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ABSTRACT

BACKGROUND: This retrospective cohort study assessed the impact of diabetes mellitus on hepatocarcinogenesis and determined the predictors of hepatocarcinogenesis in noncirrhotic, interferon-treated patients with hepatitis C virus infection.

METHODS: A total of 2058 hepatitis C virus-positive, noncirrhotic patients treated with interferon were enrolled. The median follow-up period was 6.7 years. The primary end point was the onset of hepatocellular carcinoma. The cumulative rate of new hepatocellular carcinoma cases was computed by the Kaplan–Meier method and Cox proportional hazard analysis according to diabetic state and response to interferon therapy.

RESULTS: The cumulative rates of hepatocellular carcinoma in diabetic patients (3.2% at 4 years, 8.5% at 8 years, and 24.4% at 12 years) were significantly higher than those of nondiabetic patients (1.3% at 4 years, 2.2% at 8 years, and 5.6% at 12 years, $P < .001$). In patients with a sustained virologic response, diabetes had no significant effect on the rate of hepatocarcinogenesis. In contrast, the rate in patients with a nonsustained virologic response was significantly higher in diabetic than in nondiabetic patients. Multivariate analysis identified lack of sustained virologic response (hazard ratio [HR] 7.28; 95% confidence interval [CI], 3.28-16.15; $P < .001$) and diabetes as independent risk factors for hepatocarcinogenesis (HR 2.00; 95% CI, 1.05-3.84; $P = .036$).

CONCLUSIONS: Our results highlight the enhancing effect of diabetes mellitus on hepatocarcinogenesis in noncirrhotic, interferon-treated patients with hepatitis C virus. The sustained virologic response induced by interferon therapy eliminates the influence of diabetes and markedly reduces the rate of hepatocarcinogenesis in such patients.

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KEYWORDS: Diabetes; Hepatocellular carcinoma; Interferon; Sustained virologic response

Hepatitis C virus is a common cause of chronic liver disease worldwide and a major risk of hepatocellular carcinoma.[1-10] The estimated incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis is 5% to 10% per year, and hepatocellular carcinoma is one of the major causes of death, especially in Asian countries.[10] In recent years, diabetes mellitus has attracted attention as a risk factor of hepatocarcinogenesis. Evidence suggests that in addition to various factors that affect liver fibrosis and hepatocarcinogenesis, diabetes and obesity are independent risk factors for the progression of liver fibrosis and development of hepatocellular carcinoma in chronic hepatitis C.[10-15] The majority of such clinical studies included patients with liver cirrhosis. However, for pathophysiologic reasons, liver cirrhosis increases the probability of impaired glucose tolerance. Therefore, in studies of cirrhotic patients,

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it is difficult to pinpoint the true effects of diabetes on hepatocarcinogenesis. On the other hand, we recently reported that a sustained virologic response to interferon therapy reduces the incidence of type 2 diabetes onset in chronic hepatitis C.[16] Thus, there is a gap in our knowledge on the exact effect of diabetes on hepatocarcinogenesis in interferon-treated patients.

The present retrospective study was designed to determine the effects of diabetes on hepatocarcinogenesis in noncirrhotic, interferon-treated patients with chronic hepatitis C virus infection, including the effects of viral clearance on diabetes-related hepatocarcinogenesis.

CLINICAL SIGNIFICANCE

- The hepatocarcinogenesis rate from first interferon therapy for noncirrhotic patients with chronic hepatitis C was 2 times greater in diabetic cases than in nondiabetic cases.
- Diabetes was an independent predictive factor of hepatocellular carcinoma in interferon-treated, noncirrhotic patients with chronic hepatitis C virus.
- In patients without a sustained virologic response from interferon therapy, the hepatocarcinogenesis rate of diabetic cases was approximately 15 times greater than that of nondiabetic, noncirrhotic patients with chronic hepatitis C and a sustained virologic response.

PATIENTS AND METHODS

Study Population

In this retrospective cohort study, we obtained the medical records of all patients in our database who had received interferon therapy for chronic hepatitis C between 1987 and 2007 at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these patients, 2058 satisfied the following criteria: 1) no evidence of diabetes after termination of interferon; 2) laparoscopy or liver biopsy performed before initiation of interferon therapy confirmed the lack of liver cirrhosis; 3) measurement of serologic type and hepatitis C virus viral load before initiation of interferon therapy; 4) platelet count of $\geq 10 \times 10^4$/mL; 5) negativity for hepatitis B surface antigen, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; 6) no underlying metabolic disease, such as hemochromatosis, alpha-1-antitrypsin deficiency, or Wilson disease; 7) no underlying systemic disease, such as systemic lupus erythematosus or rheumatic arthritis; 8) no evidence of hepatocellular carcinoma on ultrasonography or computed tomography before the initiation of interferon therapy; and 9) follow-up period of ≥ 24 weeks.

All patients who did not show a sustained virologic response and persistently high alanine aminotransferase level (normal range: 6-50 IU/L) received liver protection therapy, consisting mainly of glycyrrhizin and ursodeoxycholic acid (300-600 mg/d), during this research.

In all patients, the observation starting point was the time of initiation of the first interferon treatment. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The study was approved by the institutional review board of the Toranomon Hospital.

Background and Laboratory Data

Table 1 (available online) summarizes the clinical profile and laboratory data of 2058 interferon-treated patients with chronic hepatitis C. The male to female ratio was 1.78:1. Of 2058 patients, 164 (8.0%) were alcoholic (total alcohol intake > 500 kg until the initiation of interferon therapy). Before the initiation of interferon therapy, 104 patients (5.1%) were known diabetics. Furthermore, 71.2% patients had a high viral titer (low viral load; Amplicor <100 KIU/mL [Cobas Amplicor HCV Monitor Test, version 2.0, Roche Molecular Systems, Inc, Belleville, NJ] or probe <1 MEq/mL [branched DNA probe assay; version 2,0; Chiron, Daiichi Kagaku, Tokyo], high viral load; Amplicor $\geq$ 100 KIU/mL or probe $\geq$ 1 MEq/mL).

Type of Interferon and Assessment of Response to Interferon Therapy

Among 2058 patients treated with interferon, 1207 (58.6%) received interferon-α, 329 (16.0%) received interferon-β, and the remaining 522 (25.4%) received a combination therapy of interferon and ribavirin. The response to interferon therapy was assessed on the basis of sustained virologic response (sustained virologic response was regarded as elimination of hepatitis C virus-RNA at 6 months after the termination of interferon treatment). After interferon therapy, 52.5% of the patients showed sustained virologic response.

Markers of Hepatitis B and C Viruses

Anti-hepatitis C virus was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). Hepatitis C virus-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan) or the branched DNA probe assay (branched DNA probe assay; version 2.0; Chiron). Hepatitis B surface antigen was tested via radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored at -80°C at the first consultation. Diagnosis of hepatitis C virus infection was based on detection of serum hepatitis C virus antibody and hepatitis C virus RNA.

Histopathologic Examination of the Liver

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim-Silverman needle with an internal diameter of 2 mm (Tohoku University, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and pe-

Table 1 Characteristics of 2058 Noncirrhotic, Interferon-Treated Patients with Chronic Hepatitis C Virus Infection at the Initiation of Interferon and Efficacy

Parameter	(n = 2058)
Gender (M:F)	1317:741
Age (y)†	50 (15-72)
Histopathologic grade (F1-2:F3)	1916:142
Total ethanol intake (≥500 kg) (yes/no)	164:1894
Follow-up period (d)†	2443 (170-7562)
Albumin (g/dL)†	4.2 (2.3-5.3)
Total bilirubin (mg/dL)†	0.7 (0.1-11.7)
AST (IU/L)†	68 (21-488)
ALT (IU/L)†	77 (5-1212)
γ-GTP (IU/L)†	43 (5-805)
Platelet count ($\times 10^4/\mu L$)†	18.3 (10.0-48.1)
AFP ($\mu g/L$)†	4 (1.0-780)
Fasting/casual plasma glucose (mg/dL)†	96 (66-376)/100 (49-415)
Diabetes (yes/no)	104:1954
Total cholesterol (mg/dL)†	172 (102-348)
Triglyceride (mg/dL)†	89 (32-325)
LDL cholesterol (mg/dL)†	105 (39-209)
HDL cholesterol (mg/dL)†	46 (8-107)
IFN (monotherapy/combination therapy)	1536:522
HCV serologic group (1:2)	1310:748
Viral load (low:high)	592:1466
Efficacy of IFN therapy acquired viral elimination* (yes:no)	1081:977

AST = aspartate aminotransferase; ALT = alanine aminotransferase; γ-GTP = gamma-glutamyl transpeptidase; AFP = alpha-fetoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; IFN = interferon; HCV = hepatitis C virus.

*Viral elimination means sustained virologic response.

†Expressed as median (minimum, maximum).

riodic acid-Schiff after diastase digestion. 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]

Definition of Diabetes Mellitus

Diabetes was diagnosed by the use of the 2003 criteria of the American Diabetes Association.[18] These criteria include 1) casual plasma glucose ≥ 200 mg/dL; 2) fasting plasma glucose ≥ 126 mg/dL; and 3) 2-hour post-glucose (oral glucose tolerance test) ≥ 200 mg/dL.

Follow-up and Diagnosis Procedure of Hepatocellular Carcinoma

The starting time of follow-up was the point of the initiation of the first interferon treatment. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each visit together with regular checkups. Ultrasonography or computed tomography were performed every 3 to 6 months. The diagnosis of hepatocellular carcinoma was performed by biochemical examination (include alpha-fetoprotein and des-gamma carboxyprothrombin) and triple-phase dynamic computed tomography study. The number of cases lost to follow-up was 147 patients (7.1%) in this group.

Statistical Analysis

The cumulative rate of hepatocarcinogenesis (new cases of hepatocellular carcinoma) was calculated from the point of initiation of the first interferon treatment to the diagnosis of hepatocellular carcinoma using the Kaplan–Meier method. Differences in the development of hepatocellular carcinoma between different groups were tested using the log-rank test. Independent factors associated with the rate of hepatocellular carcinoma were analyzed by the Cox proportional hazard model. The following 19 variables were analyzed for potential covariates for incidence of hepatocellular carcinoma at the time of first interferon treatment initiation at Toranomon Hospital: gender, age, histologic stage of the liver, amount of total ethanol intake, existence of diabetes, viral serologic group, viral load, existence of sustained viral clearance by interferon therapy, serum concentration of albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alpha-fetoprotein, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and platelet count. A P value of less than .05 in a 2-tailed test was considered significant. Data analysis was performed using the Statistical Package for the Social Sciences version 11.0 for Windows (SPSS, Inc, Chicago IL).

RESULTS

Incidence of Hepatocellular Carcinoma in Noncirrhotic, Interferon-Treated Patients with Chronic Hepatitis C

In this cohort, hepatocellular carcinoma developed in 73 patients (3.5%) during a median observation period of 6.7 years. The cumulative rate of newly diagnosed hepatocellular carcinoma was 1.2% at 4 years, 2.6% at 8 years, and 6.8% at 12 years (**Figure 1**). The hepatocarcinogenesis rate according to interferon therapy was 2.1% at 4 years, 4.4% at 8 years, and 11.6% at 12 years in patients who did not acquire a sustained virologic response, and 0.7% at 4 years, 1.0% at 8 years, and 1.6% at 12 years in patients who acquired a sustained virologic response (**Figure 2**). The cumulative incidence rate of hepatocellular carcinoma was significantly lower in patients who acquired a sustained virologic response than in those who did not ($P < .001$).

Effect of Diabetes Mellitus on Hepatocarcinogenesis in Noncirrhotic, Interferon-Treated Patients with Hepatitis C

During the follow-up period, 58 of the 1954 nondiabetic patients (3.0%) developed hepatocellular carcinoma, and 15 of the 104

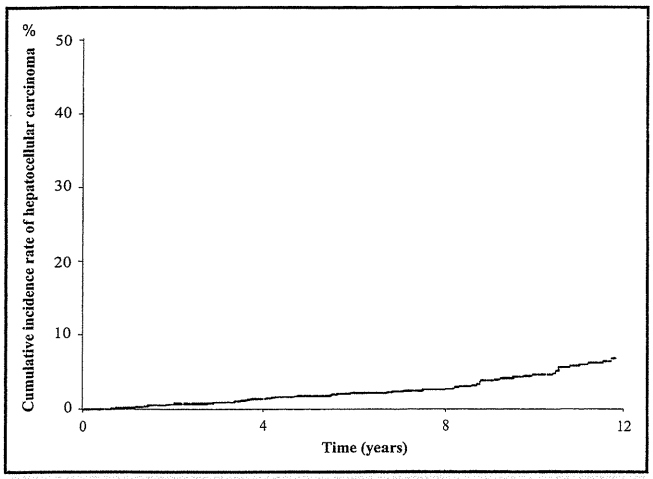

Figure 1 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection.

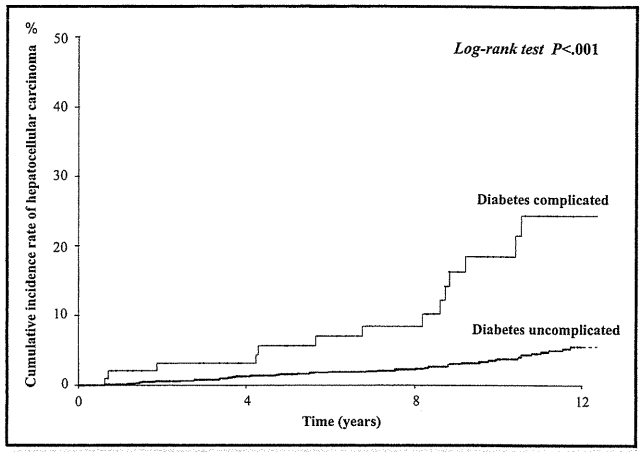

Figure 3 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection according to the presence or absence of diabetes.

diabetic patients (14.4%) developed hepatocellular carcinoma. The cumulative rate of hepatocellular carcinoma in nondiabetic patients was 1.3% at 4 years, 2.2% at 8 years, and 5.6% at 12 years. For diabetic patients, these rates were 3.2%, 8.5%, and 24.4%, respectively (Figure 3). The cumulative rate of hepatocellular carcinoma was significantly higher in patients with diabetes than those without ($P < .001$).

Effect of Sustained Virologic Response on Rate of Hepatocarcinogenesis in Noncirrhotic, Interferon-Treated Patients with Hepatitis C According to Presence of Diabetes

In the nonsustained virologic response group (n = 977), 47 (5.2%) of the nondiabetic patients (n = 906) developed hepatocellular carcinoma during the observation period, whereas 14 (19.7%) of diabetic patients (n = 71) developed hepatocellular carcinoma. In the sustained virologic response group (n = 1081), 11 (1.0%) of the nondiabetic patients (n = 1048) developed hepatocellular carcinoma during the observation period, whereas 1 (3.0%) of the diabetic patients (n = 33) developed hepatocellular carcinoma.

Analysis of data according to the efficacy of interferon therapy in diabetic and nondiabetic patients showed that in patients with nonsustained virologic response, the cumulative rate of hepatocellular carcinoma in nondiabetic patients was 1.9% at 4 years, 3.6% at 8 years, and 9.6% at 12 years, whereas in diabetic patients, these rates were 4.7%, 12.1%, and 31.0%, respectively (Figure 4). The cumulative rate of hepatocellular carcinoma was significantly higher in diabetic patients with a nonsustained virologic response than in nondiabetic patients ($P < .001$). The same analysis in

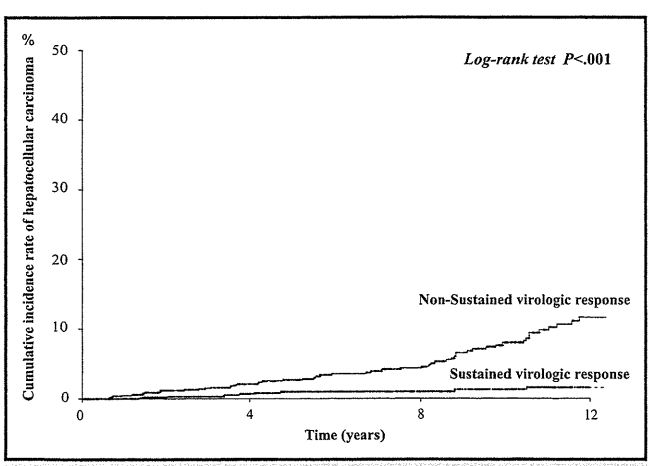

Figure 2 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection according to effect of interferon therapy.

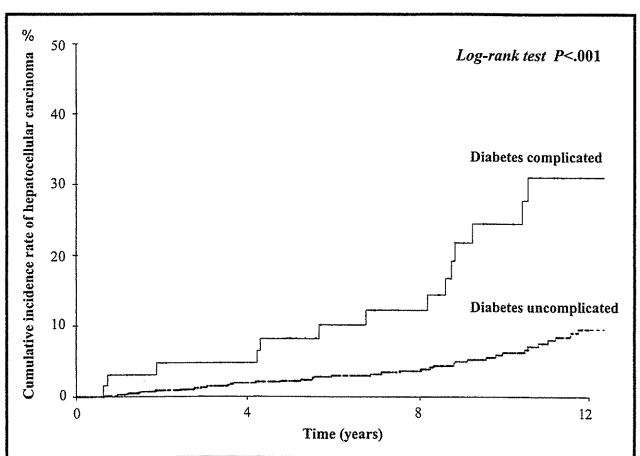

Figure 4 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection who showed nonsustained virologic response to interferon therapy according to the presence or absence of diabetes.

patients with a sustained virologic response showed a cumulative rate of hepatocellular carcinoma of 0.7%, 1.0%, and 1.7% in nondiabetic patients, and 0.0%, 0.0%, and 0.0% in diabetic patients, respectively (Figure 5). There was no significant difference between diabetic and nondiabetic groups in patients with a sustained virologic response ($P = .249$).

Factors Associated with Rate of Hepatocarcinogenesis

Multivariate Cox proportional hazard analysis revealed the following independent factors for hepatocellular carcinoma development after the initiation of the first interferon therapy in patients who showed a nonsustained virologic response (hazard ratio 7.28; 95% confidence interval [CI], 3.28-16.15; $P < .001$): male (hazard ratio 4.90; 95% CI, 2.47-9.71; $P < .001$), aged ≥ 60 years (hazard ratio 3.28; 95% CI, 1.88-5.74; $P < .001$); aspartate aminotransferase ≥ 50 IU/L (hazard ratio 3.91; 95% CI, 1.81-8.43; $P = .001$); alpha-fetoprotein ≥ 20 mg/L (hazard ratio 2.89; 95% CI, 1.43-5.84; $P = .003$); diabetes (hazard ratio 2.00; 95% CI, 1.05-3.84; $P = .036$); and platelet count $< 17 \times 10^4$/mL (hazard ratio 1.96; 95% CI, 1.11-3.48; $P = .021$) (Table 2, available online).

Rate and Prognosis of Diabetic Patients with Marked Fatty Deposition at First Interferon Initiation

Fourteen of 104 diabetic patients (13.5%) had fatty deposition in hepatic cells of ≥ 30% before the initiation of interferon therapy. Of these 14 patients, 2 were diagnosed with hepatocellular carcinoma during the observation period. One patient underwent liver resection to treat hepatocellular carcinoma, and background liver tissue was liver cirrhosis. One patient did not receive a liver resection; however, this patient's platelet count was approximately $20 \times 10^4/\mu L$ at the time of diagnosis of hepatocellular carcinoma. Thus, severe fibrosis was not suspected in view of this platelet count level.

Rate of Liver Cirrhosis at Hepatocellular Carcinoma Diagnosis

In 23 of 73 patients with hepatocellular carcinoma (31.5%), hepatic resection was performed for treatment. Five of 23 resected patients (21.7%) had liver cirrhosis in background hepatic tissue. The remaining 50 of 73 patients (68.5%) did not receive hepatic resection, and these patients received other nonresection therapy. Because the platelet count level was less than $10 \times 10^4/\mu L$ in 17 of 50 patients without resection (34.0%), liver cirrhosis was suspected. In these patients with histologic or clinical diagnosis of liver cirrhosis at the time of onset of hepatocellular carcinoma, none had a sustained virologic response by interferon therapy.

DISCUSSION

The present study described the incidence of hepatocellular carcinoma after the initiation of interferon therapy in patients with chronic hepatitis C infection. The results indicate that the annual incidence of hepatocellular carcinoma over a prolonged follow-up from first interferon therapy among noncirrhotic patients with hepatitis C virus is 0.3% to 0.5%. The present study was limited by its retrospective design. Moreover, the number of diabetic and nondiabetic patients was markedly different, which might be a potential source of bias. Another limitation of the study was that patients received different types of antiviral therapies for different duration. Thus, we did not evaluate the effect of different interferon regimens but assessed the impact of having or not having a sustained virologic response. This heterogeneity makes it somewhat difficult to interpret the results. On the other hand, the strengths of the present study are the long-term follow-up in a large number of patients treated at the same institution. The present study highlights several new findings with regard to the development of hepatocellular carcinoma after interferon therapy in noncirrhotic patients with hepatitis C virus. First, in patients with a sustained virologic response, diabetes had no significant effect on the rate of hepatocarcinogenesis. Second, in patients with a nonsustained virologic response, the rate of hepatocarcinogenesis was significantly higher in diabetics; diabetes was associated with 2-fold increase in the incidence of hepatocellular carcinoma.

In the present study, no significant difference was noted in the rate of hepatocarcinogenesis in patients with a sustained virologic response with and without diabetes. However, at least 2 studies have described a relationship between diabetes and hepatocellular carcinoma in patients without viral hepatitis.[18,19] In our study, 7.3% of the patients with a nonsustained virologic response were diabetics, compared with approximately 3.0% in the group with a sustained virologic response. These rates were lower than those in the general Japanese population (~15% for men, 9% for women), especially in those with a sustained virologic response. With regard to interferon treatment, previous studies reported that insu-

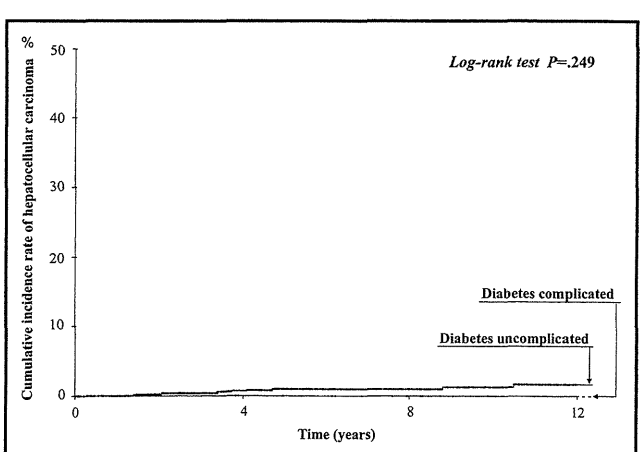

Figure 5 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection who showed sustained virologic response to interferon therapy according to the presence or absence of diabetes.

lin resistance and diabetes lower the sustained virologic response rate in patients treated with peginterferon plus ribavirin.[20,21] Therefore, interferon therapy itself may explain the different rates of diabetes in the 2 groups.

Diabetes is an independent predictor of several types of cancers, including hepatocellular carcinoma in patients with or without viral infection.[19,22,23] However, the rate of hepatocarcinogenesis in our patients with a sustained virologic response was not significantly influenced by the presence or absence of diabetes. Our retrospective study included a low rate of diabetes compared with that of the general Japanese population. This lower rate of diabetes in patients with a sustained virologic response may explain the lack of effect of diabetes on the rate of hepatocarcinogenesis.

Several studies reported the relevance of hepatitis C virus core gene to insulin resistance in patients with chronic hepatitis C.[24-26] interferon therapy is considered to worsen blood glucose control, but if the cause of insulin resistance is based on the involvement of hepatitis C virus core gene, one could consider probable improvement of insulin resistance after a sustained virologic response. Further studies are necessary to examine in these points.

CONCLUSIONS

Our retrospective cohort study is the first to examine the effects of diabetes mellitus and sustained virologic response on hepatocarcinogenesis in noncirrhotic, interferon-treated patients with hepatitis C infection. Our results indicate that a sustained virologic response induced by interferon therapy eliminates the influence of diabetes mellitus and markedly reduces the rate of hepatocarcinogenesis in noncirrhotic, interferon-treated, hepatitis C virus-positive patients.

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HCC develops even in the early stage of chronic liver disease in elderly patients with HCV infection

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Abstract. In recent years, the number of elderly patients with hepatocellular carcinoma (HCC) has been increasing. The aim of this study was to compare the liver function and the background factors of HCC patients with hepatitis C virus (HCV) infection by generation and to examine the characteristics of this disease in the elderly. A total of 1096 patients (776 men and 320 women) diagnosed with HCV-related HCC at our institution from 1995 to 2006 were divided into 4 groups as follows: D group, 75 years of age or older; C group, 65-74 years of age; B group, 55-64 years of age; A group, 54 years of age or younger, and the liver function and other clinical characteristics were compared among these 4 groups. The average age at initial diagnosis of HCV-related HCC was 66.9 years of age. The A, B, C and D groups were comprised of 87, 363, 514 and 132 patients, respectively. The rate of Child-Pugh class A patients in the D group was significantly higher than that of the other groups ($P<0.05$). The average levels of ALT, TB and PT-INR in the D group were significantly lower than the levels in the other groups ($P<0.05$). The average Alb level in the D group was significantly higher than that in the other groups ($P<0.05$). In conclusion, we found that HCV-related HCC in the elderly occurred against a background of chronic liver disease with mild inflammation and fibrosis.

Introduction

In recent years, the average age of the population of Japan has been increasing annually, and in 2006, elderly individuals over 65 years of age accounted for more than 20.8% of the entire population. The number of elderly, 65 years of age or older, who are afflicted with hepatocellular carcinoma (HCC) has also been increasing rapidly since 1990 (1,2). The average age at initial diagnosis of HCC at our institution from 1986 to 1993 was 60.9 years and 68.4 years in the period 2000 to 2006 and has been increasing annually (Fig. 1).

In Japan, more than 80% of HCC patients have hepatitis C virus (HCV) infection, and there are approximately 2 million patients with HCV-related chronic liver disease. It is estimated that there are 700,000 patients with undiagnosed HCV infection (3-5). The contributing factors include the spread of stimulant drugs, vaccinations and medical counter measures (injections, surgeries, blood transfusion) during the period after World War II when preventative measures against infection were inadequate. As a result, the incidence of HCC in patients with HCV who were infected during this period has been increasing in recent years (1,2,4,5).

Generally, 60-80% of patients develop chronic hepatitis after HCV infection, while many patients present with HCC approximately 30 years after HCV infection (6-9). On the other hand, it has been reported that the majority of patients with posttransfusion chronic HCV infection develop HCC after the age of 60 years regardless of when they acquired the HCV infection (10). Moreover, with the increase in fibrosis of transplanted livers in HCV-infected patients, it has been reported that the age of the donor is closely correlated with the rate of progression of fibrosis of the transplanted liver rather than the age of the recipient (11). Namely, the age of the patients who were infected with HCV is a more significant factor than the duration of the HCV infection in regards to the progression of HCV-persistent infection and fibrosis or carcinogenesis (10). Furthermore, in recent years the number of patients in which HCC does not occur before 60 years of age but thereafter develops in patients in their 70s or 80s has been increasing (12-16).

Therefore, it is assumed that age is the important factor contributing to hepatocarcinogenesis of HCV-related HCC. Therefore, we divided HCV-related HCC patients into 4 groups according to age. The aim of this study was to compare the liver function and the background features of the HCV-related HCC patients by generation, particularly focusing on the elderly group and examining the characteristics of this disease in the elderly.

Materials and methods

Patient population and experimental design. Among 1,404 patients with primary HCC consecutively diagnosed at our institution from January 1995 to December 2006, 1096 patients (776 men and 320 women) who were both HCV antibody-

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Key words: elderly patients, HCV-related HCC, mild inflammation and fibrosis, age

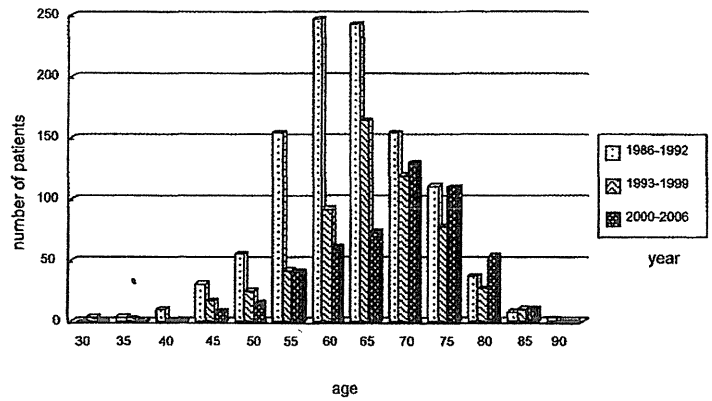

Figure 1. Changes in the average age at intitial diagnosis of HCC. The average age of onset was 60.9 years in 1986-1992, 64.9 years in 1993-1999, and 68.4 years in 2000-2006, with an annual increase in the average age.

positive and hepatitis B surface (HBs) antigen-negative were enrolled. The average age at inital diagnosis of HCC was 66±9 years (range, 42-87). The clinical characteristics of the patients are documented in Table I. These patients were divided into 4 groups as follows: D group, patients ≥75 years of age; C group, patients ≥65 and <75 years of age; B group, patients ≥55 and <65 years of age, and A group, patients ≤54 years of age. Liver function and background factors were compared among the 4 groups. A liver biopsy was also performed in noncancerous areas in 18 patients in the D group targeted consecutively from 2005 to 2006.

HCC diagnosis. The diagnosis of HCC was based on hypervascularity, confirmed by dynamic computed tomography (CT), magnetic resonance imaging (MRI), angiography or CT angiography, when the serum levels of HCC-related tumor markers, such as α-fetoprotein (AFP) or des-γ carboxy prothrombin (DCP), were increased or a mass lesion was observed by ultrasonography. When a nodule was not proven to be hypervascular, percutaneous biopsy under ultrasonography was performed for confirmation of the diagnosis of HCC, and thereafter a pathological study using hematoxylin and eosin staining was carried out. The fibrosis staging scores and activity grades were assigned according to the criteria of Desmet and colleagues (17) and the French METAVIR Cooperative Study (18-20). Staging was defined as F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), or F4 (cirrhosis), and grading was defined as A0 (no activity), A1 (mild activity), A2 (moderate activity), or A3 (severe activity). Three pathologists independently evaluated the disease stage and grade. Portal vein tumor thrombosis (PVTT) was defined as a protrusion of the tumor into the first and/or second branch, or into the main trunk of the portal vein. Tumor stage was classified according to the International Union Against Cancer (UICC) TNM classification.

Factors evaluated in the analysis. The following 19 factors were compared: gender, a history of blood transfusion, a history of habitual drinking, underlying diseases except for liver diseases, gastro-esophageal varices, Child-Pugh classification, serum albumin (Alb), serum total bilirubin (TB), prothrombin time-international normalized ratio (PT-INR),

Table I. Profile of the 1096 patients at initial diagnosis of HCV-related HCC.

Age (range)	66.9±7.6 (42-87)
Gender (male/female)	776/320
Alcohol consumption (none/light/heavy)	392/371/333
Underlying disease (none/single/multiple)	471/436/189
Gastro-esophageal varices (none/small/large)[a]	436/393/240
Blood transfusion (yes/no)	377/719
Child-Pugh classification (A/B/C)	751/290/55
Alb (g/dl)	3.48±0.47
PT-INR	1.17±0.15
TB (mg/dl)	1.17±0.66
ALT (U/l)	64.4±39.0
PLT (x10^4/μl)	10.8±5.54
HA (ng/ml)	363±385
ICG R15 (%)	32.1±17.4

Date are expressed as the median range ± standard deviation (SD). [a]Twenty seven patients did not undergo endoscopic examination. Alb, serum albumin; PT-INR, prothrombin time-international normalized ratio; TB, serum total bilirubin; ALT, serum alanine aminotransferase; PLT, platelet count; HA, hyaluronic acid; ICG R15, indocyanine green retention rate at 15 min.

serum alanine aminotransferase (ALT), platelet count (PLT), indocyanine green retention rate at 15 min (ICG R15), serum hyaluronic acid (HA), number of tumors, tumor size, portal vein tumor thrombosis (PVTT), tumor stage, α-fetoprotein (AFP) and extra-hepatic metastasis. The values at initial diagnosis of HCC were used for Alb, TB, PT-INR, ALT, PLT, ICG R15 and HA. Chronic hepatitis was included as Child-Pugh class A. Gastro-esophageal varices were classified into three types according to the endoscopic form factor grade: none, no varix; small, form 1 or 2; large, form 3, based on the classification of the Japanese Research Society for Portal Hypertension (21). The underlying diseases included hypertension, diabetes mellitus, cerebrovascular damage, cardiac disorder, renal disorder and cancer of other organs, and these were divided into three groups: none, no underlying disease; single, underlying disease by one disorder; multiple,

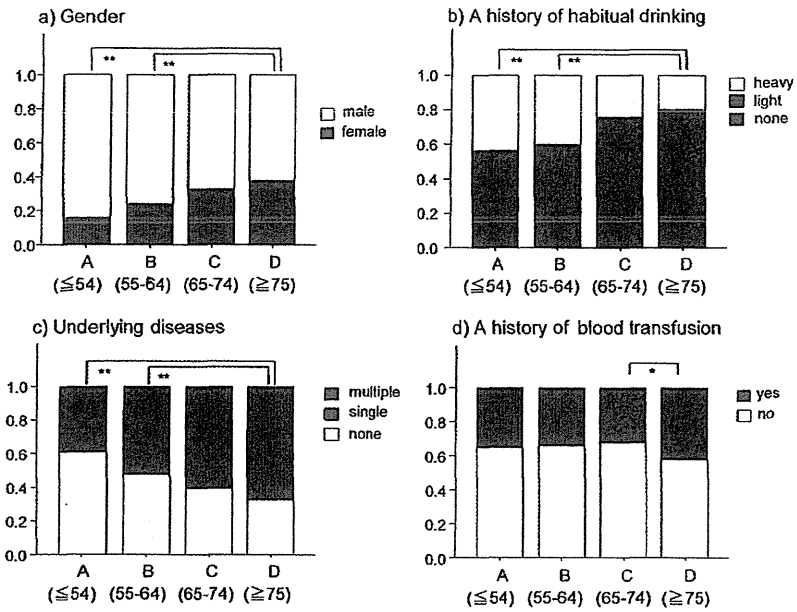

Figure 2. The age at initial diagnosis of HCC and background factors. The number of female patients, the patients without a history of habitual drinking and the patients with underlying diseases increased with age. (a) Concerning gender, the number of female patients in the D group was significantly higher than that in groups A and B (**P<0.01). (b) The number of patients without a history of habitual drinking in the D group was significantly higher than that in the A and B groups (**P<0.01). (c) The number of patients with underlying diseases in the D group was significantly higher than that in the A and B groups (**P<0.01). (d) A history of blood transfusion was noted most frequently in the D group and was significantly higher than that in the A group (*P<0.05). *P<0.05, **P<0.01 between the indicated groups.

underlying diseases by more than two disorders. A history of habitual drinking was classified into three groups: none, non-drinker; light, <84 g/day of ethanol; heavy, >84 g/day of ethanol for >5 years. The number of tumors was divided into two groups: solitary and multiple tumors. Tumor size was divided into two groups: those measuring ≤3 cm and >3 cm in size. The AFP level was divided into three categories: ≤20, 21-200 and >200 ng/ml.

Statistical analysis. Data are expressed as the mean ± standard deviation (SD). Data regarding Alb, TB, PT-INR, ALT, PLT, ICG R15 and HA levels were analyzed by one-way analysis of variance followed by the Dunnett's multiple comparison of means test. A Logistics regression analysis was used to analyze the effects of the background factors (gender, a history of blood transfusion, a history of habitual drinking, underlying diseases, degree of gastro-esophageal varices, Child-Pugh classification) and the tumor features (number of tumors, tumor size, PVTT, tumor stage, AFP, extrahepatic metastasis) on age at the initial diagnosis of HCC. The background factors and the tumor features were individually chosen as outcome variables, and the age at initial diagnosis of HCC divided into four groups were dummy coded and used as predictor variables. The statistical analysis was performed using SPSS for Windows (version 12.0). P<0.05 was considered to be statistically significant.

Results

Comparison of background factors. Among the 1096 patients, 132 (12%) were classified in the D group (patients ≥75 years of age), with the oldest patient being 87 years of age. The number of female patients increased with age, and the male:female patient ratio in the D group was 82:50. As a result, the number for female patients in the D group was significantly higher than that in the A and B groups (patients <65 years of age) (P<0.01) (Fig. 2a). The number of patients without a history of habitual drinking increased with age, and that of the D group was significantly higher than that in the A and B groups (P<0.01) (Fig. 2b). The number of patients with underlying diseases increased with age, and that of the D group was significantly higher than that in the A and B groups (P<0.01) (Fig. 2c). A history of blood transfusion was

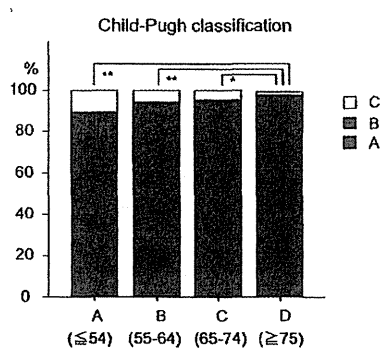

Figure 3. The age at initial diagnosis of HCC and Child-Pugh classification. The percentages of Child-Pugh class A patients were 59.8, 64.5, 70.1 and 79.5% in the A, B, C and D groups, respectively. The rate of Child-Pugh class A patients increased with age, and the rate of Child-Pugh class A patients in the D group was significantly higher than the rate in the other groups (D vs. A and B, **P<0.01; D vs. C, *P<0.05). *P<0.05, **P<0.01 between the indicated groups.

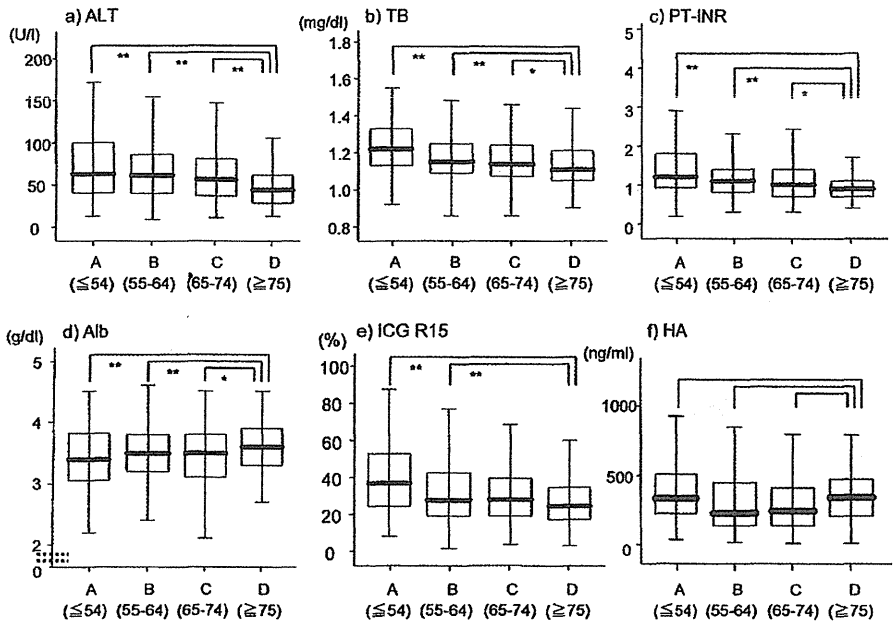

Figure 4. A comparison of age at initial diagnosis of HCC and liver functions. The average values of (a) ALT, (b) TB and (c) PT-INR in the D group were significantly lower than the values in the other groups (ALT: D vs. A, B and C, **P<0.01; TB and PT-INR: D vs. A and B, **P<0.01, D vs. C, *P<0.05). The average value of (d) Alb in the D group was significantly higher than that in the other groups (D vs. A and B, **P<0.01; D vs. C, *P<0.05). The average value of (e) ICG R15 in the D group was significantly lower than the value in the A and B groups (D vs. A and B, **P<0.01). The average value of (f) HA did not show a significant difference. *P<0.05, **P<0.01 between the indicated groups.

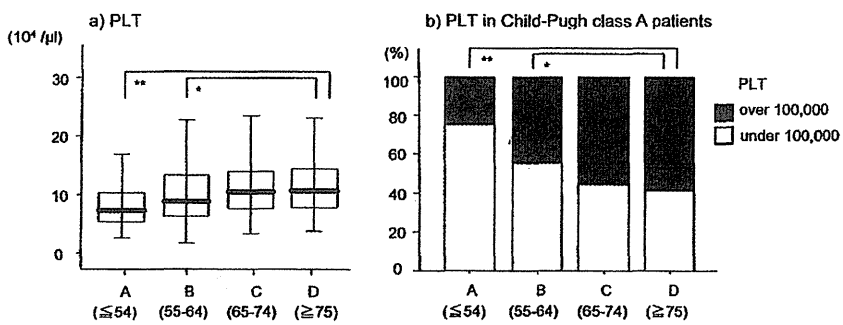

Figure 5. Comparison of age at initial diagnosis of HCC and PLT, and PLT in Child-Pugh class A patients. (a) The average value of PLT in the D group was significantly higher than the value in the A (**P<0.01) and B (*P<0.05) groups. (b) The rate of 100,000 or more of PLT in Child-Pugh class A patients of the D group was significantly higher than that in the A (**P<0.01) and B (*P<0.05) groups. *P<0.05, **P<0.01 between the indicated groups.

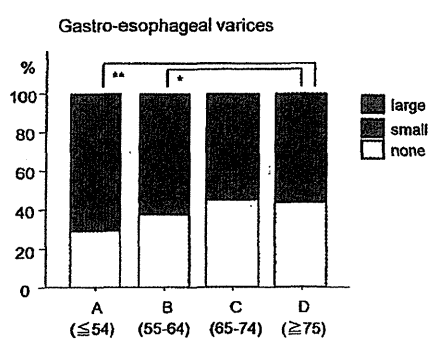

Figure 6. The age at initial diagnosis of HCC and the endoscopic form factor grade of varices. The number of patients with large varices was the lowest in the D group and the highest in the A group. The number of patients with large varices in the D group was significantly lower than that in the A (**P<0.01) and B (*P<0.05) groups. *P<0.05, **P<0.01 between the indicated groups.

noted most frequently in the D group, and it was significantly higher than that in the A group (P<0.05) (Fig. 2d).

Comparison of liver function. The percentages of Child-Pugh class A patients were 59.8, 64.5, 70.1 and 79.5%, in the A, B, C and D groups, respectively. Therefore, the percentage of Child-Pugh class A patients increased with age, and the percentage of Child-Pugh class A patients in the D group was higher than the other groups (D vs. A, B, P<0.01; D vs. C, P<0.05) (Fig. 3). The average values of ALT, TB and PT-INR were lower with age, and these average values in the D group were significantly lower than those in the other groups (ALT: D vs. A, B and C, P<0.01; TB, PT-INR: D vs. A and B, P<0.01; D vs. C, P<0.05) (Fig. 4a-c). The average value of Alb in the D group was significantly higher than that in the other groups (D vs. A and B, P<0.01; D vs. C, P<0.05)

Table II. Characteristics of the HCC tumors of the 1096 patients at initial diagnosis of HCV-related HCC.

	A group (87) 42-54 years of age	B group (363) 55-64 years	C group (514) 65-74 years	D group (132) 75-87 years	P-value
Number of tumors					<0.05, D vs. C
Solitary	37	156	217	59	
Multiple	50	207	297	73	
Tumor size					<0.05, D vs. A and B
≤3 cm	61	238	312	73	
>3 cm	26	125	202	59	
PVTT					NS
Present	7	45	66	11	
Absent	80	318	448	121	
TNM staging					NS
I	14	88	106	20	
II	37	120	171	51	
III	30	107	174	49	
IV	6	48	63	12	
AFP (ng/ml)					NS
≤20	28	120	185	53	
21-200	41	158	183	42	
>200	18	85	146	37	
Extrahepatic metastasis					NS
Yes	2	20	18	3	
No	85	343	496	129	

PVTT, portal vein tumor thrombosis; AFP, α-fetoprotein.

(Fig. 4d). The average value of ICG R15 was the lowest in the D group, and the average value of ICG R15 in the D group was significantly lower than that in the A and B groups (D vs. A and B, P<0.01) (Fig. 4e). The average value of HA was the lowest in the C group, thus indicating a strong variation and no significant difference (Fig. 4f). The average value of PLT was the highest in the D group, and the average value of PLT in the D group was significantly higher than that in the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 5a). When the Child-Pugh class A patients were divided per 100,000/μl of PLT and compared, the rate of 100,000 or more in the D group increased with age, and it was higher than the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 5b). The number of patients with large varices decreased with age, and the number of patients with large varices in the D group was lower than that in the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 6).

Comparison of characteristics of the HCC tumors. The number of patients with small HCCs ≤3 cm in the D group was lower than that in the A and B groups (D vs. A and B, P<0.05), and the number of patients with solitary HCC tumor in the D group was higher than that in the C group (D vs. C, P<0.05) (Table II). There were no statistical differences in the prevalence of PVTT, tumor stage, AFP or extrahepatic metastasis.

Pathological findings of liver biopsies from noncancerous areas. The pathological findings of patients for whom liver biopsies from noncancerous areas were performed are documented in Table III. Four of the 18 patients showed liver cirrhosis with activity ≥2 and fibrosis 4 in the Desmet classification. Fourteen of the 18 patients showed chronic hepatitis that was milder than activity 2 and fibrosis 3 in the Desmet classification.

Discussion

An elder is generally defined as anyone 65 years of age or older, but this definition is not standardized worldwide. In Japan, the average age at initial diagnosis of HCC is 65 years or older (13,14,22). Therefore, it is not appropriate to draw the dividing line at 65 years of age in order to define the characteristics of HCC that presents at old age (23). On the other hand, it has been reported that HCV-related HCC which develops in individuals in their 40s exhibits different characteristics from HCV-related HCC which develops in individuals 50 years of age or older, since HCC in individuals in their 40s is independently related to heavy drinking and the presence of HBV coinfection (24). It is therefore not appropriate that younger patients in their 40s and older patients who are 65 years of age or older are treated as a similar group, even when HCC patients are divided by a line drawn at 70 years

Table III. Pathological findings of liver biopsies from non-cancerous areas and the liver function of 18 patients with HCC 75 years of age or older.

No.	Age	Gender	Histology Desmet classification	PLT ($x10^4/\mu l$)	ALT (U/l)	TB (mg/dl)	PT-INR	Alb (g/dl)	ICG R15 (%)	HA (ng/ml)
1	78	F	A1F1	9.4	87	1.10	1.09	3.6	30.6	287
2	80	M	A2F4	7.9	79	0.70	1.03	3.3	14.3	237
3	82	F	A2F2	37.5	37	0.50	0.97	4.1	9.7	123
4	85	F	A2F2	8.3	28	0.50	1.06	4.1	16.8	470
5	75	M	A2F2	13.8	44	0.90	1.03	3.5	28.9	265
6	77	M	A2F2	17.5	64	1.30	1.13	4.0	30.3	225
7	75	M	A2F1	12.5	22	0.90	0.97	4.1	14.2	62
8	80	F	A2F2	15.3	54	1.00	1.12	3.4	45.7	1340
9	76	M	A2F4	8.3	62	0.80	1.18	3.5	24.5	557
10	87	M	A2F2	8.1	43	0.90	1.05	3.8	22.1	503
11	78	M	A1F1	16.3	33	0.95	1.05	4.5	16.8	126
12	76	M	A2F4	13.9	77	1.20	1.16	3.8	16.2	405
13	75	M	A3F4	8.9	33	1.40	1.16	3.7	48.2	619
14	76	F	A1F2	8.0	52	0.90	1.08	3.7	18.0	260
15	82	F	A2F3	11.0	37	2.20	1.20	3.1	23.8	1360
16	76	F	A2F2	11.9	45	0.70	1.02	4.2	9.6	353
17	76	M	A1F2	13.3	40	0.40	1.04	3.4	25.9	732
18	77	M	A2F3	10.1	54	1.02	1.01	4.1	45.2	163
Average	78.3			12.9	49.5	0.97	1.08	3.77	24.5	449

PLT, platelet count; ALT, serum alanine aminotransferase; TB, serum total bilirubin; PT-INR, prothrombin time-international normalized ratio; Alb, serum albumin; ICG R15, indocyanine green retention rate at 15 min; HA, serum hyaluronic acid.

of age as reported in previous studies (14,15,25,26). We therefore divided the elderly into the C group (>65 years of age and <75 years) and the D group (≥75 years of age), and the non-elderly into the A group (<54 years of age) and the B group (>55 and <65 years of age) to create 4 groups. The clinical characteristics of HCC in each group were thus examined in further detail by comparing each group, while particularly focusing on the D group.

Consequently, the most significant characteristic in patients with HCV-related HCC in the D group was that hepatic reserve was maintained and HCC occurred against a background of liver disorder with mild inflammation and fibrosis. In our study, levels of ALT and TB, which are well-known markers of inflammatory necrosis in the liver, were significantly lower in the D group than in all of the other groups. Notably, some studies found that alcohol promotes the progression of background chronic liver disease and consequently enhances carcinogenesis of the liver or that alcohol directly promotes carcinogenesis of the liver (27-29). In our study, the A group of younger HCC patients included many heavy drinkers of alcohol with severe inflammation in addition to high values of ALT and TB, as was previously found by Shimauchi *et al* (24). This finding corroborates reports that HCC frequently occurs in association with severe inflammation (30). Conversely, it was assumed to be one of the main reason that the D group had many patients with low values of ALT and TB, since the D group included many patients who did not habitually consume alcohol.

It has been reported that in chronic liver disease, changes in the platelet count correlate with the degree of fibrosis in liver histology (31-33). As a result, the platelet count is regarded as a marker for fibrosis. In our study, the platelet count in the D group was significantly higher than that in the A and B groups (younger than 65 years of age), and the D group in particular showed the highest platelet count of 100,000 or more in the Child-Pugh class A patients. The study of 18 patients in the D group was assessed by means of a liver biopsy, and the background liver showed chronic hepatitis of less than F3 except for 4 cases of F4, thus indicating that many patients with mild fibrosis had not advanced to liver cirrhosis.

Gastro-esophageal varices are the most common clinical manifestation of portal hypertension in patients with liver cirrhosis. Nakayama *et al* (34) reported that as the endoscopic form factor grade of varix increased, the incidence of the occurrence of HCC also increased, and in particular, a form 3 (large size) factor of varices was an independent predictor for HCC. In our study, many of the patients in the D group either did not have varices or only had small varices, and also the number of patients with large varices in the D group was significantly smaller than the non-elderly groups (younger than 65 years of age). Therefore, it was assumed that many of the patients in the D group had not advanced to liver cirrhosis, and many cases of HCC had thus occurred against a background of chronic liver disease that was not conducive to carcinogenesis.

Alb, PT-INR and ICG R15 values are well-known markers of hepatic reserve in the liver. In our study, the average values of Alb, PT-INR and ICG R15 in the D group were higher than in all of the other groups, showing the highest rate in the Child-Pugh class A patients. Generally, it has been reported that a decreased number of hepatocytes and decreased liver weight due to aging generally results in a decreased regeneration capacity of hepatocytes and a decreased hepatic blood flow (35-38). In addition, it has been suggested that the elderly often have a latent nutrient disturbance or metabolic anomalies, thus causing a declining trend in Alb and ICG R15 (39,40). However, in our study, many cases of HCC in the D group were present along with low values of ALT and TB, unlike the non-elderly group. Therefore, it appears that the progression of chronic liver disease was not promoted, and the hepatic reserve was consequently well-maintained in the elderly HCC group, despite the normal decline in the physiological liver function with age. As noted above, many cases of HCC in the elderly occur against a background of chronic liver disease with mild inflammation and fibrosis, and it is assumed that there are some characteristic factors which contribute to hepatocarcinogenesis in the elderly.

In recent years, age has become a point of focus in the progression of HCV-persistent infection and fibrosis or carcinogenesis. Mahmood *et al* (41) reported that age was the most important factor contributing to a high value of reactive oxygen metabolites in the blood associated with HCV-related chronic liver disease. Moreover, the involvement of oxidative stress has been previously reported in aging itself, which is believed to cause a decrease in various organ functions and immunity (42). The elderly are susceptible to oxidative stress due to a decrease in the SOD value related to the antioxidation mechanism and the decrease in NK cell activity (42,43). Therefore, hepatocarcinogenesis in the elderly may occur in association with only mild inflammation or fibrosis.

In addition, an increase in the rate of women is cited as one of the characteristics of HCC of the elderly. It has been reported that sex hormones such as estrogen and immune response play an important role in hepatocarcinogenesis (44,45). Since the levels of sex hormones decrease in ageing women, the risk of the incidence of HCC in elderly females increases.

In conclusion, we found that HCV-related HCC in the elderly occurred against a background of chronic liver disease with mild inflammation and fibrosis.

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Original Article

Adipocytokine involvement in hepatocellular carcinoma after sustained response to interferon for chronic hepatitis C

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Aim: Interferon (IFN) dramatically reduces the risk of hepatocellular carcinoma (HCC) after a sustained virological response (SVR) to chronic hepatitis C (CH-C). However, HCC still develops in some patients after SVR. To evaluate metabolic factors in patients with HCC occurring after SVR and to determine whether insulin resistance and adipocytokines were involved in this etiology.

Methods: We examined clinical and biochemical features, histological findings and serum levels of adipocytokine prior to IFN therapy and at the detection of HCC in nine patients who were diagnosed with HCC. As controls, 27 patients were included who showed SVR but had not been diagnosed with HCC for at least 5 years after SVR.

Results: Three of four patients who developed HCC within 5 years after SVR showed liver cirrhosis when HCC was diagnosed. Prior to IFN therapy, four of nine HCC patients were diagnosed as having type 2 diabetes mellitus. Serum levels of leptin and insulin, Homeostatic Model of Assessment of Insulin Resistance and body mass index (BMI) were significantly higher and serum adiponectin was significantly lower in HCC patients at the time of HCC detection than in control patients more than 5 years after SVR. Six HCC patients had increased BMI and one HCC patient had a decreased BMI during the observation period.

Conclusion: Hepatic fibrosis may be tightly related to the emergence of HCC after SVR. Insulin resistance and adipocytokine disorders may be implicated in hepatocarcinogenesis after SVR, in part by promoting hepatic fibrosis.

Key words: adipocytokine, adiponectin, hepatocellular carcinoma, insulin resistance, leptin, sustained virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide, especially in South-East Asia.[1] Hepatitis C virus (HCV) infection is a major risk factor for HCC. Seventy to eighty percent of Japanese patients with HCC are infected with HCV.[2] Interferon (IFN) is an antiviral agent against HCV that can eradicate the virus and which improves hepatic inflammation and fibrosis,[3] and is also believed to prevent clinical complications, including the development of HCC. IFN therapy for HCV was proven to lower the incidence of HCC, especially in patients who are treated successfully with IFN and show a sustained virological response (SVR) to the therapy.[4] However, previous studies have revealed that HCC still develops in 1.5–4.0% of patients with SVR.[5–11] Although advanced age,[6–11] male sex,[6,8] an advanced histological stage of hepatic damage,[6–10] higher aspartate aminotransferase levels,[11] lower platelet counts,[11] alcohol intake[7,9] and hepatic steatosis[10] before IFN therapy are thought to be risk factors for HCC, the mechanism of carcinogenesis after SVR is not yet fully understood.

The liver is one of the major organs regulating glucose metabolism. Patients with chronic liver disease

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frequently present with glucose intolerance which is referred to as hepatogenous diabetes with insulin resistance and hyperinsulinemia. The prevalence of glucose intolerance in HCV-related chronic liver disease is higher than in other liver diseases[12] and it has been suggested that HCV directly causes hepatic insulin resistance and hyperinsulinemia.[13,14] Previous studies have shown that the incidence of HCC has been increased 2–4-fold in patients with diabetes mellitus (DM)[15,16] and some reports have indicated that insulin resistance may be involved in cell growth and the carcinogenesis of HCC.[17,18] Meanwhile, several recent studies have reported that obesity increases the risk of malignancy,[19] including HCC.[20] In human obesity, intra-abdominal visceral fat content is strongly correlated with glucose intolerance.[21] In the obese state, adipose tissue promotes inflammation and macrophages migrating into adipose tissue secrete adipocytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1). Enlarged adipose cells also secrete these adipocytokines and free fatty acids (FFA). These adipocytokines and FFA can induce insulin resistance in the whole body.[22–24]

Adiponectin is an adipose tissue-specific plasma protein and is specifically secreted from fat tissue. Hypoadiponectinemia may be implicated in the pathogenesis of the various disorders which comprise metabolic disease,[25] and may be related to the pathogenesis of liver disease through cells expressing the adiponectin receptor in the liver.[26] Leptin is another circulating hormone secreted by adipocytes which acts as an important signaling molecule in energy regulation and food intake,[27] and modulates multiple physiological and pathophysiological states.[27,28]

Meanwhile, adiponectin is thought to be involved in the carcinogenesis of several cancers.[29,30] Adachi *et al.* showed that an adiponectin receptor, T-cadherin, was selectively expressed in intratumoral capillary endothelial cells in HCC, suggesting a positive role for T-cadherin in mediating angiogenesis in HCC.[31] In addition, leptin may also play an important role in the processes of initiation and progression of various human cancers.[32,33] Chen *et al.* reported that leptin induced proliferation and inhibited apoptosis in human HCC cells.[34] Zhou *et al.* showed that leptin stimulated Hep G2 cell proliferation through increased DNA synthesis and the enhancement of mitotic activity.[35] Ribatti *et al.* have reported an involvement of leptin and the leptin receptor in angiogenesis in human HCC.[36]

In this study, we investigated whether obesity, insulin resistance, leptin and adiponectin were involved in the development of HCC in patients after SVR undergoing IFN treatment for chronic hepatitis C (CH-C).

METHODS

Patients

THE SUBJECTS IN this study were nine patients who were diagnosed with HCC at Kurume University Hospital after being treated with IFN therapy for CH-C between 1993 and 2003 and exhibiting SVR. Seven patients were treated with IFN at Kurume University Hospital or affiliated hospitals, and two were treated with IFN at other hospitals. The diagnosis of HCC was based on histological examination for eight patients and on computed tomography (CT), magnetic resonance imaging (MRI) and elevation of tumor markers in one patient. One thousand one hundred and forty-three patients received IFN therapy for CH-C and 308 patients achieved SVR between 1993 and 2003 at Kurume University Hospital. Out of these patients, we selected 27 patients who met the following requirements as control: (i) periodically observed at our hospital after SVR and had no evidence of HCC for over 5 years from the time of SVR until the last outpatient visit; (ii) blood samples before IFN treatment and at following outpatient visit were stored; and (iii) hepatitis B virus surface antigen (HBsAg) was negative in the serum. All control patients and six HCC patients were followed up after SVR. These patients received ultrasonography (US), computed tomography (CT) and examination of tumor markers at least once per year. HCC were detected 1 year or later after SVR regarding six patients developing HCC with periodic observation. Another three patients developing HCC did not undergo periodic observation from the time of SVR to the detection of HCC. Intervals from SVR until detection of HCC regarding these three patients were 106, 156 and 108 months, respectively. SVR was defined as being serum HCV RNA negative for more than 6 months after the termination of IFN therapy. Serum HCV RNA was measured using the COBAS AmpliPrep/COBAS TaqMan HCV Test (Roche Molecular Systems, Branchburg, NJ, US). This newly established technique has excellent sensitivity for the detection of HCV RNA and is more sensitive than conventional methods of detection.[37]

We had less information prior to their IFN therapy about the two HCC patients treated with IFN at other hospitals. Upon detection of their HCC, both patients

were seropositive for anti-HCV antibodies, seronegative for HBsAg and negative for serum HCV RNA as determined by the COBAS AmpliPrep/COBAS TaqMan HCV Test. The other seven HCC patients and 27 control patients were all seropositive for anti-HCV antibodies, positive for serum HCV RNA and seronegative for hepatitis B antigen prior to IFN therapy. The HCV RNA load was quantitated by competitive reverse-transcription polymerase chain reaction, a branched-DNA probe assay, or by the Amplicor-HCV monitor assay.[38–40] HCV genotype was determined according to previously described methods.[41] We excluded patients who had a coexisting liver disease, such as autoimmune hepatitis or primary biliary cirrhosis. Past occurrence of HCC and treatment history for HCC were also part of the exclusion criteria. Informed consent was obtained from each patient and the study was approved by the Ethical Committee of Kurume University. The study was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki.

Metabolic parameters

We compared the clinical characteristics of the nine HCC patients and 27 control patients just before the start of IFN therapy. Body mass index (BMI) was calculated as weight in kilograms/height in meters squared. We obtained BMI values prior to IFN therapy from seven HCC patients treated with IFN in Kurume University Hospital or affiliated hospitals, and from 23 out of 27 control patients. We obtained BMI values for nine HCC patients at the time of HCC diagnosis, and for 22 of the control patients more than 5 years after SVR. The high molecular weight (HMW) form of adiponectin, leptin, insulin and glucose were measured from blood samples stored at −80°C. Blood samples were obtained from five HCC patients and all control patients within 9 months before the start of IFN therapy. Additional samples were obtained within 9 months after the time of SVR in three patients who developed HCC and from all control patients. Blood was also collected later as the last time point sample at the time of HCC diagnosis for nine HCC patients and more than 5 years after SVR for all the control patients. All samples were obtained at fasting time.

Serum insulin levels were measured using an enzyme-linked immunoassay kit (Lumipulse Presto Insulin; Fujirebio, Tokyo, Japan). Plasma glucose levels were measured using the hexokinase-glucose-6-phosphate dehydrogenase method. Insulin resistance was calculated from fasting levels of glucose and insulin using the Homeostatic Model of Assessment (HOMA) method. The formula for the HOMA model is: insulin resistance (HOMA-IR) = fasting glucose (mg/dL) × fasting insulin (μU/mL) / 405. Adiponectin is present in serum in a number of forms, and often exists as a trimer or hexamer of HMW.[42] Adiponectin in the HMW form has been reported to play a primary role in hepatic and whole-body insulin sensitivity and to have significant anti-inflammatory effects.[43] For this reason, we measured serum HMW adiponectin in this study. The levels of HMW adiponectin were determined using a HMW adiponectin assay kit (Fujirebio). Leptin levels were determined using a human leptin RIA kit (Linco Research, St Charles, MO, USA). Values of each metabolic parameter at each time point were compared between the HCC patients and controls.

Histopathology

We compared the histological features of liver tissue before IFN therapy in HCC patients and controls and also compared these with liver tissue at the time of detection of HCC. Liver biopsies were performed on 32 patients treated with IFN at Kurume University Hospital or at affiliated hospitals less than 1 year before the start of IFN therapy. One HCC patient underwent a biopsy 8 years before IFN therapy and showed cirrhosis at that point, and no biopsy was performed on one of the control patients. Assessment of the histological diagnosis was made according to the classifications of Desmet *et al.*[44] Hepatic steatosis was also graded by a modification of the Brunt scoring system as described[45,46] using the following categories: steatosis less than 5%, 5–33%, 33–66% and patients with over 66% steatosis were categorized as grades 0, 1, 2 and 3, respectively. All tissue samples were evaluated by two independent hepatologists. We also examined non-cancerous hepatic lesions resected with HCC from seven patients. These seven cases were assessed for histological tumor differentiation[47] in the resected specimen and one other case was examined using a specimen obtained by aspiration tumor biopsy.

Statistical analysis

Statistical analysis was performed using SPSS ver. 12.0J. Fisher's exact probability test was used to compare categorical data. Differences between two groups were measured using the Mann–Whitney *U*-test. A relationship between different continuous variables was measured by linear regression analysis. $P < 0.05$ was considered statistically significant.

Table 1 Characteristics of nine patients with HCC after sustained virological response

Age at detection of HCC (years)†	61 (54–75)
Maximal tumor diameter (mm)†	26.8 (16–43)
No. of tumors (1/2/3)	7/1/1
Tumor stage (I/II/III)‡	1/5/3
Differentiation (well/moderately)	3/5§
Child–Pugh score (at detection of HCC)¶	A5 for all
Therapy (resection/RFA/radiation)	7/1/1
Periodic observation	
Before detection of HCC (yes/no)	6/3

†Mean (range).
‡Based on the tumor–node–metastasis (TNM) classification.[48]
§Tumor histology was not available for one case.
¶Classified according to Child–Pugh classification.[49]
HCC, hepatocellular carcinoma; RFA, radiofrequency ablation.

RESULTS

Characteristics of nine patients with HCC after SVR

THE CHARACTERISTICS OF nine SVR patients who developed HCC are summarized in Table 1. Eight patients underwent curative therapy, including resection for seven patients and percutaneous radiofrequency ablation for one patient. Another patient was evaluated for resection, but ultimately received radiation therapy due to dementia.

Table 2 shows the clinical and histological profiles of HCC patients. Histological fibrosis worsened in three patients and did not change in one patient out of the six in whom we could compare liver histology at both time points. Four out of five alcohol consumers with HCC continued drinking alcohol after IFN therapy, and only patient 6 stopped drinking after IFN therapy. Four patients developed HCC within 5 years after SVR and five patients developed HCC more than 5 years after SVR. Three of four patients in the former group showed liver cirrhosis when HCC was diagnosed, and no patient showed liver cirrhosis in the latter group although histological data of two patients were not available.

Comparison of baseline characteristics of SVR patients with and without HCC after SVR

A comparison of the baseline characteristics of SVR patients with and without HCC is summarized in Table 3. Patients with regular smoking were significantly more in the HCC group than in the control group ($P = 0.046$). Before the start of IFN therapy, four out of nine HCC patients and none of the controls were diagnosed with type 2 DM, a significant difference between the two groups ($P = 0.002$). The average observation period from the time of SVR to the detection of HCC for the HCC group was 81.7 months (range

Table 2 Clinical and histological profiles of SVR patients with HCC

	Before IFN therapy			At the detection of HCC			DM	Alcohol (23 g/day)	Interval (months)§
	G/S†	Steatosis‡	BMI	G/S†	Steatosis‡	BMI			
Patient 1	2/2	1	23.4	1/4	0	23.7	–	–	22
Patient 2	2/4	2	26.5	1/4	1	28.9	+	–	38
Patient 3	2/1	0	19.3	1/4	1	23.1	–	–	42
Patient 4¶	2/2	1	27.8	1/1	0	29.6	–	+	48
Patient 5	2/2	0	20.2	N/A	NA	24.1	–	+	80
Patient 6	2/2	1	29.8	2/1	0	27.6	+	+	106
Patient 7¶	NA	NA	NA	NA	NA	24.5	+	–	108
Patient 8¶	1/1	2	23.3	1/2	1	24.9	–	+	135
Patient 9¶	NA	NA	NA	1/1	1	26.9	+	+	156

†G and S refer to hepatic inflammatory grading (A0–3) and fibrotic staging (F0–4), respectively.
‡Graded 0–3.
§Interval from SVR until detection of HCC.
¶Adiponectin, leptin, insulin and HOMA-IR prior to IFN therapy could not be acquired for patients 4 and 8. Detailed information for IFN therapy, laboratory and histological data, and metabolic parameters prior to IFN therapy could not be acquired for patients 7 and 9.
BMI, body mass index; DM, type 2 diabetes mellitus; HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; HCC, hepatocellular carcinoma; IFN, interferon; NA, not available; SVR, sustained virological response.

Table 3 Comparison of baseline characteristics between SVR patients with and without HCC after SVR

	HCC group	Control group	*P*-value†
Age at IFN induction (years)‡	53.0 (44–65)	51.3 (23–74)	NS
Sex (male/female)	5/4	11/16	NS
Alcohol (≥23 g/day) (+/–)	5/4	6/21	NS
Smoking (+/–)	6/3	7/20	0.046
Type 2 diabetes mellitus (+/–)	4/5	0/27	0.002
HBcAb (+/–)	5/4	11/16	NS
HCV viral load before IFN (high/low)§	2/5	8/18	NS
Virus genotype (1/2)	3/3	15/12	NS
IFN (a/b/a + b/consensus)	5/2/0/0	19/4/2/2	NS
(with RBV¶: PEG IFN††)	(3:0)	(4:1)	
Total IFN amount ($\times 10^4$) (mean)	59 800	56 392	NS
Observation period after SVR (months)‡	81.7 (22–156)	102 (61–149)	NS
AST (IU/L)‡‡	75.9 ± 49.9	62.2 ± 44.7	NS
ALT (IU/L)‡‡	69.3 ± 40.7	78.3 ± 70.4	NS
Total bilirubin (mg/dL)‡‡	1.0 ± 0.6	0.8 ± 0.2	NS
Albumin (g/dL)‡‡	3.7 ± 0.3	4.0 ± 0.2	NS
Prothrombin time (%)‡‡	93.2 ± 12.4	97.8 ± 16.4	NS
Platelet ($\times 10^4$/mL)‡‡	12.3 ± 3.4	15.4 ± 5.3	NS
Hyaluronic acid (ng/mL)‡‡	187.5 ± 153.4	122.9 ± 212.6	0.045
α-Fetoprotein (ng/mL)‡‡	42.4 ± 81.4	11.0 ± 29.2	NS
Child–Pugh score (5/6/7)	5/0/1	24/1/0	NS

All data were based on the patients from whom it was available for each characteristics.
†Comparison between patients with HCC (HCC group) and without HCC (control group) after SVR. *P*-values were calculated with Fisher's exact probability test and the Mann–Whitney *U*-test.
‡Mean (range).
§When the serum HCV RNA level was more than 1 Meq/mL by branched DNA assay, more than 10^6 copies/mL by competitive reverse transcription polymerase chain reaction, or more than 10^5 copies/mL by the Amplicor-HCV monitor assay, it was determined to be a high viral load.
¶Number of the patients in whom Ribavirin was used in combination.
††Number of patients in whom pegylated interferon was used.
‡‡Means ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBcAb, hepatitis B core antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NS, not significant.

22–156), and the average time from SVR to the outpatient visit when we obtained the last follow-up results for the control group was 102 months (range 61–149). There was no significant difference in the length of the observation period between the two groups. Serum levels of hyaluronic acid prior to IFN therapy were significantly higher in the HCC group than in the control group ($P = 0.045$).

Changes in adipocytokines during the observation period

Our findings on metabolic parameters are summarized in Table 4. Prior to IFN therapy and at the time of SVR, serum levels of adiponectin and leptin were not significantly different between the HCC group and the control group. However, adiponectin was significantly lower in the HCC group upon HCC detection than in the controls at their follow-up visit more than 5 years after SVR ($P = 0.030$), and leptin was significantly higher in HCC patients than in controls at that time ($P = 0.036$). When we only analyzed patients for whom we had data both prior to IFN therapy and at the last time point, percentage of change per year in adiponectin level was significantly lower ($P = 0.046$) and that in leptin level was significantly higher ($P = 0.003$) in the HCC patients than in controls. Figure 1(A) shows changes in adiponectin and Figure 1(B) shows changes in leptin in both groups during the observation period.

In all cases in both the HCC group and control group, serum leptin levels were positively correlated with serum insulin levels and HOMA-IR but not with adiponectin levels or BMI at the last time point (data not