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**Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2,795 patients**

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**Running title:**

**Lamivudine treatment suppresses the occurrence of HCC in chronic hepatitis**

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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 2,795 patients who satisfied criteria for analysis after treatment from any of 30 medical institutions, 657 had received lamivudine and the remaining 2,138 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 4 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 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 [3,4,5]. 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 antiviral 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 [7,8,9]. By virtue of this inhibitory ability, lamivudine was developed as an antiviral 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 [12,13,14,15,16]. 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, aspartate 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; and 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 3,022 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 hundreds twenty seven patients who had not answered the question about lamivudine treatment were excluded from the study.** This left a total of 2,795 patients for analysis. Among them, 657 patients had received lamivudine therapy and 2,138 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  $5.8 \pm 9.0$  months, and the treatment period was  $23.1 \pm 19.0$  months (range 3-96 months). For the control group, seven factors were selected on the basis of the propensity scores from the 2,138 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  $\chi^2$  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 2,138 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%/patient/year 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 inter-group 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 4.0g/dl,  $p=0.001$ ), and platelet count (less than 150,000/ $\mu$ l,  $p<0.001$ ). 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 vs. 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 vs. 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.

#### 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, 13, 14, 15, and 16].

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=2,795). 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 2,138 subjects.

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 YF 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 to 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 antiviral 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 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, 23, 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 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.

Seven HBV genotypes (A through G) have been identified to date, and their distribution shows regional variations [31, 32, 33, 34, 35, 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, HBV DNA volume). To relief these limitations, further long-term observation should be continued to clarify the conclusion.

## Appendix

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. Kunihiro Hino (Delta Clinic, Tokorozawa), Dr. Hiromasa Ishii (Keio University, School of Medicine, Tokyo), Dr. Hiromitsu Kumata (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. Hirohi Yatsunami (Nagasaki Medical Center, Omura).

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## Figure legends

### Figure 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).

