeAg) to antibody to HBeAg (HBeAb) occurs spontaneously with age. At the same time, the virus levels decrease and hepatitis abates. Some patients, however, remain positive for HBeAg, and in those patients the hepatitis virus persists at high levels, resulting in the progression to hepatic cirrhosis, and the onset of hepatocellular carcinoma (HCC) in a high percentage of such patients [3–5]. The number of HBV carriers is decreasing in Japan and some other countries as a result of the prevention of mother–child transmission through the use of HBV vaccines and/or high-potency antibody to hepatitis B surface antigen (HBsAb) human immunoglobulin (HBIG) [6]. Even in these countries, however, only persons born after 1986 are protected by vaccination, and many chronic hepatitis B patients still need treatment. In the past, it was not easy to manage chronic hepatitis B using anti-viral agents such as interferon. In recent years, however, the development of lamivudine, a nucleoside analogue that inhibits reverse transcriptase, has drastically changed the treatment of hepatitis B [7–9]. By virtue of this inhibitory ability, lamivudine was developed as an anti-viral agent against human immuno-deficiency virus (HIV). It was later also found to be effective against HBV because HBV is a member of the Hepadnaviridae family, which utilizes reverse transcriptase in its replication process [10]. Lamivudine was found to inhibit the replication of HBV, reduce hepatitis, and improve liver histological findings in long-term treatment [11]. It is also useful when hepatitis B becomes severe due to acute exacerbation, as well as in the treatment of liver cirrhosis associated with symptoms of hepatic failure, such as ascites and edema [12–16]. However, a number of problems are associated with lamivudine therapy, such as relapse of hepatitis due to the appearance of YMDD mutant viruses and the difficulty of estimating the optimal time to discontinue the treatment [17,18]. In addition, until recently no adequate studies had been conducted to determine whether or not lamivudine inhibits the onset of hepatic cancer, even though it is known to slow the progression of histological changes in the liver. This lack of research is attributable partly to the need for long-term follow-up of a large number of patients and partly to the difficulty of conducting clinical trials. We conducted a multicenter study of a large number of registered patients to evaluate the effects of lamivudine on the course of hepatitis B and the onset of HCC. The data obtained were analyzed in a matched case-controlled study.

2. Materials and methods

2.1. Study design

The Inuyama Hepatitis Study Group designed this multicenter retrospective study to determine whether or not lamivudine is effective in preventing HCC. The subjects were Japanese patients with hepatitis B who were diagnosed with chronic liver disease by liver biopsy after 1980 and were followed up until March 2002. Each patient completed a questionnaire containing 16 items in four categories: background factors: date of birth, sex, family clustering of hepatitis B, and alcohol consumption during follow-up (80 g or more per day as ethanol); examination and test items: date of liver biopsy, grade and stage of histological findings of the liver, hepatitis Be antigen (HBeAg), antibody to HBeAg (HBeAb), albumin, asparate aminotransferase (AST), alanine aminotransferase (ALT), and platelet counts; clinical outcomes: the presence or absence of HCC during the follow-up period and the date of onset if present; lamivudine therapy: the presence or absence of lamivudine therapy during the follow-up period, and the date of initiation and duration of therapy if provided. The study was allowed by the review board of each participating institution. The names, ID numbers, and all other information that would directly identify individual patients were deleted to protect their privacy.

2.2. Patients

The present study included 3022 patients with chronic hepatitis B who underwent liver biopsy at any of 30 medical institutions after 1980. No patient had superinfection with hepatitis C virus and HIV. Two hundred and twenty-seven patients who had not answered the question about lamivudine treatment were excluded from the study. This left a total of 2795 patients for analysis. Among them, 657 patients had received lamivudine therapy and 2138 patients had not.

Histological findings of the liver were scored with respect to the grade of inflammation and stage of hepatic fibrosis according to the New Inuyama Histological Criteria [19] by a pathologist at each institution.

2.3. Lamivudine treatment

The lamivudine treatment group consisted of 657 patients who had received lamivudine therapy (100 mg/day). The median lamivudine treatment period was 18.9 months. Lamivudine therapy was continued until the end of the follow-up period in 45% of the patients.

2.4. Matched case-controlled study

In our analysis of the relationship between lamivudine therapy and hepatic carcinogenicity, the starting point was the day of liver biopsy. However, many patients in the lamivudine group (279 patients or 41.4%) initiated lamivudine therapy more than 2 years after liver biopsy, making them inappropriate subjects for the evaluation of the effects of lamivudine on hepatic carcinogenicity. For this reason, 377 patients who started lamivudine therapy within 2 years after liver biopsy were selected for analysis from the 657 patients in the lamivudine group. The interval from liver biopsy to lamivudine therapy was 5.8 ± 9.0 months, and the treatment

period was 23.1 ± 19.0 months (range 3–96 months). For the control group, seven factors were selected on the basis of the propensity scores from the 2138 patients who had not received lamivudine: age at the time of liver biopsy, gender, family clustering of hepatitis B, stage of hepatic fibrosis, serum albumin level, and platelet count. On that basis, 377 matching patients were selected for the control group [20].

2.5. Statistical analyses

A series of analyses was conducted using the day of liver biopsy as the starting point. Background factors at the time of liver biopsy were compared by the Student's *t*-test (numerical data) or the χ^2 test (categorical data), and differences were regarded as significant if $p < 0.05$ on both sides. Factors related to HCC were analyzed using a Cox regression model. The incidence of HCC was reported as an annual incidence rate (%/(patient/year)).

Because of the large differences in background factors between the lamivudine and control groups, the groups were matched for further analysis of HCC-related factors. For this analysis, all patients who had started lamivudine therapy within 2 years after liver biopsy were selected. The propensity score method was used to select patients from the control group [20]. Matching was done with respect to the HCC-related factors selected using the Cox regression model. After the matching, the incidence of HCC was shown by the Kaplan–Meier method and compared between the groups by the log-rank test. Differences were regarded as significant if $p < 0.05$ on both sides.

3. Results

3.1. Comparison of background factors

Table 1 demonstrates the comparison of background factors at the time of liver biopsy between the lamivudine and control groups. Significant differences were found in the mean age ($p < 0.001$), duration of follow-up ($p < 0.001$), history of IFN therapy ($p < 0.001$), inflammation of the liver ($p < 0.001$), HBeAg ($p < 0.001$), HBeAb ($p = 0.001$), serum albumin level ($p < 0.001$), AST level ($p = 0.011$), and platelet count ($p < 0.001$).

3.2. Evaluation of factors related to hepatic carcinogenicity by univariate analyses

HCC occurred in 31 of the 657 patients (4.7%) in the lamivudine group and in 239 of the 2138 patients (11.2%) in the control group. The mean follow-up periods after liver biopsy were 4.9 and 6.2 years in the lamivudine and control groups, respectively. Thus, the crude incidence of HCC determined was 1.0 and 1.8%/(patient/year) in the lamivudine and control groups, respectively.

Table 2 shows the incidences of HCC in the lamivudine and control groups in an analysis stratified with respect to background factors. In the lamivudine group, HCC did not occur in patients whose histological findings were grade 0 in inflammation and stage 0 in fibrosis, and significant intergroup differences were noted in this respect. No significant differences were observed other than in the histological findings.

3.3. Evaluation of factors related to hepatic carcinogenicity using a multivariate Cox regression model

Factors contributing to the incidence of HCC were analyzed using a Cox regression model (Table 3). The following variables were selected by the forward–backward stepwise selection method: lamivudine therapy (no therapy, $p = 0.002$), gender (male, $p < 0.001$), family history of hepatitis B (present, $p = 0.015$), age at the time of liver biopsy (older than 40 years, $p < 0.001$), stage of liver fibrosis (more than F2, $p < 0.001$), serum albumin level (less than 4.0 g/dL, $p = 0.001$), and platelet count (less than 150,000/μL, $p < 0.001$)). This analysis showed that lamivudine reduces the risk of HCC.

3.4. Evaluation of factors related to hepatic carcinogenicity by a six-factor matched case-controlled study

Matched case-control analyses were performed for six factors (sex, family history of hepatitis B, age at the time of liver biopsy, stage of liver fibrosis, serum albumin level, and platelet count). There were no significant differences in background factors between the groups, as shown in Table 4. The mean follow-up period in the control group (5.3 years) was about twice that in the lamivudine group (2.7 years). In the lamivudine group, HCC occurred in 4 of 377 patients (1.1%), with an annual incidence rate of 0.4%/(patient/year), compared to 50 of 377 patients (13.3%) and 2.5%/(patient/year), respectively, in the control group. A comparison of the cumulative HCC incidence between the two groups by the Kaplan–Meier method showed a significantly lower incidence in the lamivudine group ($p < 0.001$) (Fig. 1).

Next, the background factors were compared between patients with HCC and those without it in the lamivudine and control groups. In the lamivudine group (Table 5), the mean age was significantly higher in patients with HCC than in those without it (55.0 years versus 41.3 years, $p = 0.024$), but there were no significant differences in the other factors. In the control group (Table 6), the mean age was significantly higher in patients with HCC than in those without it (50.6 years versus 40.0 years, $p < 0.001$). Significant differences were also noted in the stage of liver fibrosis ($p < 0.001$), serum albumin level ($p < 0.001$), and platelet count ($p < 0.001$), suggesting that underlying liver disease was more advanced in patients who developed HCC.

Table 1
Comparison of background factors between lamivudine group and control group assessed at the time of liver biopsy

Parameter	Lamivudine group ($n=657$)	Control group ($n=2138$)	p-Value
Gender[a]			
Male	503 (76.6%)	1583 (74.0%)	0.194
Female	154 (23.4%)	555 (26.0%)	
Age (years)[b]	40.9 ± 11.0	37.3 ± 12.4	<0.001
Follow-up period (years)[b]	4.9 ± 4.4	6.2 ± 5.5	<0.001
Family clustering of hepatitis B[a]			
Yes	376 (57.2%)	1085 (50.7%)	0.011
No	242 (36.8%)	924 (43.2%)	
Unknown	39 (5.9%)	129 (6.0%)	
Drinking during the course of the study (>ethanol 80 g/day)			
Yes	69 (10.5%)	359 (16.8%)	<0.001
No	557 (84.8%)	1708 (79.9%)	
Unknown	31 (4.7%)	71 (3.3%)	
IFN therapy[a]			
Yes	269 (40.9%)	812 (38.0%)	<0.001
No	369 (56.2%)	1306 (61.1%)	
Unknown	19 (2.9%)	20 (0.9%)	
Liver histology			
Grade of inflammation[a]			
A0	15 (2.3%)	84 (3.9%)	<0.001
A1	194 (29.5%)	642 (30.0%)	
A2	283 (43.1%)	996 (46.6%)	
A3	142 (21.6%)	389 (18.2%)	
Unknown	23 (3.5%)	27 (1.3%)	
Stage of fibrosis[a]			
F0	12 (1.8%)	49 (2.3%)	0.491
F1	201 (30.6%)	721 (33.7%)	
F2	167 (25.4%)	524 (24.5%)	
F3	171 (26.0%)	491 (23.0%)	
F4	98 (14.9%)	331 (15.5%)	
Unknown	8 (1.2%)	22 (1.0%)	
HBeAg[a]			
+	355 (54.0%)	1272 (59.5%)	<0.001
−	280 (42.6%)	723 (33.8%)	
Unknown	22 (3.3%)	143 (6.7%)	
HBeAb[a]			
+	215 (32.7%)	642 (30.0%)	0.001
−	418 (63.6%)	1330 (62.2%)	
Unknown	24 (3.7%)	166 (7.8%)	
Albumin (g/dL)[b]	4.01 ± 0.49 ($n=629$)	4.14 ± 0.49 ($n=1941$)	<0.001
AST (IU/L)[b]	110.2 ± 131.8 ($n=593$)	94.5 ± 131.5 ($n=2023$)	0.011
ALT (IU/L)[b]	183.4 ± 211.1 ($n=641$)	163.5 ± 234.3 ($n=2022$)	0.056
Platelet count ($\times 1000/mm^3$)[b]	165.4 ± 54.9 ($n=629$)	176.9 ± 59.6 ($n=1931$)	<0.001

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.

4. Discussion

It is clear that this study has several limitations: it is not prospective, it is not randomized, there is no single regimen of lamivudine, and there is a lack of virological analysis (including that of the HBV genotype and that of YMDD mutations). It would be desirable to conduct a well-designed prospective study using controls. However, because lamivudine has been used in general practice under the insurance system in Japan, it is difficult to conduct a prospective and randomized control study of lamivudine therapy for chronic hepatitis B. In addition, it is ethically unacceptable to leave patients untreated for a long period of time in a control group, because lamivudine has been shown to abate hepatitis and improve histological findings of the liver [12–16].

Table 2
Comparison of the incidence of HCC in relation to each background factor between lamivudine group and control group

Parameter	Category	Group	Total number of patients (number)	No. of patients with HCC (number)	Average follow-up period (year)	Adjusted incidence of HCC (%/year)
Gender	Male	Lamivudine group	503	27	5.0	1.07
		Control group	1583	191	6.4	1.89
	Female	Lamivudine group	154	4	4.3	0.60
		Control group	555	48	5.6	1.54
Age (years)	<30	Lamivudine group	110	2	4.7	0.39
		Control group	642	8	5.9	0.21
	30≦ and <40	Lamivudine group	192	9	5.7	0.82
		Control group	646	52	6.8	1.18
	40≦ and <50	Lamivudine group	206	9	5.3	0.82
		Control group	491	75	6.7	2.28
	50≦	Lamivudine group	149	11	3.3	2.24
		Control group	359	104	5.3	5.47
Duration of lamivudine treatment (years)	<1	Lamivudine group	178	7	5.0	0.79
		Control group	–	–	–	–
	1≦ and <2	Lamivudine group	215	13	4.4	1.37
		Control group	–	–	–	–
	2≦ and <3	Lamivudine group	145	7	4.6	1.05
		Control group	–	–	–	–
	3≦	Lamivudine group	107	4	5.9	0.63
		Control group	–	–	–	–
Family clustering of hepatitis B	No	Lamivudine group	242	10	4.8	0.86
		Control group	924	100	6.4	1.69
	Yes	Lamivudine group	376	20	5.0	1.06
		Control group	1085	128	5.9	2.00
	Unknown	Lamivudine group	39	1	4.4	0.58
		Control group	129	11	8.2	1.04
Drinking during the course of the study (>ethanol 80 g/day)	No	Lamivudine group	557	23	4.8	0.86
		Control group	1708	158	5.8	1.59
	Yes	Lamivudine group	69	7	5.6	1.81
		Control group	359	76	7.8	2.71
	Unknown	Lamivudine group	31	1	3.8	0.85
		Control group	71	5	7.7	0.91
IFN therapy	No	Lamivudine group	369	19	4.2	1.23
		Control group	1306	167	6.0	2.13
	Yes	Lamivudine group	269	12	6.0	0.74
		Control group	812	70	6.5	1.33
	Unknown	Lamivudine group	19	0	2.6	0.00
		Control group	20	2	7.9	1.27
Liver histology Grade of inflammation	A0	Lamivudine group	15	0	9.3	0.00
		Control group	84	8	6.6	1.44
	A1	Lamivudine group	194	4	5.4	0.38
		Control group	642	59	6.4	1.44
	A2	Lamivudine group	283	15	4.9	1.08
		Control group	996	109	6.3	1.74
	A3	Lamivudine group	142	10	3.4	2.07
		Control group	389	52	5.5	2.43
	Unknown	Lamivudine group	23	2	6.1	1.43
		Control group	27	11	8.7	4.68

Table 2 (*Continued*)

Parameter	Category	Group	Total number of patients (number)	No. of patients with HCC (number)	Average follow-up period (year)	Adjusted incidence of HCC (%/year)
Stage of fibrosis	F0	Lamivudine group	12	0	7.2	0.00
		Control group	49	3	5.7	1.07
	F1	Lamivudine group	201	6	6.0	0.50
		Control group	721	29	6.7	0.60
	F2	Lamivudine group	167	8	4.7	1.02
		Control group	524	38	5.8	1.25
	F3	Lamivudine group	171	11	4.0	1.61
		Control group	491	61	6.0	2.07
	F4	Lamivudine group	98	6	3.6	1.70
		Control group	331	99	6.2	4.82
	Unknown	Lamivudine group	8	0	6.7	0.00
		Control group	22	9	8.3	4.93
HBeAg	−	Lamivudine group	280	10	4.2	0.85
		Control group	723	83	6.4	1.79
	+	Lamivudine group	355	19	5.3	1.01
		Control group	1272	134	6.0	1.76
	Unknown	Lamivudine group	22	2	6.2	1.47
		Control group	143	22	7.4	2.08
HBeAb	−	Lamivudine group	418	19	4.9	0.93
		Control group	1330	137	6.0	1.72
	+	Lamivudine group	215	10	4.7	0.99
		Control group	642	75	6.3	1.85
	Unknown	Lamivudine group	24	2	6.1	1.37
		Control group	166	27	7.4	2.20
Albumin (g/dL)	<4.0	Lamivudine group	257	19	4.5	1.64
		Control group	619	113	5.7	3.20
	4.0≦	Lamivudine group	372	9	4.9	0.49
		Control group	1322	90	6.1	1.12
AST (IU/L)	<50	Lamivudine group	187	7	5.7	0.66
		Control group	905	82	6.1	1.49
	50≦ and <100	Lamivudine group	200	14	4.7	1.49
		Control group	572	81	5.9	2.40
	100≦ and <200	Lamivudine group	142	7	5.1	0.97
		Control group	367	31	6.2	1.36
	200≦	Lamivudine group	64	2	4.4	0.71
		Control group	179	15	6.0	1.40
ALT (IU/L)	<50	Lamivudine group	117	5	4.7	0.91
		Control group	570	69	6.1	1.98
	50≦ and <100	Lamivudine group	155	7	4.9	0.92
		Control group	506	60	5.8	2.04
	100≦ and <150	Lamivudine group	109	9	4.7	1.76
		Control group	297	36	5.9	2.05
	150≦	Lamivudine group	260	9	4.8	0.72
		Control group	649	44	6.2	1.09
Platelet count ($\times 1000/mm^3$)	<150	Lamivudine group	254	18	3.8	1.86
		Control group	629	125	5.8	3.43
	150≦	Lamivudine group	375	11	5.3	0.55
		Control group	1302	67	6.1	0.84

Table 3
Estimation of effects of covariates following selection of regressor in Cox regression model

Category	Hazard ratio	95% Confidence interval (CI)	p-Value
Lamivudine therapy			
No	1		
Yes	0.49	0.31–0.77	0.002
Gender			
Male	1		
Female	0.42	0.28–0.62	<0.001
Family clustering of hepatitis B			
No	1		
Yes	1.44	1.08–1.94	0.015
Age at liver biopsy			
<40 y.o.	1		
≧40 y.o.	2.09	1.77–2.48	<0.001
Stage of liver fibrosis			
F0 or F1	1		
F2, F3, or F4	1.43	1.24–1.64	<0.001
Serum albumin level			
<4.0 g/dL	1		
≧4.0 g/dL	0.58	0.43–0.79	0.001
Platelet count			
<150 × 1000/μL	1		
≧150 × 1000/μL	0.53	0.38–0.73	<0.001

In the analysis of retrospective studies, great precautions are required in order to eliminate any bias between lamivudine-treated and non-treated groups. To minimize inter-group bias, we conducted with the cooperation of multiple medical institutions and a large number of patients ($n = 2795$). The effect of lamivudine on HCC was ultimately analyzed in a matched case-controlled study. Because the time of liver biopsy was used as the starting point in our analysis, the analytical results were not expected to appropriately reflect lamivudine's effect if the therapy was started a long time after the biopsy. Therefore, from among the 657 patients who received lamivudine therapy, we selected 377 patients who started lamivudine therapy within 2 years after biopsy. For a control group, the same number of patients ($n = 377$) without lamivudine therapy was selected from the 2138 subjects.

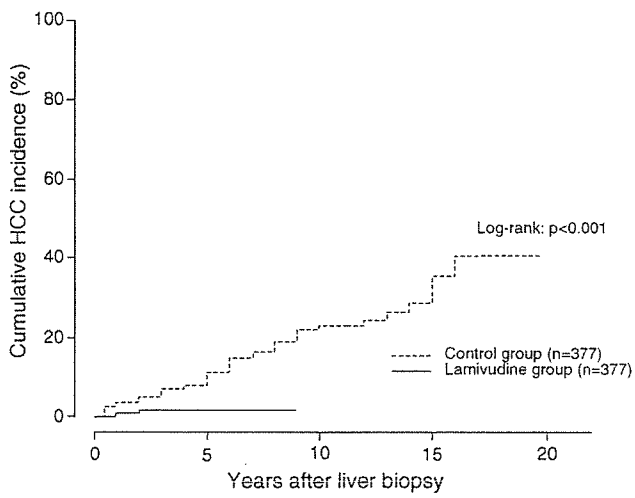

Fig. 1. Comparison of the cumulative HCC incidence between the lamivudine group (solid line) and the control group (broken line) by the Kaplan–Meier method in a case-matched control study. A significant difference was seen between the two groups ($p < 0.001$, log-rank test).

The regimen was not the same in all patients who have been treated by lamivudine. It was transiently discontinued before being recommenced later in some patients, whereas it was uninterrupted throughout the follow-up period in the majority (63%) of subjects in the matched case-controlled study. The duration of lamivudine regimen was not taken into account in the design of our study. Some patients received lamivudine for relatively short periods to improve acute exacerbation of their clinical course in chronic hepatitis B. On the other hand, some patients received lamivudine for the long-term to suppress the development of HCC. In the analysis by a multivariate Cox regression model in all unmatched patients, lamivudine therapy was selected as one of the factors inhibiting the occurrence of HCC. In the matched case-controlled study, the annual occurrence rate of HCC was significantly lower (0.4%/(patient/year)) in the lamivudine group than in the control group (1.8%/(patient/year)), suggesting that lamivudine treatment is effective for inhibiting the occurrence of HCC.

Recently, Liaw et al. conducted a multicenter, centrally randomized, double-blind, placebo-controlled, parallel group study to evaluate the effects of lamivudine on the progression of chronic hepatitis B to hepatic cancer [21]. They randomized 651 patients with histologically confirmed (F3 and F4), compensated hepatic cirrhosis to receive either lamivudine or a placebo at a ratio of 2:1 and continued the treatment for up to 5 years. The study was terminated after a median treatment duration of 32.4 months (range 0–42) owing to a significant difference between the groups in the number of end points reached. The end points were reached by 7.8% of the patients receiving lamivudine and 17.7% of those receiving placebo (hazard ratio for disease progression, 0.45; $p = 0.001$). The Child–Pugh score increased in 3.4% of the patients receiving lamivudine and in 8.8% of those receiving placebo (hazard ratio, 0.45; $p = 0.02$), whereas HCC occurred in 3.9% of those in the lamivudine group and in 7.4% of those in the placebo group (hazard ratio, 0.49: $p = 0.047$). The results of our analysis, which included patients with F0 through F2 hepatic fibrosis, were similar to those of Liaw et al. [21]. Thus, two studies demonstrated that the use of potent anti-viral agents such as lamivudine represents a major advance in the treatment of chronic hepatitis B and slows the progression of severe liver disease to liver cirrhosis as well as HCC.

Both hepatitis B and C are caused by persistent infection with hepatitis viruses, and both have a high probability of resulting in HCC. For this reason, these two diseases have a number of common traits, but some differences have been noted in their relationships with HCC. Among both

Table 4
Comparison of background factors between lamivudine group and control group assessed at the time of liver biopsy (matched case-controlled study)

Parameter	Lamivudine group ($n=377$)	Control group ($n=377$)	p-Value
Gender[a]			
Male	276 (73.2%)	273 (72.4%)	0.806
Female	101 (26.8%)	104 (27.6%)	
Age (years)[b]	41.5 ± 12.0	41.4 ± 12.2	0.950
Follow-up period (years)[b]	2.7 ± 2.1	5.3 ± 4.7	<0.001
Family clustering of hepatitis B[a]			
Yes	238 (63.1%)	242 (64.2%)	0.762
No	139 (36.9%)	135 (35.8%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	38 (10.1%)	62 (16.4%)	0.007
No	333 (88.3%)	314 (83.3%)	
Unknown	6 (1.6%)	1 (0.3%)	
IFN therapy[a]			
Yes	129 (34.2%)	143 (37.9%)	0.046
No	236 (62.6%)	231 (61.3%)	
Unknown	12 (3.2%)	3 (0.8%)	
Liver histology			
Grade of inflammation[a]			
A0	6 (1.6%)	18 (4.8%)	0.001
A1	110 (29.2%)	101 (26.8%)	
A2	157 (41.6%)	186 (49.3%)	
A3	98 (26.0%)	72 (19.1%)	
Unknown	6 (1.6%)	0 (0.0%)	
Stage of fibrosis[a]			
F0	7 (1.9%)	6 (1.6%)	0.647
F1	103 (27.3%)	117 (31.0%)	
F2	95 (25.2%)	97 (25.7%)	
F3	107 (28.4%)	90 (23.9%)	
F4	65 (17.2%)	67 (17.8%)	
HBeAg[a]			
+	193 (51.2%)	220 (58.4%)	0.005
−	178 (47.2%)	141 (37.4%)	
Unknown	6 (1.6%)	16 (4.2%)	
HBeAb[a]			
+	126 (33.4%)	121 (32.1%)	0.030
−	245 (65.0%)	237 (62.9%)	
Unknown	6 (1.6%)	19 (5.0%)	
Albumin (g/dL)[b]	4.00 ± 0.51	4.00 ± 0.52	0.989
AST (IU/L)[b]	118.5 ± 155.4	95.5 ± 126.4	0.031
ALT (IU/L)[b]	191.7 ± 234.8	151.5 ± 180.5	0.009
Platelet count ($\times 1000/mm^3$)[b]	161.7 ± 52.7	164.3 ± 59.5	0.523

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.

hepatitis B patients and hepatitis C patients, HCC occurs mainly in those with advanced hepatic fibrosis, but the incidence of liver cirrhosis as a background of liver disease is lower in patients with B than in those with C. Furthermore, among hepatitis C patients HCC occurs mainly in those 60 years or older, while among hepatitis B patients it occurs mainly in those under 60 [22–24]. Studies on the cumulative incidence of HCC in hepatitis B patients showed that the HCC incidence increases linearly during the initial 12 years, plateaus, and then increases again in the 17th or 18th year [24,25]. In hepatitis C patients, on the other hand, the HCC incidence shows a continuous, linear increase [26,27]. Various findings obtained to date suggest that these clinical differences are related not only to differences in the hepatitis viral infection route and the timing of infection but also to differences in the mechanisms underlying cancer associated with hepatitis B and C. HCV is an RNA virus, and viral genes are not integrated into the host's genes, whereas HBV is a DNA virus with reverse-transcriptase activity. Thus, HBV genes are often integrated into the host's chromosomes

Table 5
Comparison of distribution of background factors between patients who developed HCC and those who did not in the lamivudine group (matched case-controlled study)

Parameter	Patients with HCC ($n=4$)	Patients without HCC ($n=373$)	p-Value
Gender[a]			
Male	3 (75.0%)	273 (73.2%)	1.000[c]
Female	1 (25.0%)	100 (26.8%)	
Age (years)[b]	55.0 ± 19.5 ($n=4$)	41.3 ± 11.9 ($n=373$)	0.024
Follow-up period (years)[b]	1.5 ± 0.6 ($n=4$)	2.7 ± 2.1 ($n=373$)	0.236
Family clustering of hepatitis B[a]			
Yes	2 (50.0%)	236 (63.3%)	0.628[c]
No	2 (50.0%)	137 (36.7%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	1 (25.0%)	37 (9.9%)	0.393[c]
No	3 (75.0%)	330 (88.5%)	
Unknown	0 (0.0%)	6 (1.6%)	
IFN therapy[a]			
Yes	0 (0.0%)	129 (34.6%)	0.387[c]
No	4 (100.0%)	232 (62.2%)	
Unknown	0 (0.0%)	12 (3.2%)	
Liver histology			
Grade of inflammation[a]			
A0	0 (0.0%)	6 (1.6%)	0.458[c]
A1	0 (0.0%)	110 (29.5%)	
A2	3 (75.0%)	154 (41.3%)	
A3	1 (25.0%)	97 (26.0%)	
Unknown	0 (0.0%)	6 (1.6%)	
Stage of fibrosis[a]			
F0	0 (0.0%)	7 (1.9%)	0.918[c]
F1	1 (25.0%)	102 (27.3%)	
F2	1 (25.0%)	94 (25.2%)	
F3	2 (50.0%)	105 (28.2%)	
F4	0 (0.0%)	65 (17.4%)	
HBeAg[a]			
+	3 (75.0%)	190 (50.9%)	0.648[c]
−	1 (25.0%)	177 (47.5%)	
Unknown	0 (0.0%)	6 (1.6%)	
HBeAb[a]			
+	2 (50.0%)	124 (33.2%)	0.632[c]
−	2 (50.0%)	243 (65.1%)	
Unknown	0 (0.0%)	6 (1.6%)	
Albumin (g/dL)[b]	4.23 ± 0.45 ($n=4$)	4.00 ± 0.51 ($n=373$)	0.384
AST (IU/L)[b]	47.0 ± 22.8 ($n=4$)	119.4 ± 156.2 ($n=326$)	0.356
ALT (IU/L)[b]	46.3 ± 24.2 ($n=4$)	193.2 ± 235.5 ($n=372$)	0.213
Platelet count ($\times 1000/mm^3$)[b]	141.0 ± 27.0 ($n=4$)	161.9 ± 52.9 ($n=373$)	0.431

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.
[c] Fisher's exact test.

and play an important role in hepatic carcinogenesis [28,29]. It is known that the repeat of necrosis and regeneration of liver might accelerate the mutation of oncogenes. In addition, de novo carcinogenesis is thought to be promoted in hepatitis B patients as a result of the increased genetic instability caused by the integration of the HBV genome into the host's chromosomes. When administered to patients with hepatitis B, lamivudine decreases the blood HBV-DNA concentration and markedly improves ALT levels, with consequent improvement of liver histological findings [7,11,13,14]. An early in vitro study showed that lamivudine decreases the amount of free HBV-DNA in hepatocytes but does not affect integrated HBV genes [30]. Therefore, lamivudine is thought to inhibit HCC by abating hepatitis and not by inhibiting viral gene integration. In fact, as shown in the matched case control study, all four patients who developed HCC in the lamivudine group had non-cirrhotic liver disease, whereas 23 (46%) of 50 patients who developed HCC had liver cirrhosis. Due to the small number of patients included, however, further studies are necessary to confirm this finding.

Table 6
Comparison of distribution of background factors between patients who developed HCC and those who did not in the control group (matched case-controlled study)

Parameter	Patients with HCC ($n=50$)	Patients without HCC ($n=327$)	p-Value
Gender[a]			
Male	40 (80.0%)	233 (71.3%)	0.236[c]
Female	10 (20.0%)	94 (28.7%)	
Age (years)[b]	50.6 ± 10.1	40.0 ± 11.9	<0.001
Follow-up period (years)[b]	5.3 ± 4.3	5.2 ± 4.8	0.951
Family clustering of hepatitis B[a]			
Yes	29 (58.0%)	213 (65.1%)	0.345[c]
No	21 (42.0%)	114 (34.9%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	14 (28.0%)	48 (14.7%)	0.050[c]
No	36 (72.0%)	278 (85.0%)	
Unknown	0 (0.0%)	1 (0.3%)	
IFN therapy[a]			
Yes	16 (32.0%)	127 (38.8%)	0.578[c]
No	34 (68.0%)	197 (60.2%)	
Unknown	0 (0.0%)	3 (0.9%)	
Liver histology			
Grade of inflammation[a]			
A0	2 (4.0%)	16 (4.9%)	0.026[c]
A1	6 (12.0%)	95 (29.1%)	
A2	27 (54.0%)	159 (48.6%)	
A3	15 (30.0%)	57 (17.4%)	
Stage of fibrosis[a]			
F0	0 (0.0%)	6 (1.8%)	<0.001[c]
F1	7 (14.0%)	110 (33.6%)	
F2	8 (16.0%)	89 (27.2%)	
F3	12 (24.0%)	78 (23.9%)	
F4	23 (46.0%)	44 (13.5%)	
HBeAg[a]			
+	26 (52.0%)	194 (59.3%)	0.564[c]
−	22 (44.0%)	119 (36.4%)	
Unknown	2 (4.0%)	14 (4.3%)	
HBeAb[a]			
+	20 (40.0%)	101 (30.9%)	0.319[c]
−	27 (54.0%)	210 (64.2%)	
Unknown	3 (6.0%)	16 (4.9%)	
Albumin (g/dL)[b]	3.63 ± 0.59	4.06 ± 0.49	<0.001
AST (IU/L)[b]	96.9 ± 100.8	95.3 ± 130.0	0.934
ALT (IU/L)[b]	132.8 ± 165.5	154.4 ± 182.7	0.431
Platelet count ($\times 1000/mm^3$)[b]	126.8 ± 50.7	170.0 ± 58.7	<0.001

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.
[c] Fisher's exact test.

Seven HBV genotypes (A–G) have been identified to date, and their distribution shows regional variations [31–36]. In Japan, genotypes C, B, and the other five account for 85, 12, and 3% of hepatitis B patients [36]. The virological differences between HBV genotype B and genotype C might influence not only on the natural course of hepatitis B but also the efficacy by lamivudine. The patients with HBV genotype B are frequently negative for HBeAg, have lower ALT levels and a better prognosis. In contrast, the patients with HBV genotype C tend to remain HBeAg-positive for a longer duration and tend to have elevated ALT levels and more advanced liver disease, such as liver cirrhosis and HCC. This indicates that the analysis of HBV genotypes will be needed in this study.

In conclusion, our multicenter, retrospective, matched case study indicated that lamivudine treatment might suppress the risk of HCC in patients with chronic hepatitis B. However, the study has several limitations, such as the relatively short duration of treatment and the lack of virological analyses (HBV genotype, YMDD mutation, and HBV-DNA volume). To relief these limitations, further long-term observation should be continued to clarify the conclusion.

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Appendix A

The Inuyama Hepatitis Study Group consists of the following 30 institutions and members: Dr. Sumio Watanabe (Akita University School of Medicine, Akita, Yamagata), Dr. Sumio Kawada (Yamagata University School of Medicine, Yamagata), Dr. Osamu Yokosuka (Chiba University, Graduate School of Medicine, Chiba), Dr. Kunihiko Hino (Delta Clinic, Tokorozawa), Dr. Hiromasa Ishii (Keio University, School of Medicine, Tokyo), Dr. Hiromitu Kumada (Toranomon Hospital, Tokyo), Dr. Gotaro Toda (Jikei University School of Medicine, Tokyo), Dr. Yasuyuki Arakawa (Nihon University School of Medicine, Tokyo), Dr. Nobuyuki Enomoto (Yamanashi University, School of Medicine, Kofu), Dr. Kendo Kiyosawa (Shinshu University School of Medicine, Matsumoto), Dr. Takafumi Ichida (Niigata University, Graduate School of Medical and Dental Science, Niigata), Dr. Tomoteru Kamimura (Niigata Saiseikai Hospital Dai-2, Niigata), Dr. Masashi Mizogami (Nagoya City University Graduate School of Medical Science, Nagoya), Dr. Shinichi Kakumu (Aichi Medical University, Nagoya), Dr. Hisataka Moriwaki (Gifu University School of Medicine, Gifu), Dr. Shuichi Kaneko (Kanazawa University, Graduate School of Medical Science, Kanazawa), Dr. Takeshi Okanoue (Kyoto Prefectural University, Graduate School of Medical Science, Kyoto), Dr. Norio Hayashi (Osaka University Graduate School of Medicine, Osaka), Dr. Masatoshi Kudo (Kinki University School of Medicine, Sayama), Dr. Yasushi Shiratori (Okayama University, Graduate School of Medicine and Dentist[r]y, Okayama), Dr. Gotaro Yamada (Kawasaki Hospital, Kawasaki Medical School, Okayama), Dr. Kazuaki Chayama (Hiroshima University, Graduate School of Biomedical Science, Hiroshima), Dr. Kiwamu Okita (Yamaguchi University, School of Medicine, Ube), Dr. Shigeki Kuriyama (Kagawa Medical University, Takamatsu), Dr. Morikazu Onji (Ehime University School of Medicine, Juushin-cho), Dr. Saburo Ohnishi (Kochi University School of Medicine, Nangoku), Dr. Michio Sata (Kurume University School of Medicine, Kurume), Dr. Shigetoshi Fujiyama, and Dr. Hiroshi Sasaki (Kumamoto University, Faculty of Medical and Pharmaceutical Science, Kumamoto), Dr. Hirohito Tsubouchi (Miyazaki University School of Medicine, Miyazaki), and Dr. Hiromi Ishibashi and Dr. Hiroshi Yatsuhashi (Nagasaki Medical Center, Omura).

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CLINICAL RESEARCH STUDY

Long-Term Outcome after Hepatitis B Surface Antigen Seroclearance in Patients with Chronic Hepatitis B

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ABSTRACT:

PURPOSE: The aim of this study was to elucidate the long-term outcome after hepatitis B surface antigen (HBsAg) seroclearance in a large number of Japanese patients.

METHODS: We studied the biochemical, virologic, histologic, and prolonged prognoses of 231 Japanese patients with HBsAg seroclearance (median follow-up, 6.5 years). Serum alanine aminotransferase, serum hepatitis B virus (HBV) markers, liver histology, and clinical aspects were monitored. HBV-DNA levels were measured with the qualitative polymerase chain reaction assay. The mean age of patients with HBsAg seroclearance was 52 years.

RESULTS: After HBsAg seroclearance, 203 patients (87.9%) had normal alanine aminotransferase levels 1 year after HBsAg seroclearance. HBV-DNA showed positive results in 4 patients (1.7%) 1 year after HBsAg seroclearance. Thirteen patients were examined for histologic changes of the liver after HBsAg seroclearance. All patients showed marked improvement of necroinflammation of the liver, but only 2 of the 13 patients showed no liver fibrosis. Liver cirrhosis and hepatocellular carcinoma did not develop in any of the 164 patients without evidence of liver cirrhosis at the time of HBsAg seroclearance. Hepatocellular carcinoma developed in 2 of the 67 patients with liver cirrhosis at the time of HBsAg seroclearance. During the observation period, 15 patients died. However, the cause of death of these 15 patients was not related to liver disease, such as hepatocellular carcinoma, decompensated liver cirrhosis, and rupture of esophageal varices.

CONCLUSION: Our results suggest that HBsAg seroclearance confers favorable long-term outcomes in patients without hepatocellular carcinoma or decompensated liver cirrhosis at the time of HBsAg seroclearance

KEYWORDS: Chronic hepatitis B; HBV-DNA; Seroclearance of hepatitis B surface antigen

Chronic hepatitis B virus (HBV) is a serious liver disease with significant mortality. In patients with chronic HBV infection, persistent viral replication is associated with ongoing necroinflammation in the liver and progressive liver damage.[1-3] Yang et al[4] reported that the relative risk of hepatocellular carcinoma was 9.6 among men who were positive for HBsAg alone and 60.2 among men who were positive for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), compared with men who were negative for both. Epidemiologic studies have shown that positivity for HBsAg is one of the most important risk factors for hepatocellular carcinoma. However, in patients with HBeAg seroclearance and marked reduction of serum HBV-DNA, the prognosis of the disease is generally improved.[5-7] Therefore, marked reduction of HBV replication can possibly prevent hepatocellular carcinoma development. Moreover, HBsAg

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seroclearance has been associated with a good prognosis, including liver histology and liver function improvement and even prolonged survival.[8-10]

However, spontaneous remissions occur in a small proportion of patients during the natural history of chronic HBV infections. Seroclearance of HBsAg in patients with chronic HBV infection is unusual (0.4%-2% per year in white patients[11-13] and 0.1%-0.8% per year in Chinese patients[9]). Thus, until now, few studies have dealt with prognosis in Japanese patients with seroclearance of HBsAg.

A few previous studies have had conflicting results, with some suggesting that adverse complications are not rare in patients with HBsAg clearance,[14,15] and others suggesting that spontaneous HBsAg seroclearance is excellent.[16] These discrepancies might depend on concurrent hepatitis infection, age, and other factors. The present study excluded patients with concurrent hepatitis virus infection. Moreover, the main focus of this article is the survival time of patients with HBsAg seroclearance. Thus, we performed this study to elucidate the long-term outcome after HBsAg seroclearance in a large number of Japanese patients.

CLINICAL SIGNIFICANCE

- HBsAg seroclearance confers favorable long-term outcomes in patients without hepatocellular carcinoma or decompensated liver cirrhosis at the time of HBsAg seroclearance.
- Patients with liver cirrhosis at the time of HBsAg seroclearance should be closely monitored for predictable complications such as hepatocellular carcinoma.
- Some patients had a trace of hepatitis B virus DNA at the fifth and/or tenth year after seroclearance of HBsAg and were followed on with the administration of steroids and/or immunosuppressive agents.

MATERIALS AND METHODS

Patients

From 1972 to 2002, a total of 5055 chronic HBsAg carriers, who were known to be seropositive for HBsAg for at least 6 months, were studied at Toranomon Hospital in Tokyo, Japan. After a mean follow-up period of 4 years (range 0.5-30 years), 231 patients were noted to have delayed HBsAg seroclearance, which is defined as persistent absence of HBsAg antigenemia by radioimmunoassay for at least 1 year and until the last examination. We excluded from the study all patients with: concurrent hepatitis C virus and hepatitis D virus; a history of alcohol abuse or autoimmune liver disease; clinical evidence of hepatocellular carcinoma at entry into the study on the basis of ultrasonography, alpha-fetoprotein levels (<200 ng/mL), and/or histology; or history or clinical evidence of complications of decompensated cirrhosis at enrollment (ie, ascites, encephalopathy, or icterus).

A total of 156 of 231 patients had spontaneous seroclearance of HBsAg; 46 patients had been given interferon monotherapy for 1 to 90 months; 14 patients had been given steroid withdrawal monotherapy; and 12 patients had been treated with both steroids and interferon. The remaining 3 patients had been given 100 mg of lamivudine daily for more than 1 year. The total median dose of interferon monotherapy was 336 MU (range 168-1890 MU). The patients treated with steroids were generally given prednisolone for 4 weeks, given in a single dose of 40 mg/day for 1 week, 30 mg/day for 1 week, 20 mg/day for 1 week, and then 10 mg/day for 1 week until it was abruptly withdrawn (total dose 700 mg). A total of 231 patients were followed up for more than 1 year after HBsAg seroclearance.

Methods

The time of entry into the study was defined as the time of serum HBsAg clearance as measured by radioimmunoassay. After HBsAg seroclearance, patients were followed up every 3 or 6 months or more frequently when their levels of alanine aminotransferase and α-fetoprotein were elevated. Follow-up studies included clinical, biochemical, and virologic aspects, and hepatocellular carcinoma screening with ultrasonography and alpha-fetoprotein. Biochemical tests were measured using routine automated techniques and performed in the clinical pathology laboratories of Toranomon Hospital. HBsAg, anti-HBs, and antibody to hepatitis D virus were

Table 1 Characteristics of subjects at the seroclearance of HBsAg

Characteristic	
N	231
Sex (male/female)	186/45
Age (years)	51 (23-66)
Body weight (kg)	67.5 (46.9-82.4)
HBV-genotype (A/B/C/D/F)	5/23/118/2/1
US (non-LC/LC)	164/67
Total protein (g/dl)	7.5 (6.5-9.3)
Albumin (g/dl)	4.2 (3.1-5.1)
Total bilirubin (g/dl)	0.7 (0.1-2.0)
AST (IU/L)	21 (10-219)
ALT (IU/L)	19 (6-946)
Hb (g/dl)	15.2 (12.0-17.4)
Platelet ($\times 10^4/mm^3$)	16.8 (8.4-32.5)
Follow-up period after disappearance of HBs antigen (year)	6.5 (1-23.6)

Data are number of patients or median (range)
ALT = alanine aminotransferase; AST = aspartate aminotransferase; Hb = hemoglobin; US = ultrasonography

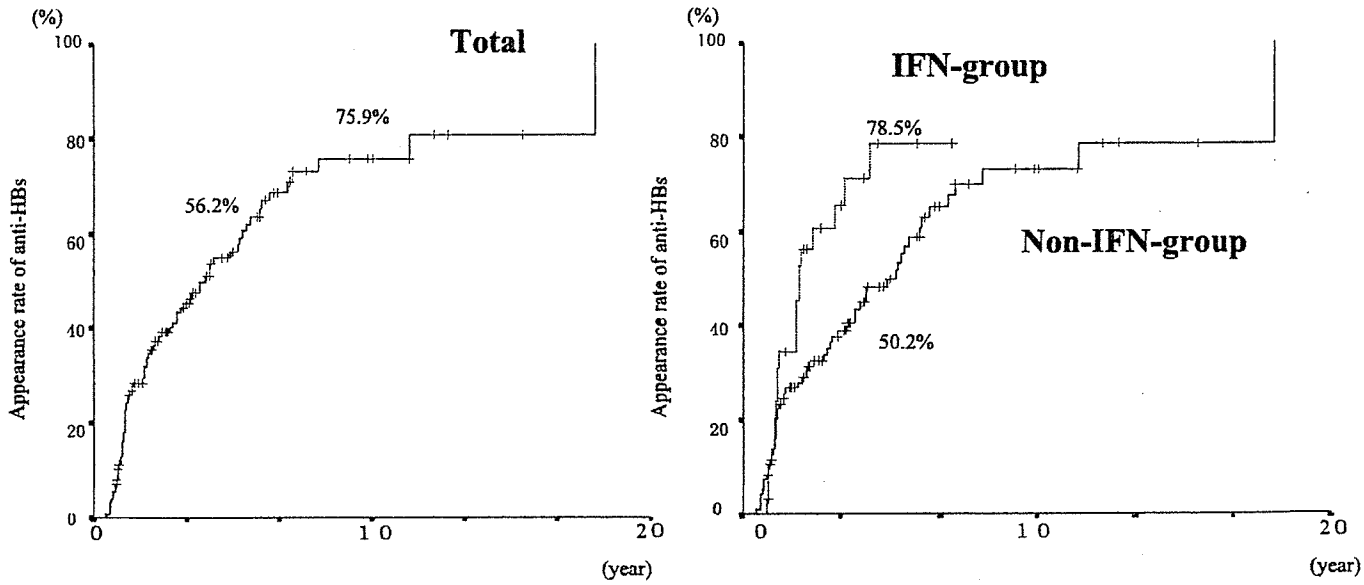

Figure 1 Cumulative appearance rate of the antibody to hepatitis B surface antigen (HBsAg) after seroclearance of HBsAg. IFN = interferon.

all assayed with commercially available radioimmunoassay kits. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay (Ortho Diagnostic Japan, Tokyo, Japan). HBV genotype was determined with a previously reported method.[17]

Serum HBV-DNA level was measured with a commercially available quantitative polymerase chain reaction assay (Amplicor HBV, monitor, Roche Diagnostics, GmbH, Mannheim, Germany)[18] 1 year after seroclearance of serum HBsAg. The sensitivity of HBV-DNA according to the manufacturer is approximately 400 copies/mL in quantitative polymerase chain reaction. Serum samples were conserved at −80° until use.

Status of liver cirrhosis was determined on the basis of liver biopsy and/or ultrasonographic findings. Ultrasonography was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo, Japan: Logic 700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). The diagnosis of liver cirrhosis was defined as having a score of more than 8 in an ultrasonographic scoring system based on liver surface, liver parenchyma, hepatic vessel, and spleen size, as reported by Lin et al.[19]

The diagnostic accuracy of ultrasonography for liver cirrhosis was at least 80%. This study was approved by the institutional review board of our hospital. The physicians in charge explained the purpose and method of this clinical trial to each patient, who gave their informed consent for participation.

Liver Histology and Ultrasonographic Findings

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in

Table 2 Predictive factors for appearance of anti-HBsAg

Factor	Category	Odds ratio	95% CI	*P* value
Interferon therapy	(−)/(+)	1/1.90	1.13-3.21	.016
Prednisolone withdrawal therapy	(−)/(+)	1/2.25	1.07-4.72	.032
Age (years)	<60/≥60	1/0.500	0.248-1.01	.052
Total protein (g/dl)	<8/≥8	1/1.84	0.90-3.76	.096
US	Non-LC/LC	1/0.71	0.430-1.16	.167
HBV-genotype	B/C	1/1.63	0.58-4.55	.350
Sex	Male/Female	1/1.34	0.72-2.50	.355
AST (IU/L)	≥38/<38	1/1.59	0.57-4.43	.375
Platelet ($\times 10^4/mm^3$)	≤20/>20	1/1.20	0.700-2.06	.504
ALT (IU/L)	≥50/<50	1/1.28	0.46-3.55	.634

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; Hb = hemoglobin; HCV = hepatitis C virus; US = ultrasonographic findings; LC = liver cirrhosis; HBV = hepatitis B virus.

Table 3 Histological features of the 13 patients with HBsAg seroclearance

Patients	Age of HBsAg clearance, y	Histologic activity index before HBsAg clearance*				Histologic activity index after HBsAg clearance*							
		Periportal bridging necrosis	Intralobular degeneration and focal necrosis	Portal inflammation	Fibrosis	Interval-1† 1† mo	Interval-2† 2† mo	Periportal bridging necrosis	Intralobular degeneration and focal necrosis	Portal inflammation	Fibrosis		
1	24	3	3	1	1	16.0	12.1	0	0	0	0		
2	29	3	3	3	3	72.5	43.1	0	0	0	3		
3	36	3	3	3	3	105.4	52.8	0	0	0	2		
4	40	5	3	4	3	47.3	12.0	0	0	0	1		
5	42	4	3	4	2	101.6	2.3	1	1	1	1		
6	42	3	3	3	2	87.5	12.1	0	0	1	2		
7	45	5	4	3	3	183.0	34.5	0	0	1	1		
8	46	3	3	1	3	180.2	36.5	0	0	1	2		
9	48	3	3	3	4	204.0	69.8	0	0	0	4		
10	59	5	4	4	3	207.7	2.3	0	1	1	0		
11	61	4	3	4	3	72.1	8.9	0	1	1	1		
12	61	3	3	1	4	124.1	18.9	0	0	0	2		
13	61	3	3	3	2	98.6	40.0	0	0	1	2		

*Histologic activity index score: 0-10 for periportal bridging necrosis and 0-4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.
†Interval-1: interval between first biopsy before HBsAg clearance and last biopsy after HBsAg clearance. Interval-2: interval between HBsAg clearance and last biopsy after HBsAg clearance.

10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than 6 portal areas. Histopathologic interpretations of these 3- to 4-μm–thick sections were made independently by experienced liver pathologists (YA and HK) who had no clinical information or knowledge of chronologic order of the biopsies in each pair. The biopsy specimens were scored according to the system of Knodell et al.[20]

Patient Follow-Up

Clinical evaluation and biochemical and hematologic tests were performed at 2- to 6-month intervals. Thirty patients were lost to follow-up. Because the appearance of hepatocellular carcinoma was not identified in these 30 patients, they were considered as censored data in statistical analysis.[21] Hepatocellular carcinoma was diagnosed by histology or the typical hypervascular characteristics observed on angiography, in addition to certain features of computed tomography and ultrasonography.

Statistical Analysis

Statistical analysis was performed with Fisher's exact test, Kaplan-Meier estimate, log-rank test, and a Cox proportional hazard model where appropriate. *P* values less than .05 were considered statistically significant. The SPSS software package (SPSS Inc., Chicago, Ill) was used to perform statistical analysis.

RESULTS

Changes of Liver Biochemistry After HBsAg Seroclearance

Table 1 shows the characteristics of the 231 patients who had seroclearance of HBsAg. These patients were classified into a liver cirrhosis group or a non-liver cirrhosis group by ultrasonographic findings. A total of 67 patients showed a finding of liver cirrhosis. Histologic evidence of liver cirrhosis before HBsAg seroclearance was seen in 47 patients.

The alanine aminotransferase test showed that 203 of 231 patients (87.9%) had normal alanine aminotransferase levels 1 year after seroclearance of HBsAg. Twenty-eight patients had elevated alanine aminotransferase levels (18 with fatty infiltration of liver, 3 with alcohol abuse, and 8 with unknown origin).

Changes of HBV Marker after HBsAg Seroclearance

The cumulative appearance of anti-HBs is shown in Figure 1. A Cox proportional hazards model was used to analyze the factors contributing to the appearance of anti-HBs (Table 2). The patients treated with interferon showed the high cumulative appearance of anti-HBs by

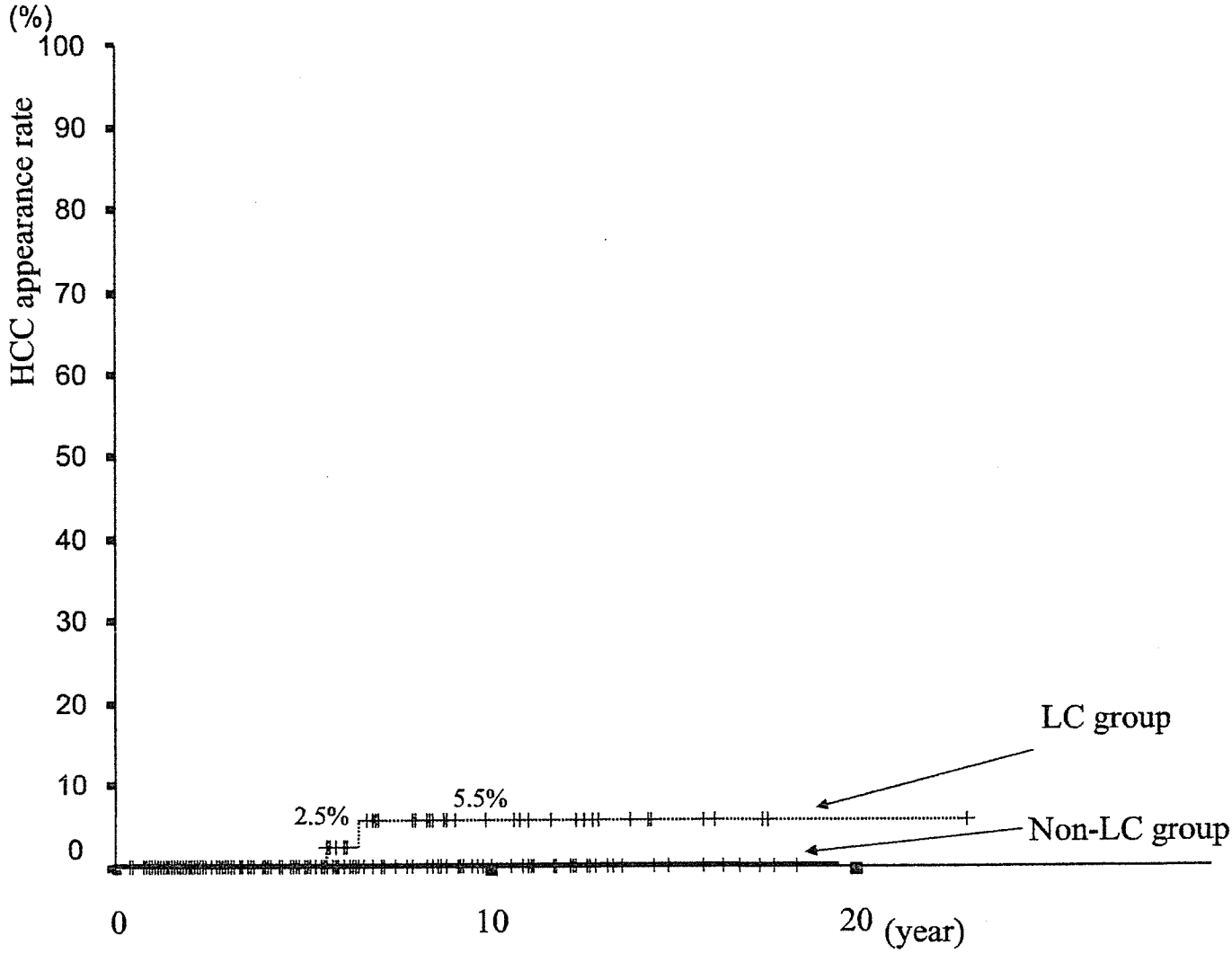

Figure 2 Hepatocellular carcinoma (HCC) appearance rate after seroclearance of HBsAg. LC = liver cirrhosis.

log-rank test. Anti-HBs became detectable in 50.2% of patients with spontaneous seroclearance of HBsAg and in 78.5% of patients treated with interferon at the fifth year after HBsAg seroclearance.

Next, we examined serum HBV-DNA level with the qualitative polymerase chain reaction assay (Amplicor HBV monitor). HBV-DNA showed positive results in 1.7% (3/231) 1 year after seroclearance of HBsAg.

Table 4 Recent studies on the outcomes following HBsAg clearance

Source	Status at clearance	No of cases	No of HBV alone	Follow-up, mo	Mean age, y	Outcomes	
						Decompensated LC	HCC
Fattovich et al[14]	LC	32	30*	55	44	6	1§
Huo et al[15]	Non-LC	55	32*	23	54	6	1§
Chen et al[16]	Non-LC	189	146*	65.4	43	0	2§
	LC	29	17*	50.8	54	4†	1§
Yuen et al[10]	LC or non-LC	92	92	51.1	42.6		5
Present	Non-LC	167	167	61.1	51	0	0
	LC	67	67	74.1	52.5	0	2

LC = liver cirrhosis; HCC = hepatocellular carcinoma.
*Remaining patients had concurrent virus of hepatitis C virus and/or HDV.
†Two of 4 patients had concurrent virus.
§These patients had concurrent virus.