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INTERNAL MEDICINE

CASE REPORT

Prolonged Hepatitis after Acute Infection with Genotype H Hepatitis B Virus

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Abstract

We present a case report of a Japanese patient who showed prolonged infection after acute hepatitis B with genotype H. The patient was a 60-year-old man who underwent an annual health care check every year for several years and was never pointed out to have any liver damage, and markers for hepatitis B and C were negative. He was found to be positive for hepatitis B surface antigen (HBsAg) at his health care check in December 2005. After one month, he had an elevated aminotransferase level with hepatitis B e antigen and a high level of serum HBV DNA. He was diagnosed as having acute hepatitis B. On HBV genotype, he had genotype H by the direct sequence method, and he was given a 100 mg of lamivudine daily. However, his acute hepatitis tended to go toward prolonged infection. After two months, he was treated with interferon daily for 28 days. He had negative HBsAg in August 2006. Genotype H, the newest type of hepatitis B, could be the type which shows a poor response to lamivudine. The present paper is the first report, describing the clinical course of acute hepatitis B with genotype H from onset to remission.

Key words: acute hepatitis B, HBV genotype H, prolonged infection

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Introduction

Hepatitis B virus (HBV) infection is related to many liver diseases, acute or fulminant hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. It is estimated that approximately 350 million are chronically infected. Annual mortality rate is 500 000-700 000 (1, 2). Many of the adult patients with acute hepatitis B are cured through the natural course (2). However, there are some individuals who are continuously with HBV and developed to cirrhosis or hepatocellular carcinoma.

Phylogenetic analysis has classified HBV into eight genotypes, designated A to H. The genotypes have different biological properties; these differences affect the clinical outcome and response to antiviral therapy (3). Genotype H has been newly found in Nicaragua and in the U.S.; it seems to be distributed in Central America (4). The prolonged prognosis and response to antiviral therapy in acute hepatitis B patients with genotype H is still obscure. We present here a patient who was suffered from acute hepatitis B with genotype H and had a prolonged clinical course after receiving intensive treatment for hepatitis B. This is the first report to describe the whole clinical course, including the period before onset, of a patient with acute hepatitis B due to HBV genotype H.

Case Report

The patient was a 60-year-old man. He underwent an annual health exam for several years. He had never been pointed out to have any liver damage, and he was negative for markers of hepatitis B or C. At the annual health care check in December 2005, he was found to be positive for serum hepatitis B surface antigen (HBsAg), along with aspartate aminotransferase (AST) 31 IU/l, alanine aminotransferase (ALT) 39 IU/l and was referred to the Toranomon Hospital. He did not have any complaints at that time. One

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Table 1. Laboratory Findings on Admission*

Parameter	Value	Parameter	Value
Hematology		K	4.2 mEq/l
White blood cells	5000/μl	Cl	107mEq/l
Hemoglobin	15.1g/dl	CRP	1.0mg/d
Platelets	25.6×10⁴/μl	Coagulation test	
Blood chemistry		Prothrombin test	89.2%
Total protein	7.2g/dl	Viral markers	
Albumin	3.9 g/dl	IgM anti-HAV(EIA)	0.2(-)
Total bilirubin	0.5mg/dl	IgM anti-HBV(CLIA)	21.9(+)
AST	150IU/l	HBsAg (RPHA)	2048(+)
ALT	434IU/l	HBeAg (CLIA)	1460(+)
LDH	221IU/l	Anti-HBe(CLIA)	0%(-)
ALP	293	HBV DNA(TMA)	8.2 LEG/ml
γ-GTP	127 IU/l	HBV genotype	H
Creatinine	0.8 mg/dl	Anti-HCV(CLEIA)	0.3(-)
Na	143mEq/l	Anti-HIV(CLEIA)	(-)

* AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase; CRP, C-reactive protein; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg.

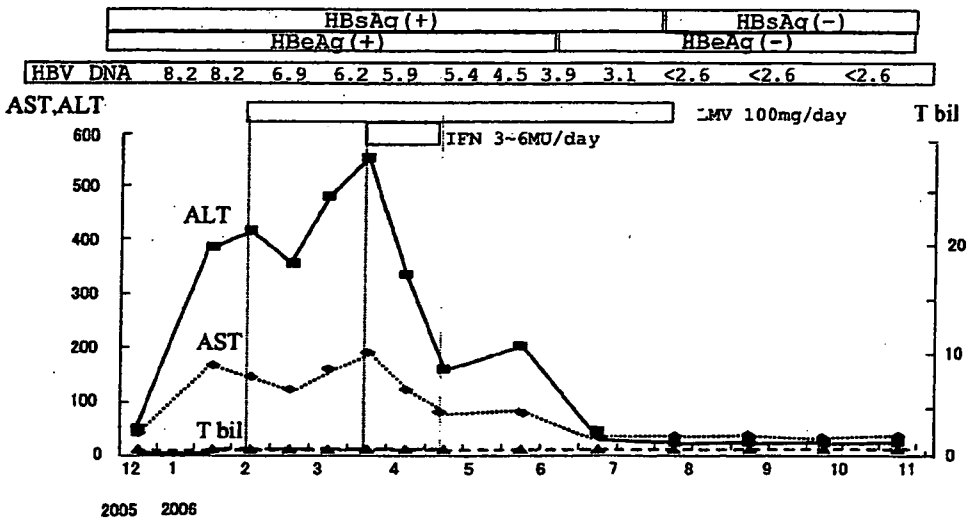

Figure 1. Clinical course of the patient with acute hepatitis B and genotype H.

month later, he just complained of slight fatigue and showed elevated AST and ALT.

He was admitted to our hospital for suspected acute hepatitis B in January, 2006. On admission he showed no jaundiced and was relatively healthy. He was positive for hepatitis B e antigen (HBeAg) and 8.2 LGE/ml of serum HBV-DNA as measured by transcription-mediated amplification and hybridization protect assay [Chugai Daiagnostics Science Co., Tokyo, Japan (5)]. Serum levels of AST and ALT were relatively low. Serological markers for HBsAg, HBeAg were strongly positive and serum level of HBV-DNA was high. IgM antibody to hepatitis B core antigen was high (21.9 S/CO) by the CLIA method (Abbott Japan Co., Ltd., Tokyo, Japan) as shown in Table 1. Therefore, he was diagnosed as having acute hepatitis B. No personal or family history of liver disease was recorded. Serological markers for antibodies to hepatitis C virus and antibodies to HIV type 1 and 2 were negative. However, he was a homosexual habit and went to a 'meeting' two to three times each month near his residence. In the meeting he had sexual contacts with unknown persons.

Lamivudine (LMV), a nucleoside analogue, was prescribed for him to reduce activity in the liver and HBV-DNA serum levels. He was given 100 mg of LMV daily. One month later from the initiation of lamivudine, his transaminase level began to increase, and natural interferon (IFN) beta (Toray Industiries, Inc., Tokyo, Japan) was started by intravenous injection from one more week later. Interferon was started at 6 MU daily. But neutropenia was seen in one week. The dose was then decreased to 3 MU. Unfortunately, three more weeks later, he had complained of depression which was suspected to be an interferon related side effect and IFN therapy was discontinued within one month. Over that time, HBV-DNA had gradually decreased (Fig. 1). Mu-

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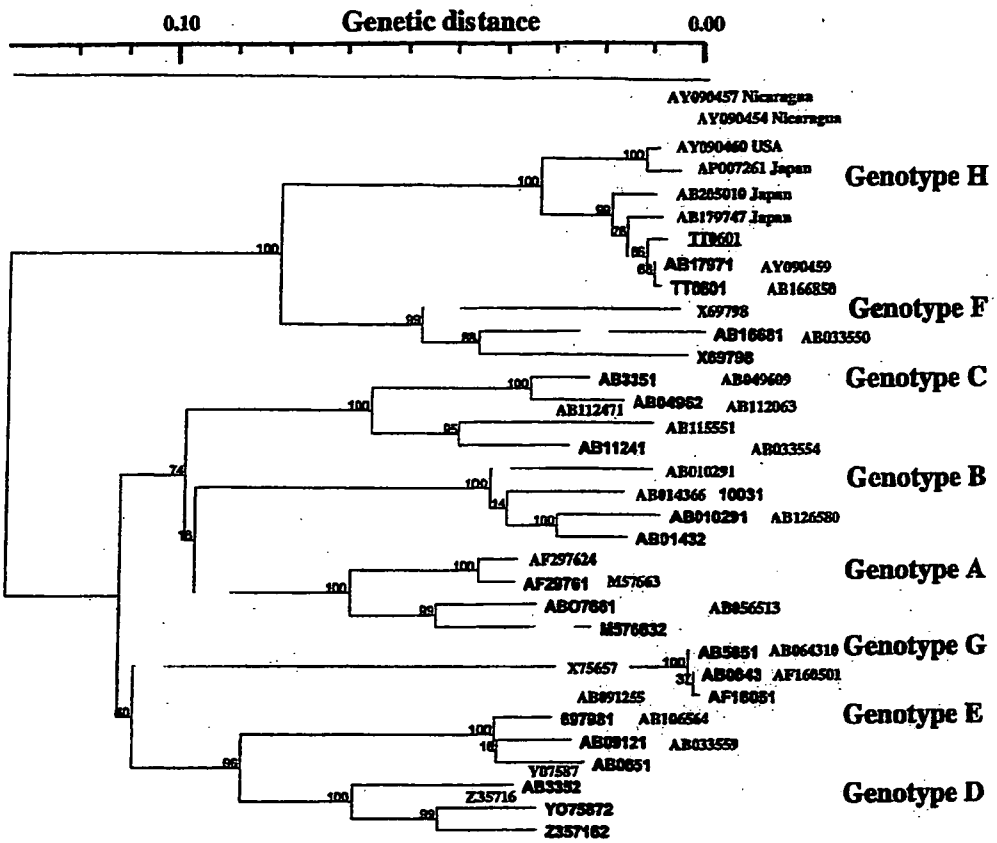

Figure 2. Phylogram generated by neighbor-joining analysis of genetic distance in the full-length sequence of HBV. Thirty strains (without TT0601; indicated by underline) were retrieved from the GenBank/EMBL/DDBJ database.

tation of the HBV DNA polymerase gene (rtM204I/V, L180M) was determined using polymerase chain reaction and restriction fragment length polymorphism as described previously (6). This patient did not show mutations at rt180 or 204 in the HBV DNA polymerase gene at the initiation of IFN therapy.

Full genome sequence analysis by PCR direct sequencing technique before treatment revealed that the patient was infected with genotype H virus (Fig. 2). The sequence was named HBV-TT0601. When compared with previously reported HBV isolates with full genome sequences, ST0404 showed high overall identity (99.2%) with a prototype of the Los Angeles strain (AY090460) and 97.5% identity with a Nicaragua strain (AY090457) of the genotype H group at the nucleotide level. Moreover, ST0404 showed higher overall identity (99.8%, 99.4% and 98.8%) with Japanese strains (AB179747, AB205010 and AP007261 respectively) (7-9).

Five months after the onset, needle liver biopsy under laparoscopy was performed. Portal Tracts had edematous enlargement with lymphocytic infilitration and increased collagen fiber. Moreover, the lobular area showed necroinflammatory activity. Inflammatory changes remained within the liver five months after the onset of acute hepatits B (Fig. 3). With the continuous treatment by LMV, eight months after onset from acute hepatitis, serum HBsAg converted to negative.

Discussion

Here, we report a 60-year-old man infected with genotype H HBV, who had a prolonged clinical course after onset of acute hepatitis B. The present case was suspected for infection from homosexual contact. The genotype H of this patient was reported three times in Japan previously (7-9).

This patient had several features. First, he showed a low level of serum aminotransferase and total bilirubin in spite of a high titer of serum HBV DNA level. In our previous report, we described that patients with a low serum level of aminotransferase and total bilirubin in acute hepatitis B have a high possibility of persistence (10). Low maximum ALT levels (<500 IU/l) and high baseline HBV-DNA levels (>8.7 LGE/ml) were going to persistent in patients with genotype A. Thus, we selected the intensive care for the present case of acute hepatitis B in order to prevent disease progression from acute to the chronic phase.

Second, acute hepatitis B with genotype H has the possibility of being prolonged or persistent in spite of intensive treatment. Generally, acute hepatitis B with HBV genotype A tends to be persistent (11). On the other hand, most patients with acute hepatitis B due to genotype B and C are

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Figure 3-1. Picture of liver biopsy. Panacinar necrosis of the portal tracts and parenchymal remnants leads to disruption of the lobular architecture. Portal tracts exhibited increased fibrosis.

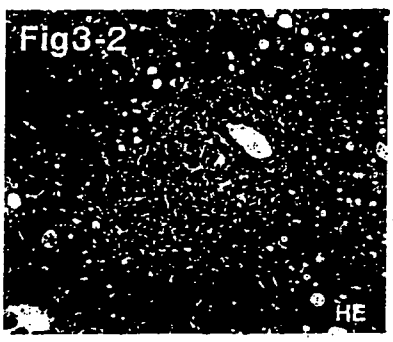

Figure 3-2. Picture of liver biopsy. Edematous enlargement with light lymphocytic infiltration of the portal tracts and parenchymal remnants was clear.

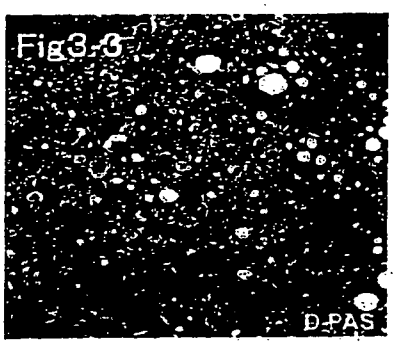

Figure 3-3. Picture of liver biopsy. Kupffer cells underwent hypertrophy and hyperplasia and were laden with lipofuscin pigment: red spot in D-PAS stain, indicating inflammatory persistence.

usually cured without antiviral drugs. The present patient showed a prolonged course after the onset of acute hepatitis by histological examination. HBV replicates by reverse transcription of an RNA intermediate, pregenomic RNA (pgRNA). For pgRNA to be encapsulated, its 5′ end is folded into a stem-loop structure, known as the encapsidation signal. PgRNA is transcribed from the distal Precore region and proximal C gene and consists of 60 nucleosides (positions 1847-1906, numbering from the EcoR1 site) (12-14). In general, the patients with HBV genotype A show adenosine at position 1858 in sequence. On the other hand, the patients with HBV genotype B or C show uracil at position 1858 in sequence. The present patient with genotype H had adenosine at position 1858 in sequence. We suggest that stability of pgRNA in HBV genotype A and H is associated with the clinical course after the onset of acute hepatitis B.

Thirdly, the present patient did not show a good response after lamivudine therapy. In most cases, acute hepatitis is cured with rest and observe. Therefore, antiviral treatment is rarely used for such cases. When antiviral drugs, such as lamivudine, are given the patients with acute hepatitis B in one or two months after onset, most patients show a decrease in the serum levels of ALT and HBV DNA level decrease (9). However, the present patient responded poorly to LMV treatment and had prolonged hepatitis. The serum level of ALT decreases slowly after the initiation of IFN therapy. IFN therapy may aid in decreasing aminotransferase.

Eight genotypes (A-H) of HBV have now been described. In brief, genotypes B and C are prevalent in Asia and the Far East, while genotype A is prevalent in northwestern Europe, North America and Africa. Genotype D is predominant in the Mediterranean area and India (15), while genotype E circulates in sub-Saharan Africa (16). Genotype F is found in Central and South America (17). Genotype G has been reported from France and North America (18). Genotype H has been described only recently, and the first report was from Central America (4). The strain in the present case showed high homology with those reported in Japan (7-9) and Los Angeles (4). However in the future, acute hepatitis B due to genotype H could be spread. Moreover, based on the difference of HBV-genotype, persistence rate is different (2, 10). Limitation of this case was other immunosuppressive factors. The patient was a homosexual. Homosexual men can be associated with poor responsibility for treatment of hepatitis (19).

In conclusion, the acute hepatitis B patients in Japan have shown various genotypes recently. We encountered a rare case of acute hepatitis B with genotype H which led to a prolonged state of acute hepatitis. LMV and IFN were effective for changing HBsAg to negative.

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Glycyrrhizin injection therapy prevents hepatocellular carcinogenesis in patients with interferon-resistant active chronic hepatitis C

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Aim: There is no useful and effective treatment for patients with non-sustained response to interferon, from the viewpoint of cancer prevention. Our aim was to elucidate the influence of a glycyrrhizin therapy on hepatocarcinogenesis rate in interferon-resistant hepatitis C

Methods: We retrospectively analyzed 1249 patients with chronic hepatitis with or without cirrhosis. Among 346 patients with high alanine transaminase values of twice or more of the upper limit of normal, 244 patients received i.v. glycyrrhizin injection and 102 patients did not, after judgment of interferon resistance.

Results: Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the fifth year, and 21.5% and 35.5% at the 10th year, respectively ($P = 0.021$). Proportional hazard analysis using time-dependent covariates disclosed that fibrotic stage, gender and glycyrrhizin treatment were significantly associated with future carcinogenesis. A long-term glycyrrhizin injection therapy decreased the hepatocarcinogenesis rate (hazard ratio, 0.49; 95% confidence interval, 0.27–0.86, $P = 0.014$) after adjusting the background features with significant covariates. Cancer preventive activity was also found in a subgroup of older patients of 60 years or more.

Conclusions: Glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with interferon-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of the upper limit of normal after interferon.

Key words: cancer prevention, chronic hepatitis, glycyrrhizin, hepatitis C virus, hepatocellular carcinogenesis

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers in the world. Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of HCC aside from hepatitis B virus (HBV).[1–5] The annual incidence of HCC in patients with HCV RNA-positive cirrhosis ranges 5–7%.[5–7] The carcinogenesis rate was higher in those patients with cirrhosis caused by HCV than in those with HBV-related cirrhosis.[5]

Interferon (IFN) is effective in reducing HCC rate through suppression of necroinflammatory process serum alanine aminotransferase (ALT) and in eliminating HCV in some patients with chronic HCV and cirrhosis. Although IFN proves to be valuable in suppression of the risk of carcinogenesis, it is not effective in every patient with HCV-related disease. Oka *et al.*[8] reported in a randomized controlled trial that a kind of medicinal herb, "Sho-saiko-to", could significantly decrease hepatic carcinogenesis rate in patients without hepatitis B surface antigen (HBsAg)-negative cirrhosis. Tarao *et al.*[9] showed that the HCC appearance rate was significantly higher in HCV-related cirrhotics with a high ALT value of 80 IU or more than that of those with lower ALT value, and also suggested that treatment of cirrhosis and prevention of HCC should be directed to suppress the necroinflammation of HCV-related hepatitis.

In Japan, a glycyrrhizin-containing herbal medicine, Stronger Neo-Minophagen C (SNMC), is widely used in Japan for the treatment of chronic hepatitis. It is used in the form of an i.v. solution, comprised of 0.2% glycyrrhizin, 0.1% cysteine and 0.2% glycine in physiological solution. It is made by dissolving glycyrrhizin (200 mg),

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Table 1 Patients profiles and laboratory data at the time of judgment of interferon-resistance

	Glycyrrhizin group ($n = 453$)	Untreated group ($n = 796$)	*P*-value
Demography			
Sex (M/F)	283/170	495/301	0.92‡
Age (year)†	54 (25–81)	52 (18–77)	<0.001
Liver histology			
F1/F2/F3/F4	146/193/38/69	502/192/52/38	<0.001‡
Laboratory data†			
Aspartic transaminase (IU/L)†	81 (19–446)	54 (11–355)	<0.001
Alanine transaminase (IU/L)†	122 (12–630)	83 (10–822)	<0.001
HCV serological group 1/2	360/73	582/165	0.032‡

†Expressed by median (range). ‡χ^2 test or Mann–Whitney *U*-test.

Imaging diagnosis with ultrasonography (US) and/or computerized tomography (CT) was made three or more times per year in a majority of patients with cirrhosis, and once a year in patients without cirrhosis.

The numbers of cases lost to follow up were 121 (9.7%): 28 patients (6.2%) in the glycyrrhizin group and 93 (11.7%) in the untreated group. Because the eventual outcomes regarding appearance of HCC were not identified in these patients, they were dealt as censored data in the following statistics. Death unrelated to HCC was also classified as withdrawal and regarded as a censored case. The median observation period of the total number of patients was 5.7 years with a range of 0.1–16.1 years.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics of the patients, including Mann–Whitney *U*-test and χ^2 method. HCC appearance rates were calculated from a period between the judgment of IFN ineffectiveness and the appearance of HCC in each group, using the Kaplan–Meier technique.[17] The differences in carcinogenesis curves were tested using the log-rank test. Independent factors associated with the appearance rate of HCC were studied using time-dependent Cox regression analysis.[18] An interaction term of IFN treatment and "waiting time" to the therapy was introduced in the analysis as a time-dependent covariate. The independence of treatment factor from "waiting time" was also confirmed by a log-minus-log plot of a proportional hazard model.

All data analysis was performed using the computer program SPSS version 11 (SPSS, Chicago, IL, USA).

RESULTS

Initial aminotransferase and carcinogenesis rates

BECAUSE AMINOTRANSFERASE LEVEL is likely to affect future disease progression, entire patients of the cohort were classified into six categories according to average ALT value during the first year after cessation of IFN therapy: (i) normal ALT; (ii) less than 1.5 times of upper limit of normal (ULN); (ii) 1.5–2 times of ULN; (iv) 2–3 times of ULN; (v) 3–4 times of ULN; and (vi) more than 4 times of ULN. Hepatocellular carcinogenesis rates were 2.5%, 5.0%, 8.1%, 11.8%, 12.0% and 12.7% at the end of the fifth year, and 6.6%, 7.2%, 19.6%, 15.1%, 21.0% and 39.3% at the 10th year, respectively. There was a significant statistical difference among the six subgroups (log-rank test, $P < 0.0001$). The higher the average ALT, the higher the carcinogenesis rate was.

Glycyrrhizin therapy was usually performed in patients with a high ALT value and high hepatitis activity. In this retrospective study, average ALT values were significantly different between the treated and the untreated groups: (i) normal average ALT was found in 38 among patients with glycyrrhizin therapy and in 188 among patients without therapy; (ii) ALT of less than 1.5 times of ULN was found 42 and 331; (iii) 1.5 times to 2 times of ULN 84 and 138; (iv) 2–3 times of ULN in 143 and 92; (v) 3–4 times in 53 and 29; and (vi) ALT of more than 4 times of ULN in 93 of the glycyrrhizin group and 18 of the untreated group, respectively. The rate of a high ALT value of twice or more of ULN in the glycyrrhizin treated group (64.2%, 289/453) was significantly higher than that of the untreated group (16.2%, 129/796).

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untreated group. Of patients in the treated group, some of them received glycyrrhizin injection therapy several months or a few years after judgment of IFN ineffectiveness. In order to elucidate the cancer preventive activity of glycyrrhizin in active HCV-related liver disease, we further stratified the treated patients into two groups: (i) early treatment group of glycyrrhizin within 2 years after judgment of IFN ineffectiveness; and (ii) late treatment group after 2 years. Because the latter patients were observed without therapy for a considerable period inspite of the "treated group", they were regarded as partly and insufficiently treated with glycyrrhizin from a viewpoint of the entire observation period. We therefore compared the carcinogenesis rates between the treated and untreated patients, excluding those patients of a late treatment group.

The hepatocellular carcinogenesis rate of the patients with a sufficient period of glycyrrhizin injection was significantly lower than that of those without therapy ($P = 0.038$). In the treated group, median ALT values significantly decreased after initiation of the glycyrrhizin injection, suggesting that suppression of the necroinflammatory process was the principal mechanism of the anti-carcinogenic activity of the medicine. The current study dealing with a large cohort ($n = 1249$), showed that the carcinogenesis rate reduces when glycyrrhizin therapy is started at an early time after judgment of IFN ineffectiveness. Cancer preventive activity of glycyrrhizin was also found in a subgroup of elderly patients 60 years or older. Because glycyrrhizin therapy has few side-effects, it should be taken into account for the treatment of aged patients with chronic hepatitis C, from the viewpoint of cancer prevention. Survival rate is likely to increase in those patients undergoing long-term glycyrrhizin injection therapy through suppression of aggressive necroinflammatory process and suppression of liver-related morbidity and mortality.

CONCLUSIONS

AS CARCINOGENESIS IS not a single-step event, but a complex, multistep process, the exact mechanism of the glycyrrhizin activity in suppression of liver carcinogenesis still remains unknown. One of the principal roles of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate seemed to be anti-inflammatory ones, which would retrieve an active carcinogenic process with ALT elevation and continuous hepatic necroinflammation. Glycyrrhizin may only postpone the time of HCC appearance in the clinical course of cirrhosis. Because the entire process of hepatocellular carcinogenesis from initial transformation of a hepatocyte to detectable growth is considered to take at least a few years, the influence of glycyrrhizin on the carcinogenesis rate will not be evaluated in a short period of a few years. Future studies should therefore be aimed at defining the basic oncogenic mechanisms and roles of long-term administration of glycyrrhizin in carcinogenesis in patients with cirrhosis caused by HCV.

In conclusion, a long-term intermittent glycyrrhizin therapy for a few years or more successfully reduced hepatocellular carcinogenesis in patients with HCV-related chronic liver disease. A randomized control study with a larger number of cases, with or without glycyrrhizin therapy, is expected to confirm the effectiveness of this therapy.

CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the author.

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CLINICAL RESEARCH STUDY

Viral Elimination Reduces Incidence of Malignant Lymphoma in Patients with Hepatitis C

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ABSTRACT

PURPOSE: A high prevalence of malignant lymphoma among patients with hepatitis C virus (HCV) infection has been reported. The aim of this retrospective study was to determine the incidence of malignant lymphoma and the relationship between malignant lymphoma and viral elimination in patients with HCV.

METHOD: We studied 501 consecutive HCV-infected patients who had never received interferon therapy and 2708 consecutive HCV-infected patients who received interferon therapy.

RESULTS: In the non-interferon group, the cumulative rates of malignant lymphoma development were 0.6% at the 5th year, 2.3% at the 10th year, and 2.6% at the 15th year. The cumulative rates of malignant lymphoma development in interferon-treated patients with sustained virologic response were 0% at the 5th year, 0% at the 10th year, and 0% at the 15th year. The cumulative rates of malignant lymphoma development with persistent infection were 0.4% at the 5th year, 1.5% at the 10th year, and 2.6% at the 15th year. The malignant lymphoma development rate was higher in patients with persistent infection than in patients with sustained virologic response ($P = .0159$). The hazard ratio of lymphomagenesis in 1048 patients with sustained virologic response was significantly lower than in patients with persistent infection (hazard ratio: 0.13; $P = .049$).

CONCLUSION: Our retrospective study is the first to determine the annual incidence of malignant lymphoma among patients with HCV at 0.23%. Our results indicate that sustained virologic response induced by interferon therapy protects against the development of malignant lymphoma in patients with chronic HCV.

KEYWORDS: Cohort study; Hepatitis C virus; Hepatocellular carcinoma; Interferon; Malignant lymphoma; Sustained virologic response; Viral elimination\

Hepatitis C virus (HCV) is a major risk for hepatocellular carcinoma.[1-10] The incidence of hepatocellular carcinoma in patients with HCV-related cirrhosis is estimated at 5% to 10% per year, and it is one of the major causes of death, especially in Asian countries.[10] On the other hand, HCV has been detected not only within infected hepatocytes but also in blood cells, such as lymphocytes,[11] and has been implicated as a putative agent of cryoglobulinemia.[12] The virus sustains clonal expansion of B lymphocytes in HCV-infected patients.[13] Moreover, the prevalence of HCV infection in B-cell non-Hodgkin's lymphoma also is high,[14] and anti-HCV seropositivity is a risk factor of malignant lymphoma.[15] Zuckerman et al[16] suggested that HCV might induce clonal proliferation of B-cell and t(14;18) translocation. However, the mechanism of lymphomagenesis is not well known in patients with HCV.

There are many reports on the prevalence of HCV infection in malignant lymphoma,[14] but there is little or no information on the cumulative incidence and influence of

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interferon therapy on the development rate of malignant lymphoma. In our hospital, we evaluate a large number of patients with HCV-related hepatitis and often find hepatocellular carcinoma among our cases. We also find a proportion of patients with HCV-related hepatitis in whom malignant lymphoma develops. In the present retrospective study, we examined the incidence of malignant lymphoma among HCV-infected patients and determined the relationship between malignant lymphoma and interferon therapy in such patients.

CLINICAL SIGNIFICANCE

- The annual incidence of malignant lymphoma in patients with HCV infection who have never received interferon therapy is 0.23% per year.
- The risk of malignant lymphoma in patients with persistent HCV infection is approximately 7 times that in patients with sustained virologic response induced by interferon therapy.
- The risk of malignant lymphoma is low in patients with chronic HCV who show a sustained virologic response to interferon therapy.

PATIENTS AND METHODS

Study Population

In the retrospective cohort study, we analyzed all patients in our database of chronic HCV between 1969 and 2006 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan: 511 consecutive patients who did not receive interferon therapy (non-interferon group) and 2960 consecutive patients who received interferon therapy (interferon group). The patients were positive for anti-HCV antibody and HCV-RNA, and negative for hepatitis B surface antigen. Among them, 10 patients of the non-interferon group and 252 patients of the interferon group were excluded for the following reasons: possible association with hepatocellular carcinoma; possible association with malignant lymphoma and other hematologic malignancy; association with hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, α-1-antitrypsin deficiency, or Wilson disease; or a short follow-up period of 6 months or less. Consequently, 501 patients of the non-interferon group and 2708 patients of the interferon group were retrospectively evaluated for the malignant lymphoma development rate and the efficacy of interferon therapy. All patients who did not show a sustained virologic response and persistently high alanine aminotransferase level (normal range of alanine aminotransferase: 6-50 IU/L) received liver protection therapy, consisting mainly of glycyrrhizin and ursodeoxycholic acid (300-600 mg/d), during this research.

In these groups, the observation starting point was the time of the first medical examination at our hospital.

Background and Laboratory Data

Table 1 summarizes the profiles and laboratory data of the 2708 patients who received interferon therapy and the 501 patients who did not receive interferon therapy. Patients of the interferon group were younger than those of the non-interferon group. The observation period was significantly shorter in the interferon group than in the non-interferon group (median 4.5 vs 14 years; $P < .0001$). Although all patients were HCV-RNA positive during the clinical course, the serum concentration of HCV-RNA using initial sera was analyzed in 2976 patients (92.7%). HCV subtype was analyzed in every patient. Serologic grouping of HCV showed that the percentage of HCV-2 in the interferon group was significantly higher than in the non-interferon group. The initial serum concentration of HCV-RNA was assessed in 2878 patients (89.7%). There was no significant difference between the 2 groups with

Table 1 Patient Profiles and Laboratory Data at the Time of the First Medical Examination at Our Hospital

	Non-IFN Group	IFN Group	*P* Value
No. of patients	501	2708	
Sex (M/F)	300/201 (1.49:1)	1735/973 (1.78:1)	.077
Age (y)	53 (21-79)	51 (10-83)	<.0001
Observation period (y)	14 (0.7-35.8)	4.5 (0.5-17.9)	<.0001
AST (IU/L)	66 (12.8-704)	59 (9-1266)	<.0001
ALT (IU/L)	96 (12-832)	92 (1-1620)	.927
HCV serologic group			
1	256 (84%)	1749 (66%)	<.001
2	50 (16%)	921 (34%)	
Viral load*			
Low	72 (28%)	807 (31%)	.566
High	183 (72%)	1816 (69%)	
Chronic hepatitis/liver cirrhosis	449/52	2533/175	.003

IFN = interferon; AST = aspartate aminotransferase; ALT = alanine aminotransferase; HCV = hepatitis C virus.
*Viral load: low; Amplicor <100 KIU/mL or Probe < 1 MEq/mL, high; Amplicor ≥ 100 KIU/mL or Probe ≥ 1 MEq/mL.

regard to the initial viral load (low viral load; Amplicor < 100 KIU/mL [Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc, Belleville, NJ] or Probe < 1 MEq/mL, high viral load [branched DNA probe assay; version 2.0; Chiron, Dai-ichi Kagaku, Tokyo, Japan]; Amplicor ≥ 100 KIU/mL or probe ≥ 1 MEq/mL). In this study, the percentage of patients with chronic hepatitis was significantly higher in the interferon group than in the non-interferon group.

Type of Interferon and Judgment of Interferon Effect

Among 2708 patients with interferon therapy, 1675 patients received interferon-alpha, 415 patients received interferon-beta, 33 patients received both interferon-alpha and interferon-beta, and the remaining 585 patients received a combination therapy of interferon and ribavirin. The response to interferon therapy was based on a sustained virologic response (elimination of HCV-RNA at 6 months after the end of treatment). Among patients treated with interferon, 1048 patients (38.7%) acquired a sustained virologic response. Among 1660 patients in the nonsustained virologic response group, there were 1012 patients with a relapse of HCV RNA after temporal viral clearance, and the remaining 648 patients had nonviral clearance during treatment.

Viral Markers of Hepatitis B and C Viruses

Diagnosis of HCV infection was based on the detection of serum HCV antibody and positive RNA. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc, Belleville, NJ) or the branched DNA probe assay (branched DNA probe assay; version 2.0; Chiron, Dai-ichi Kagaku, Tokyo, Japan). Hepatitis B surface antigen was tested by radioimmunoassay (Austria, Abbott Laboratories, Detroit, Mich). The used serum samples were stored −80°C at the first consultation.

Histopathologic Examination of Liver

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim-Silverman needle with an internal diameter of 2 mm. All specimens for examination contained at least 6 portal areas. Chronic hepatitis was diagnosed on the basis of histopathologic assessment according to the scoring system of Desmet et al.[17] Patients who did not undergo liver biopsy were diagnosed with chronic hepatitis on the basis of the presence of irregular liver surface, portal-hypertension, and/or ascites by ultrasonography, computed tomography (CT), and/or endoscopy.

Follow-up, Diagnosis, and Classification of Malignant Lymphoma

Patients were followed up monthly to trimonthly after the first medical examination at our hospital. Physical examination and biochemical tests were conducted at each examination together with a regular checkup with CT or ultrasonography imaging in each patient. When a patient had any symptoms in relation to malignant lymphoma (unexplained weight loss, fever, and lymphadenopathy), we further explored possible malignant lymphoma. Malignant lymphoma was diagnosed by histopathologic examination. Classification was based on the Revised European-American Classification of lymphoid neoplasms/new World Health Organization classification[18] revised by Harris.[19] Staging and extranodal involvement were determined according to the Ann Arbor classification by physical examination, total body CT scan, and bone marrow biopsy. The number of cases lost to follow-up included 78 patients (15.6%) in the non-interferon group and 184 patients (6.8%) in the interferon group.

Statistical Analysis

Nonparametric procedures were used for the analysis of background features of the patients, including the Mann-Whitney *U* test and chi-square method. The cumulative appearance rate of malignant lymphoma was calculated from the period between the first medical examination at our hospital to the appearance of malignant lymphoma, using the Kaplan-Meier method. Differences in lymphomagenesis curves were tested using the log-rank test. Independent factors associated with the incidence rate of malignant lymphoma were analyzed by a time-dependent Cox proportional hazard model, using the term of interferon therapy with "waiting time" as a time-dependent variable. The following 9 variables were analyzed for potential covariates for incidence of malignant lymphoma at the time of first medical examination at our hospital: age, sex, state of liver disease (chronic hepatitis or liver cirrhosis), viral serotype, viral load, history of interferon therapy, efficacy of viral clearance by interferon therapy, serum concentrations of aspartate aminotransferase, and alanine aminotransferase. A *P* value of less than .05 in a 2-tailed test was considered significant. Data analysis was performed using the computer program SPSS version 11.0 (SPSS Inc, Chicago, Ill). The physicians in charge explained the purpose and method of this clinical trial to each patient, who gave their informed consent for participation. This study was approved by the institutional review board of our hospital.

RESULTS

Incidence of Malignant Lymphoma in Patients Without Interferon Therapy

In the interferon group, malignant lymphoma developed in 12 patients (2.4%) during a median observation period of 14 years. The cumulative rate of newly diagnosed malignant lymphoma was 0.62% at the end of the 5th year, 2.26% at the 10th year, and 2.62% at the 15th year (Figure 1). Table 2 summarizes the characteristics of patients who developed malignant lymphoma. The period between the first medical

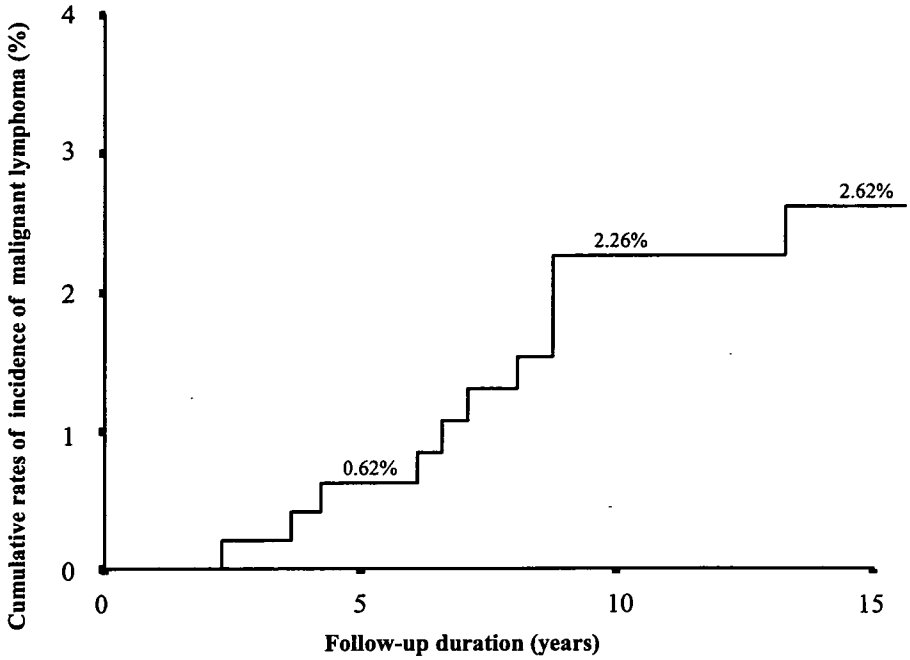

Figure 1 Cumulative rate of the incidence of malignant lymphoma from the first medical examination at our hospital in patients with chronic HCV who did not receive interferon therapy.

examination at our hospital and development of malignant lymphoma ranged from 2.2 to 26.1 years (median of 7.5 years). The patients who develop malignant lymphoma included 4 men and 8 women, aged 45 to 79 years (median, 70 years). With regard to the histologic type of malignant lymphoma, diffuse large cell lymphoma was found in 7 patients, follicular lymphoma was found in 3 patients, Hodgkin disease (nodular lymphocyte predominant) was found in 1 patient, and unclassified lymphoma was found in 1 patient. With regard to the background liver tissue, 6 patients had chronic hepatitis and 6 patients had cirrhosis at the time of malignant lymphoma development.

In our cohort, hepatocellular carcinoma developed in 102 patients (20.4%). The hepatocarcinogenesis rate in this cohort was 4.7% at the end of the 5th year, 11.9% at the 10th year, and 21.0% at the 15th year.

Incidence of Malignant Lymphoma in Patients with Interferon Therapy

In the interferon group, 14 patients (0.49%) developed malignant lymphoma during a median observation of 3.9 years. The cumulative rates of newly diagnosed malignant lymphoma were 0.16% at the end of the 5th year, 0.61% at the 10th year, and 1.81% at the 15th year. There was no significant difference in the incidence rate of malignant lymphoma between the non-interferon and interferon groups (Figure 2). Table 3 summarizes the characteristics of pa-

Table 2 Characteristics of Patients Not Treated with Interferon

Case	Sex	Age (y)	Histology	Stage	Extranodal	Serologic Group	Viral Load*	Liver Disease
1	F	45	Follicular	IV	BM	1	High	CH
2	F	50	Diffuse large cell	III	None	2	High	CH
3	F	59	Follicular	II	None	1	High	CH
4	F	67	Diffuse large cell	IV	BM	1	High	LC
5	F	69	Follicular	II	None	1	High	LC
6	F	69	ND	IV	Lung	ND	Low	LC
7	F	73	Hodgkin disease (nodular LP)	I	None	2	High	LC
8	F	74	Diffuse large cell	II	None	1	High	CH
9	M	71	Diffuse large cell	IV	BM	1	High	CH
10	M	71	Diffuse large cell	IV	Liver	ND	ND	CH
11	M	76	Diffuse large cell	IIE	Stomach	1	High	LC
12	M	79	Diffuse large cell	II	None	2	High	LC

IFN = interferon; BM = bone marrow; CH = chronic hepatitis; LC = liver cirrhosis; ND = not determined.
*Viral load: low; Amplicor < 100 KIU/mL or Probe < 1 MEq/mL, high; Amplicor ≥ 100 KIU/mL or Probe ≥ 1 MEq/mL.

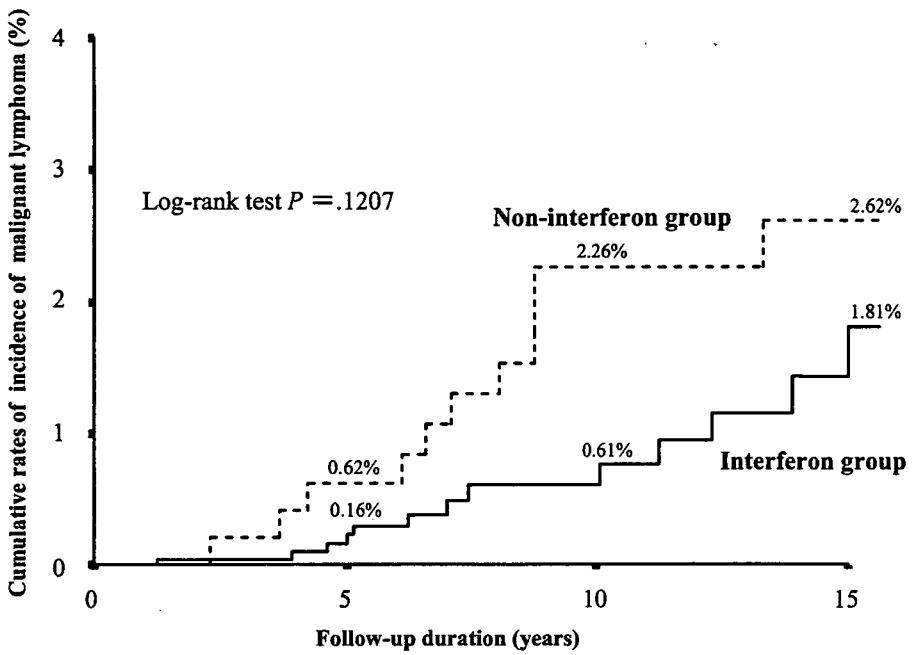

Figure 2 Cumulative rate of the incidence of malignant lymphoma from the first medical examination at our hospital in patients with chronic HCV who were treated or not treated with interferon.

tients who developed malignant lymphoma. Their median age was 61 years, and the period between the start of first interferon therapy and development of malignant lymphoma ranged from 0.7 to 14.5 years, with a median of 6.1 years. They included 7 men and 7 women aged 45 to 76 years (median, 67.5 years). Histologically, diffuse large cell lymphoma was found in 8 patients, follicular lymphoma was found in 3 patients, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue was found in 1 patient, extranodal natural killer/T-cell lymphoma was found in 1 patient, and angioimmunoblastic T-cell lymphoma was found in 1 patient. With regard to the background liver disease, 10 patients had chronic hepatitis and 4 patients had cirrhosis at the time of malignant lymphoma development.

In our cohort, hepatocellular carcinoma developed in 154 patients (5.7%), and the rate of hepatocarcinogenesis was 2.5% at the end of the 5th year, 7.3% at the 10th year, and 15.3% at the 15th year.

Impact of Viral Elimination on the Incidence of Malignant Lymphoma

Among all 3209 patients, during the observation period, 1 patient developed malignant lymphoma among those with a

Table 3 Characteristics of Patients Treated with Interferon

Case	Sex	Age (y)	Histology	Stage	Extranodal	Serologic Group	Viral Load*	Liver Disease	Effect of IFN Treatment
1	F	46	Follicular	IV	BM	1	High	CH	Non-SVR
2	F	52	Diffuse large cell	III	None	2	High	CH	Non-SVR
3	F	59	MALT type	IE	Trachea	2	High	CH	Non-SVR
4	F	68	Diffuse large	II	None	1	High	LC	Non-SVR
5	F	70	Diffuse large	IV	Liver	1	High	LC	Non-SVR
6	F	74	Extranodal NK/T cell	IE	Nose	1	Low	LC	Non-SVR
7	F	76	Follicular	II	None	2	High	LC	Non-SVR
8	M	45	Diffuse large cell	IIS	Spleen	1	High	CH	Non-SVR
9	M	61	Diffuse large cell	II	None	2	High	CH	Non-SVR
10	M	64	Diffuse large cell	IVE	Omentum	1	ND	CH	Non-SVR
11	M	67	Diffuse large cell	IV	BM	1	High	CH	Non-SVR
12	M	68	Follicular	IIIE	Left pleural effusion	1	High	LC	Non-SVR
13	M	70	Diffuse large cell	IV	Lung	ND	High	LC	Non-SVR
14	M	73	Angioimmunoblastic T-cell lymphoma	III	None	2	Low	CH	SVR

IFN = interferon; BM = bone marrow; CH = chronic hepatitis; LC = liver cirrhosis; SVR = sustained virologic response; MALT = mucosa-associated lymphoid tissue; NK = natural killer; ND = not determined.

*Viral load: low; Amplicor < 100 KIU/mL or Probe < 1 MEq/mL, high; Amplicor ≥ 100 KIU/mL or Probe ≥ 1 MEq/mL.

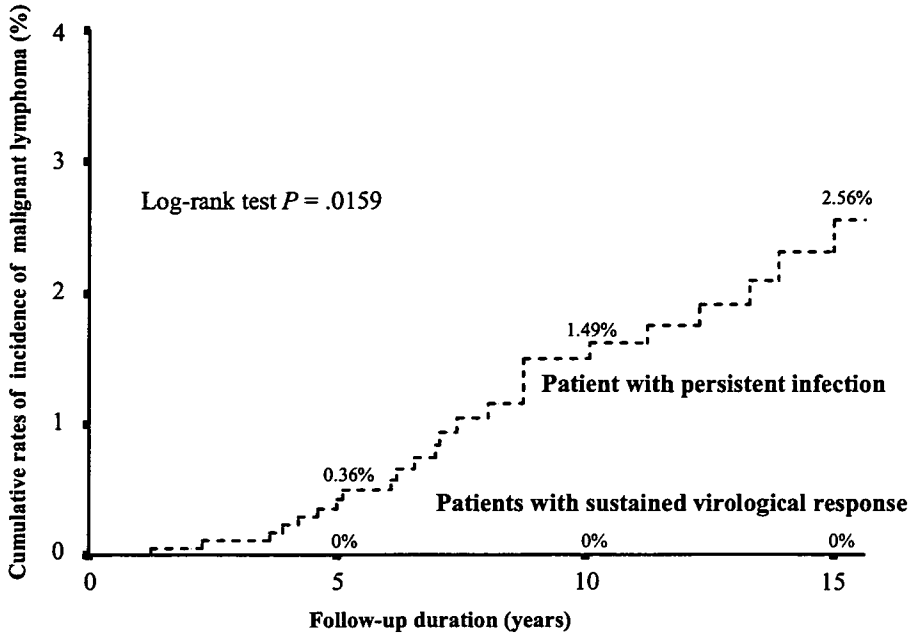

Figure 3 Cumulative rate of the incidence of malignant lymphoma from the first medical examination at our hospital in patients with sustained virologic response and those with persistent chronic HCV infection.

sustained virologic response, and 25 patients developed malignant lymphoma among those with persistent infection. The malignant lymphoma development rates were 0% at the end of the 5th year, 0% at the 10th year, and 0% at the 15th year among patients with a sustained virologic response, and 0.36% at the 5th year, 1.49% at the 10th year, and 2.56% at the 15th year among patients with persistent infection (Figure 3). Among patients with a sustained virologic response, 1 patient developed malignant lymphoma after 19.8 years from the first medical examination and after 466 days from the end of interferon therapy. In patients with persistent infection, the development rate of malignant lymphoma was significantly high ($P = .0159$).

Determinants of Malignant Lymphoma Incidence

We then investigated the factors associated with the incidence of malignant lymphoma in all 3209 patients. Univariate analysis identified the following 6 factors that influenced incidence of malignant lymphoma: age (<60/≥60) ($P < .0001$), alanine aminotransferase (<100/≥100) ($P = .0006$), viral elimination (yes/no) ($P = .016$), sex (male/female) ($P = .025$), state of liver disease (chronic hepatitis/liver cirrhosis) ($P = .045$), and viral load (low/high) ($P = .060$).

These 6 parameters were entered into multivariate Cox proportional hazard analysis (time-dependent model). The incidence rate of malignant lymphoma was significantly higher for patients with persistent infection (hazard ratio: 7.49; $P = .049$), aged 60 years or more (hazard ratio: 3.25; $P = .005$), and with serum alanine aminotransferase less than 100 IU/L (hazard ration: 3.02; $P = .030$) (Table 4).

Mortality and Causes of Death

During the observation period, 102 patients (3.18%) died: 65 of the non-interferon group and 37 of the interferon group. The estimated 5-year survivals of the non-interferon and interferon groups were 98.3% and 99.8%, 10-year survivals were 96.0% and 98.5%, and 15-year survivals were 88.4% and 90.4%, respectively. There was no significant difference in the overall survival between the non-interferon and interferon groups (log-rank test, $P = .60$). When examined according to the curative effect, the estimated 5-year survivals for patients with sustained virologic response and patients with persistent infection were 99.8% and 99.3%, 10-year rates were 99.8% and 97.1%, and 15-year rates were 98.7% and 88.9%, respectively. The survival of patients with sustained virologic response was significantly higher than that of patients with persistent infection (log-rank test, $P = .0005$). There were 2 and 3 malignant lym-

Table 4 Factors Associated with Malignant Lymphoma in Patients with Hepatitis C-related Hepatitis (Multivariate Cox Proportional Hazard Analysis: Time-Dependent Model)

Factors	Category	Hazard Ratio (95% CI)	*P* Value
Viral elimination*	1: Yes	1	.049
	2: No	7.488 (1.01-55.8)	
Age	1: <60 y	1	.005
	2: ≤60 y	3.247 (1.42-7.42)	
ALT	1: ≤100 IU/L	1	.030
	2: >100 IU/L	3.02 (1.11-8.20)	

ALT = alanine aminotransferase; CI = confidence interval.
*Viral elimination means sustained virologic response.

phoma-related deaths in the non-interferon group and the interferon group, respectively.

DISCUSSION

The reported significant incidence of HCV infection in B-cell non-Hodgkin's lymphoma in several areas of the world indicates a link between viral infection and this subset of lymphoproliferative disorder. The controversial results of the different research groups may be explained by the low probability of HCV carriers who develop lymphoma; thus, an accurate assessment of the exact risk could only come from a large cohort study.[20] Recent reports attesting to the efficacy of interferon therapy for HCV-related, low-grade B-cell non-Hodgkin's lymphoma[21] support the hypothesis of a link between HCV infection and B-cell lymphoma.

Little is known about the relationship between the incidence of malignant lymphoma and interferon therapy. The aim of this research was to clarify the relationship in patients with HCV. Our retrospective cohort study showed that 12 of 501 cases without interferon therapy (non-interferon group) developed malignant lymphoma and 14 of 2708 cases with interferon treatment (interferon group) developed malignant lymphoma. This epidemiologic study demonstrates the malignant lymphoma occurrence rate in HCV-positive patients: The annual appearance rate was 0.23% in the non-interferon group. The annual appearance rate was higher than that in the general Japanese population (~0.008%). Furthermore, our results clearly indicate that the hazard ratio for malignant lymphoma development in patients with HCV elimination is 0.133 compared with that of patients with persistent infection (Table 4).

Multivariate analysis identified age, HCV elimination, and alanine aminotransferase level as significant determinants of malignant lymphoma development. Interpretation of this finding requires further examination and analysis (Table 4). Zuckerman et al[16] reported that chromosome translocation of B-cell improved after interferon therapy in patients with malignant lymphoma complicating HCV infection. Our results are in agreement with those of a previous study that showed interferon-induced improvement of HCV-related lymphoma.[21] In our study, the incidence rate of malignant lymphoma was significantly lower among patients with a sustained virologic response than in patients with persistent infection for both the non-interferon and interferon groups (Figure 3). Thus, our results indicate that interferon therapy coupled with sustained virologic response reduces the likelihood of development of malignant lymphoma. We again emphasize the high prevalence of malignant lymphoma in HCV-positive patients without interferon therapy and the significance of viral elimination by interferon in regard to the suppression of lymphomagenesis.

Although it is safe to conclude that malignant lymphoma is not a risk in patients who achieve a sustained virologic response by interferon therapy, malignant lymphoma developed in 1 patient after achieving an interferon-induced sustained virologic response. However, the cause of malignant lymphoma is not clear, that is, whether it is de novo, mutation of genome by infection of HCV, or other factors. Studies are under way in our laboratories to investigate this issue.

We also analyzed the incidence of malignant lymphoma according to the subtype of malignant lymphoma. The results showed a significant prevalence of diffuse large-cell lymphoma ($P = .029$) and follicular lymphoma ($P = .005$) in our cohort compared with the distribution of the same subtypes of malignant lymphoma in Japan.[22] Malignant lymphoma cases with HCV-related hepatitis may develop a specific subtype of non-Hodgkin's lymphoma. However, our cohort included only a small number of malignant lymphoma cases, and this finding should be confirmed in another study with a large number of malignant lymphoma cases with HCV-related hepatitis.

Although the design of our study was retrospective in nature, multicenter prospective studies are needed to confirm the results described in this report.

CONCLUSION

Our retrospective cohort study reported for the first time the cumulative incidence rate of malignant lymphoma in HCV-infected patients and indicated that interferon therapy reduces the incidence of malignant lymphoma in patients with HCV-related hepatitis.

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ORIGINAL RESEARCH ARTICLE

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Virological Response in Patients with Hepatitis C Virus Genotype 1b and a High Viral Load

Impact of Peginterferon-α-2a plus Ribavirin Dose Reductions and Host-Related Factors

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Abstract

Background and objective: In Japan the prevalence of the hepatitis C virus (HCV) antibody is highest in the elderly population. Therefore, it is important for elderly patients to undergo interferon (IFN) therapy. In patients with HCV genotype 1b and a high viral load, the sustained virological response (SVR) rate is lower in older compared with younger patients receiving combination antiviral therapy. In addition, inadequate adherence to combination therapy is often seen in elderly patients, and is associated with reduced response rates. The aim of this retrospective analysis was to evaluate the effects of host-related factors (i.e. sex, age, baseline HCV RNA level, bodyweight and fibrosis stage) and peginterferon (PEG IFN)-α-2a plus ribavirin dose reductions on SVR rates.

Methods: A total of 192 treatment-naive patients with a HCV genotype 1b infection and a high viral load were included in the analysis. Patients had been enrolled into a phase III trial of 48 weeks of treatment with PEG IFN-α-2a plus ribavirin or PEG IFN-α-2a plus placebo. All patients were evaluated for effect of drug exposure on SVR. In addition, the impact of host-related factors or dose reductions on SVR was assessed.

Results: Approximately 30% of patients were considered elderly (≥60 years of age). The overall SVR rate was significantly higher in patients treated with combination therapy versus monotherapy (59.4% vs 24.0%, $p < 0.001$). Attainment of an SVR following combination therapy was not influenced by any factor evaluated in the analysis, although elderly males were associated with decreased SVR rates. Younger age (odds ratio [OR] 1.081; 95% CI 1.125, 1.034; $p = 0.0009$), lower baseline HCV RNA levels (OR 1.003; 95% CI 1.006, 1.001;

p = 0.006) and a severe fibrosis stage (F3/4) [OR 6.194; 95% CI 1.037, 37.000; p = 0.0455] significantly increased the likelihood of achieving an SVR with monotherapy. In the combination therapy group, patients maintaining a full dosage schedule of PEG IFN-α-2a and ribavirin and those requiring dose reductions of either study drug had similar SVR rates (64.5% vs 61.9%). However, the SVR rate was reduced to 33.3% among patients who discontinued combination therapy. Three out of the 31 patients who received the full dosage schedule were elderly patients. In addition, of the 15 patients who discontinued combination therapy, three were <50 years of age and six were ≥60 years of age. The SVR rate was reduced in patients with cumulative PEG IFN-α-2a and ribavirin doses of <60%; the majority of these patients were elderly.

Conclusion: The attainment of an SVR following PEG IFN-α-2a plus ribavirin combination therapy was not influenced by any of the host-related factors evaluated in this analysis, although elderly males were associated with a decreased SVR rate. Younger age, male sex and lower baseline HCV RNA levels significantly increased the likelihood of achieving an SVR with monotherapy. In addition, dose reductions appeared to have a negative impact on SVR in elderly patients. Therefore, it is important to minimize PEG IFN-α-2a and ribavirin dose reductions by effectively managing treatment-related adverse events in elderly patients.

Introduction

In Japan, the prevalence of the hepatitis C virus (HCV) antibody is highest in the elderly population. In a recent analysis,[1] the average age of HCV-positive patients in Japan was found to be greater than that of US patients by approximately 20 years. Results of the analysis suggested that the introduction of HCV into the Japanese population occurred >100 years ago, followed by wide dissemination in the 1930s and 1940s. In contrast, HCV was introduced into the US 100 years ago, followed by wide dissemination in the 1960s. This extended period of exposure to HCV was the likely reason for the considerably higher prevalence of hepatocellular carcinoma in Japan.

To date, it is unclear if genetic and/or environmental factors have an influence on the incidence of hepatocellular carcinoma in Japan. The duration of HCV infection appears to be an important factor for the development of hepatocellular carcinoma, although the age of patients with post-transfusion HCV has been reported to be a significant factor, regardless of the duration of exposure to HCV.[2] Therefore, it appears to be important for elderly patients to undergo interferon (IFN) therapy in the absence of serious complications such as uncontrolled hypertension or insulin-dependent diabetes mellitus.

Combination therapy with peginterferon (PEG IFN)-α-2a plus ribavirin was found to be more effective than PEG IFN-α-2a monotherapy in Japanese patients with HCV genotype 1b.[3] However, a recent study showed that sustained virological response (SVR) rates were lower in older (≥40 years of age) compared with younger patients with HCV genotype 1b and a high viral load.[4] In addition, inadequate adherence to combination therapy with IFN-α-2b and ribavirin was independently associated with increasing patient age and a reduction in SVR response rates.[5] There are insufficient numbers of clinical trials evaluating the use of PEG IFN plus ribavirin in elderly patients, and an effective dose and treatment period has not been established.

The aim of this retrospective analysis was to investigate the effects of host-related factors (i.e. sex, age, baseline HCV RNA level, bodyweight and fibrosis stage) and PEG IFN-α-2a plus ribavirin

dose reductions on SVR rates in patients with a difficult-to-treat form of chronic hepatitis C.

Patients and Methods

We retrospectively analysed data from a phase III, randomized, double-blind clinical trial conducted at 43 Japanese centres between June 2002 and September 2004.[3]

Patients

A total of 192 treatment-naive patients were included in the analysis. Inclusion criteria were Japanese adults aged ≥20 years with an HCV genotype 1b infection, a serum HCV RNA level of $\geq 1 \times 10^5$ IU/mL, an elevated serum alanine aminotransferase (ALT) level of ≥45 IU/L within 6 months of screening, and chronic hepatitis C confirmed by liver biopsy. Patients were excluded if they had neutropenia (<1500 neutrophils/mm^3), leucopenia (<3000 cells/mm^3), thrombocytopenia (<90 000 platelets/mm^3), anaemia (haemoglobin <12 g/dL), a hepatitis B virus co-infection, decompensated liver disease, organ transplant, a creatinine clearance <50 mL/min, poorly controlled psychiatric disease, poorly controlled diabetes, malignant neoplastic disease, severe cardiac or chronic pulmonary disease, immunologically mediated disease, or retinopathy.

Study Design

Patients were randomized according to a 1 : 1 ratio to 48 weeks of treatment with subcutaneous PEG IFN-α-2a (Pegasys®, Roche, Tokyo, Japan)[1] 180 μg/week in combination with either twice daily oral ribavirin tablets (Copegus®, Roche, Basle, Switzerland) or placebo, followed by 24 weeks of untreated follow-up. The ribavirin dosage was 600, 800 or 1000 mg/day in patients with a bodyweight of ≤60, 60–80 or >80 kg, respectively; these dosages were based on the currently used dosages of ribavirin in Japan. Patients were stratified according to HCV RNA level.

Virological Methods

Qualitative and quantitative serum HCV RNA assessments were conducted using the Cobas Amplicor HCV Test PCR assay (version 2.0; limit of detection 50 IU/mL) and the Cobas Amplicor HCV Monitor Test (version 2.0; limit of quantitation, 500 IU/mL), respectively. HCV genotyping was performed according to the method described by Okamoto et al.[6] The presence of serum anti-HCV antibodies was not assessed.

Histology

Liver biopsies were taken within 12 months of enrolment. An independent pathologist evaluated, graded and staged liver biopsy specimens according to the Ishak modified hepatic activity index and the new European classification.[7,8]

Assessment of Efficacy

The primary efficacy end point of the study was the SVR rate, which was defined as a HCV RNA level of <50 IU/mL after 24 weeks of untreated follow-up.

Statistics

The Cochran-Mantel-Haenszel test was used to compare treatment groups, with a significance level of $p < 0.05$.

Host-related factors associated with an SVR were evaluated using stepwise and multiple logistic-regression models. The following pretreatment factors were considered: sex, age, bodyweight, serum HCV RNA and fibrosis stage (F1/2: mild/moderate; F3/4: severe/cirrhosis). Factors such as the maintenance of a full dosage schedule, the requirement of dose reductions, and treatment discontinuation were also considered.

All patients receiving at least one dose of study drug were included in the efficacy analysis. Patients without follow-up data were considered not to have attained an SVR.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

Results

Patient Demographics

A total of 192 patients were randomized to treatment and patient characteristics were similar at baseline in the two treatment groups (table I). Approximately 30% of patients were considered elderly (≥60 years of age).

Virological Response

The overall SVR rate was significantly higher in patients who received combination therapy with PEG IFN-α-2a plus ribavirin (57/96 patients; 59.4%; 95% CI 48.9, 69.3) versus PEG IFN-α-2a monotherapy (23/96 patients; 24.0%; 95% CI 15.8, 33.7), resulting in an odds ratio (OR) of 4.65 (95% CI 2.49, 8.69; $p < 0.001$).

Factors Associated with Sustained Virological Response (SVR)

Following combination therapy, the attainment of an SVR was not influenced by any pretreatment factor (including fibrosis stage, age, sex, HCV RNA level and bodyweight) evaluated in this analysis (table II). In the combination therapy group, the SVR rate tended to be higher in younger males (<50 years of age) versus males aged ≥60 years (figure 1).

Multiple logistic-regression analyses found no significant correlations between host-related factors

Table I. Baseline characteristics of study patients

Characteristic	Peginterferon-α-2a + placebo (n = 96)	Peginterferon-α-2a + ribavirin (n = 96)
Sex [no. (%)]		
Male	59 (61.5)	61 (63.5)
Female	37 (38.5)	35 (36.5)
Age (y)		
Mean	50.8	52.1
Range	20–73	20–69
Age groups (y) [no. (%)]		
≤29	7 (7.3)	4 (4.2)
30 to 39	13 (13.5)	9 (9.4)
40 to 49	22 (22.9)	22 (22.9)
50 to 59	24 (25.0)	36 (37.5)
60 to 69	27 (28.1)	25 (26.0)
≥70	3 (3.1)	
Weight (kg) [mean]	61.9	62.9
ALT activity (IU/L) [mean]	101.0	100.9
Serum HCV RNA (IU/mL) [no. (%)]		
1 to $<5 \times 10^5$	26 (27.1)	21 (21.9)
5 to $<8.5 \times 10^5$	27 (28.1)	32 (33.3)
$\geq 8.5 \times 10^5$	43 (44.8)	43 (44.8)
Fibrosis staging[a,b] [no. (%)]		
F1	23 (24.0)	18 (18.8)
F2	58 (60.4)	60 (62.5)
F3	15 (15.6)	16 (16.7)
F4		1 (1.0)

a F1 = mild, F2 = moderate, F3 = severe, F4 = cirrhosis.

b One patient was not classified in the combination therapy group.

ALT = alanine aminotransferase; **HCV** = hepatitis C virus.