Sustained Virological Response Reduces Incidence of Onset of Type 2 Diabetes in Chronic Hepatitis C

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Diabetes is present in patients with chronic hepatitis C virus infection. The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for type 2 diabetes after the termination of interferon therapy in Japanese patients positive for hepatitis C virus (HCV). A total of 2,842 HCV-positive patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin were enrolled. The mean observation period was 6.4 years. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses during follow-up. The primary goal was the onset of type 2 diabetes. Evaluation was performed by using the Kaplan-Meier method and Cox proportional hazard analysis. Of 2,842 HCV patients, 143 patients developed type 2 diabetes. The cumulative development rate of type 2 diabetes was 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years. Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), the patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was ≥50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). *Conclusion:* Our results indicate sustained virological response causes a two-thirds reduction in the risk of type 2 diabetes development in HCV-positive patients treated with IFN. (HEPATOLOGY 2009;49:739-744.)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20% to 50% of cases over a period of 10 to 30 years.[1-3] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[4-8] Moreover, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, porphyria cutanea tarda, membranoproliferative glomerulonephritis, autoimmune thyroiditis, sialadenitis, and cardiomyopathy.[9-13] Lately, data supporting a link between type 2 diabetes mellitus (T2DM) and chronic hepatitis C infection have been reported.[14,15]

Although there is growing evidence to support the concept that HCV infection is a risk factor for developing T2DM, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients with high risk of developing diabetes. Thus, prospective studies including metabolic evaluations are clearly needed to clarify these issues.

With this background in mind, the cohort study was initiated to investigate the cumulative incidence and risk factors of T2DM after prolonged follow-up in HCV-infected patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. There were 5,890 patients diagnosed with chronic HCV infection and treated with IFN mono-

Abbreviations: CI, confidence interval; FPG, fasting plasma glucose; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response; T2DM, type 2 diabetes mellitus.

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therapy or combination IFN + ribavirin therapy between September 1990 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 2,842 patients satisfied the following criteria: (1) no evidence of diabetes mellitus for 3 months after the termination of IFN (plasma glucose concentration <126 mg/dL [6.9 mmol/L] in the fasting state, <200 mg/dL [11.0 mmol/L] in casual state and/or 2 hours after a 75-g oral glucose load); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy before the initiation of IFN therapy; (3) positivity for serum HCV RNA before the initiation of IFN therapy; (4) period of ≤1 year of IFN therapy; (5) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined via radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown on ultrasonography and/or computed tomography; and (7) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatic arthritis.

Patients who were taking medications known to alter glucose tolerance or had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial were excluded from the study. Patients were classified as having normal glucose or pre-diabetes based on fasting plasma glucose (FPG), casual plasma glucose, or 2-hour plasma glucose. The normal glucose group was regarded as having an FPG of <100 mg/dL, casual plasma glucose of <140 mg/dL, and/or 2-hour plasma glucose of <140 mg/dL. The pre-diabetes group was regarded as having an FPG of 100-125 mg/dL, casual plasma glucose of 140-200 mg/dL, and/or 2-hour plasma glucose of 140-200 mg/dL.[16]

Next, we assessed predictive factors for T2DM in chronic hepatitis C patients treated with IFN. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or the patient's family. Informed consent was obtained from all living patients included in the present cohort study. The study was approved by the Institutional Review Board of our hospital.

Outcome Measures. The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association.[16] These criteria include (1) casual plasma glucose ≥200 mg/dL; (2) FPG ≥126 mg/dL; (3) 2-hour post-glucose (oral glucose tolerance test) ≥200 mg/dL.

Laboratory Investigation. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). 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 HCV infection was based on detection of serum HCV antibody and positive RNA. Height and weight were recorded at baseline, and the body mass index was calculated as weight (in kg)/height (in m^2).

Evaluation of Liver Cirrhosis. Liver status of the 2,842 patients was mainly determined via peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained 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 periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[17]

Follow-up. The starting time of follow-up was 3 months after the termination of IFN therapy. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-up. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses. These included aminotransferase activities, total cholesterol, platelet counts, and serum HCV RNA level. Three hundred twenty-four patients were lost to follow-up; because the appearance of T2DM and death was not identified in these patients, they were considered as censored data in the statistical analysis.[18] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical Analysis. The cumulative appearance rate of T2DM was calculated from 3 months after the termination of IFN treatment to the appearance of T2DM using the Kaplan-Meier method. Differences in the development of T2DM were tested using the log rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following 11 variables were analyzed for potential covariates for incidence of T2DM at the time of termination of IFN therapy at our hospital: age, sex, state of liver disease (chronic hepatitis or liver cirrhosis), body mass index, glucose level, aspartate aminotransferase level, alanine aminotransferase level, type of IFN, total dose of IFN, efficacy of IFN therapy, hypertension, triglyceride level, and total cholesterol level. A P value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1 shows the characteristics of the 2,842 HCV-positive patients treated with

Table 1. Patient Characteristics

N	2,842
Sex (male/female)	1,778/1,064
Age (years)	51.8 ± 9.0
Height (cm)	163.8 ± 9.1
Body weight (kg)	62.7 ± 11.7
Body mass index	23.3 ± 3.2
Blood pressure (systolic/diastolic, mm Hg)	128 ± 18/77 ± 12
HCV genotype (1b/2a/2b/other)	744/752/290/56
HCV RNA level (KIU/mL)	593 ± 540
Staging (non-LC/LC)	2,649/193
Blood glucose level (normal/prediabetes)	2,601/241
Fasting plasma glucose (mg/dL)	87 ± 24
Triglyceride (mg/dL)	166 ± 31
Total bilirubin (g/dL)	102 ± 56
AST (IU/L)	74 ± 63
ALT (IU/L)	116 ± 102
IFN monotherapy*/combination therapy†	2,417/425
Efficacy of treatment (SVR/non-SVR)	1,175/1,667
Follow-up period (years)	6.4 ± 5.0

Data are expressed as the number of patients or mean ± standard deviation.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis; SVR, sustained virological response.

*Outbreak of IFN monotherapy: recombinant IFN-α2a, 304 cases; recombinant IFN-α2b, 235 cases; natural IFN-bα, 1,355 cases; natural IFN-β, 522 cases; total dose of IFN = 598 ± 170 MU.

†Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, 175 cases; total dose of IFN = 537 ± 196 MU; total dose of ribavirin = 182 ± 69 g; pegylated IFN-α2b + ribavirin, 250 cases; total dose of pegylated IFN = 4.28 ± 1.17 mg; total dose of ribavirin = 232 ± 60 g.

IFN monotherapy or combination therapy with IFN and ribavirin. The sustained virological response (SVR) rate was 36.7% (886/2417) in IFN monotherapy and 68% (289/425) in IFN + ribavirin therapy. Thus, the number of patients with SVR was 1,175. The mean period after the termination of antiviral drugs was 6.4 years.

Incidence of T2DM in Patients with HCV. A total of 143 patients (102 men and 41 women) developed T2DM during a mean observation period of 6.4 years. Of these, 26 were SVR and 117 were non-SVR. The cumulative development rate of T2DM was determined to be 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years using the Kaplan-Meier method (Fig. 1). The factors associated with the incidence of T2DM in all 2,842 patients treated with IFN therapy are shown in Table 2.

Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was >50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). SVR causes a two-thirds reduction of development of T2DM in patients treated with IFN. In addition to SVR, age ≥50 years, liver cirrhosis, and pre-diabetes contribute to a high risk of developing diabetes. The cumulative development rates of T2DM based on difference of age, efficacy of the IFN therapy, histological diagnosis, and glucose level at the starting time of follow-up are shown in Fig. 2.

Fig. 3 shows the impact of reduction due to SVR on the incidence of T2DM in patients with ≥50 years, liver cirrhosis, or pre-diabetes. When patients with age ≥50 years, liver cirrhosis, and pre-diabetes have SVR after IFN therapy, SVR could statistically reduce the onset of T2DM compared with those without SVR.

Discussion

We have described the development incidence of diabetes after the termination of antiviral therapy in HCV-positive patients treated with IFN therapy in the present study. Diabetes has been reported in less than 0.08% of patients treated with IFN[19,20]; thus, to exclude diabetes originating from IFN-related side effects, patients without diabetes for 3 months after the termination of IFN were enrolled in the present study. The present study indicates that the annual incidence of T2DM for a prolonged follow-up after the termination of IFN therapy among HCV patients is 0.8% to 1.0%. The present study was limited by a retrospective cohort trial. We started the present study in 1991 based on the diabetes mellitus criteria published by Fajans.[21] However, after that, diabetes mellitus criteria were revised. We thus rechecked the diagnosis of T2DM based on the diabetes mellitus criteria of 2003 in patients seen prior to 2003.[16] Because of rechecking the diagnosis of T2DM on the basis of diabetes mellitus criteria in 2003, the present study was regarded as a retrospective cohort study. However, the patients were

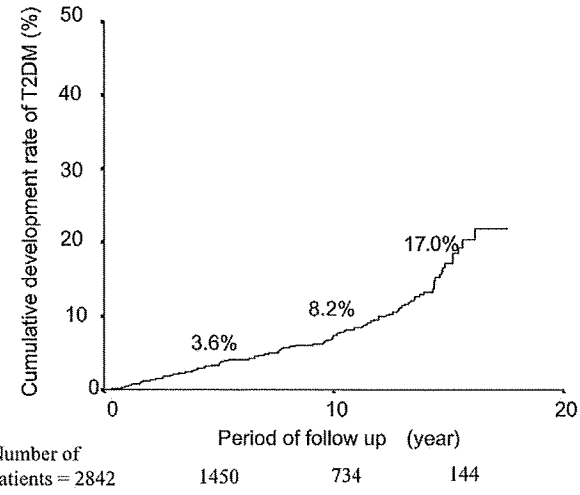

Fig. 1. Cumulative development rate of T2DM in patients treated with IFN.

Table 2. Predictive Factors for T2DM Development

Variables	Univariate Analysis		Cox Regression	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age, years (≥50/<50)	2.55 (1.74-3.73)	<0.001	2.10 (1.38-3.18)	<0.001
Sex (female/male)	0.84 (0.59-1.19)	0.318		
Body mass index (≥25/<25)	1.44 (0.98-2.08)	0.057		
HCV load (KIU/mL, ≥1,000/<1,000)	0.67 (0.43-1.03)	0.069		
Genotype (1/2)	0.73 (0.50-1.06)	0.098		
ALT (IU/L, ≥50/<50)	1.83 (1.14-2.94)	0.012		
Glucose level (prediabetes/normal)	2.25 (1.53-3.33)	<0.0001	2.19 (1.43-3.37)	<0.001
Triglyceride (mg/dL, ≥150/<150)	1.66 (0.93-2.98)	0.088		
Cholesterol (mg/dL, ≥220/<220)	1.56 (0.62-3.95)	0.346		
Histological diagnosis (LC/non-LC)	4.03 (2.55-6.36)	<0.0001	3.30 (2.06-5.28)	<0.001
Combination of ribavirin (−/+)	1.53 (0.99-2.38)	0.058		
Type of IFN (α/β)	0.88 (0.57-1.35)	0.882		
Total dose of IFN (MU, ≥500/<500)	0.91 (0.59-1.40)	0.672		
Efficacy (non-SVR/SVR)	2.73 (1.77-4.20)	<0.0001	2.78 (1.75-4.41)	<0.001

Data are expressed as the median (range).
Abbreviations: ALT, alanine aminotransferase; HR, hazard ratio; LC, liver cirrhosis.

prospectively followed. Another limitation of the study was that patients were treated with different types of antiviral therapy (IFN monotherapy or combination IFN + ribavirin therapy) for different duration (4 to 52 weeks). This heterogeneity makes it difficult to interpret the results of the study. On the other hand, the strength of the present study is the long-term follow-up in the large numbers of patients included.

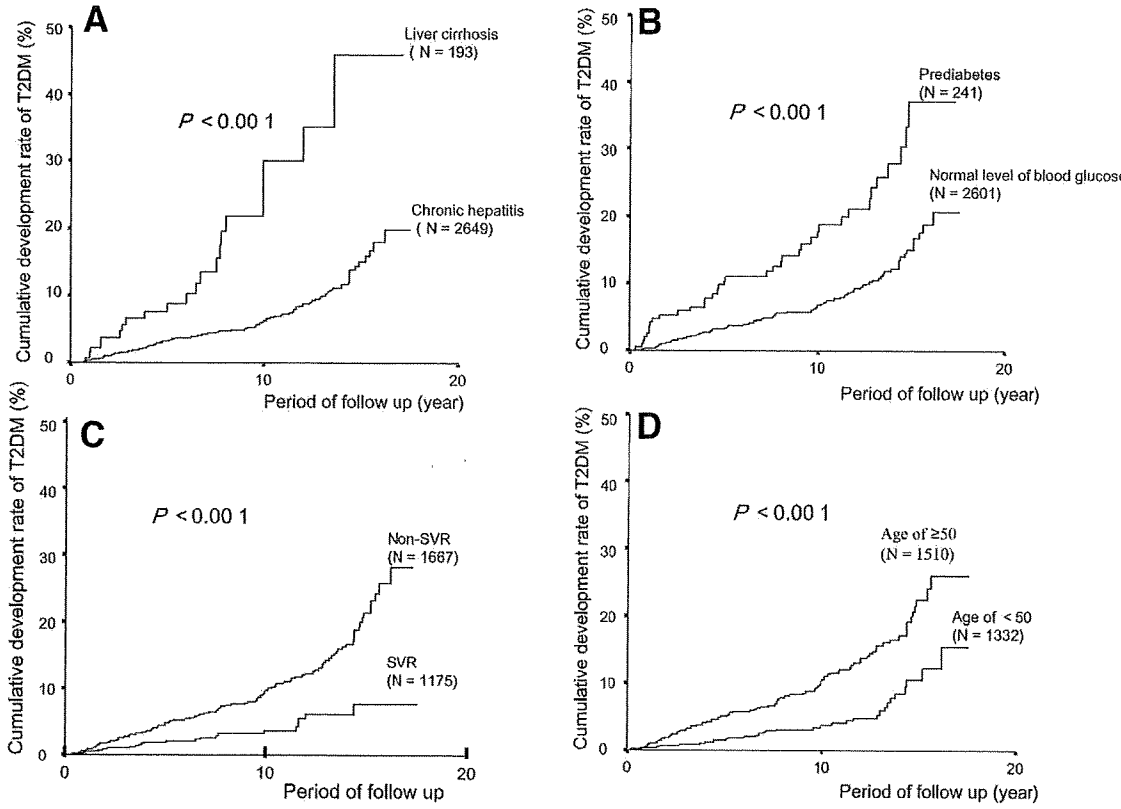

Fig. 2. Cumulative development rate of T2DM in patients treated with IFN. (A) Cumulative development rate of T2DM based on difference of hepatic fibrosis. (B) Cumulative development rate of T2DM based on the difference of glucose level. (C) Cumulative development rate of T2DM based on the difference of efficacy. (D) Cumulative development rate of T2DM based on the difference of age.

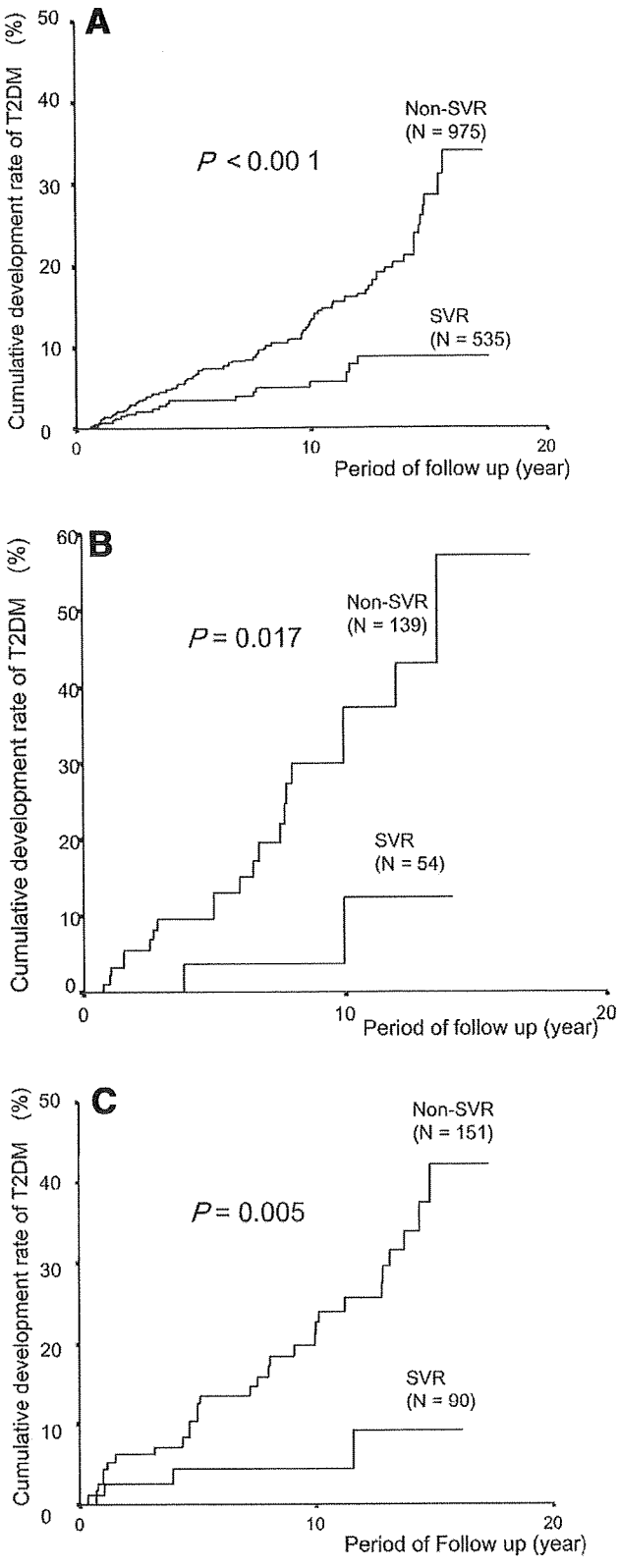

Fig. 3. Cumulative development rate of T2DM in patients with SVR or without SVR after IFN therapy. (A) Cumulative development rate of T2DM based on SVR or non-SVR in patients with age ≥50 years. (B) Cumulative development rate of T2DM based on SVR or non-SVR in patients with liver cirrhosis. (C) Cumulative development rate of T2DM based on the difference of SVR or non-SVR in patients with pre-diabetes.

The present study shows several findings with regard to development of T2DM after the termination of antivirus agents for HCV positive patients. First, the T2DM development rate in the non-SVR group was higher than that in the SVR group. The SVR caused a two-thirds reduction in the onset of T2DM in the course of posttreatment follow-up. That SVR reduced the onset of diabetes mellitus in HCV patients is in accordance with the data reported by Simó et al.[22] and Romero-Gómez et al.[23] Though the role of HCV in the pathogenesis of diabetes mellitus remains speculative, the following possible mechanisms have been reported: (1) patients with HCV have a tendency to attain insulin resistance[24]; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance and T2DM development[25]; and (3) SVR in HCV patients reduces insulin resistance and onset of the incidence of abnormal glucose value.[26] Thus, it is accepted that clearance of HCV reduces the onset of T2DM.

Second, in addition to persistence of HCV, the present study suggests that aging, histological progression, and pre-diabetes enhanced the onset of T2DM in patients with HCV infection. However, when HCV was eradicated even in patients with age ≥50 years, pre-diabetes, or liver cirrhosis, the cumulative development rate of T2DM decreased.

T2DM is increasing dramatically in many Asian nations, including Japan, over the past decades.[27] It is widely accepted that 7 to 8 million people are affected by diabetes mellitus in Japan. Approximately 8% to 10% of adults in Japan have T2DM. In general, T2DM is associated with a genetic predisposition, but it is also strongly influenced by lifestyle-related factors, such as eating habits and/or physical activity.[28-33] The risk factors associated with T2DM include family history, age, sex, obesity, smoking, and physical activity. T2DM occurred in elderly patients compared to young patients. Life expectancies are long in Japan; thus, in the near future, a large number of patients with HCV will be >60 years of age. Therefore, it is apparent that the incidence of T2DM will increase in HCV-positive patients.

T2DM is a serious, costly disease. Treatment for T2DM may prevent some of its devastating complications, but does not usually restore normoglycemia or eliminate all the adverse consequences.[28,29] Moreover, HCV patients with T2DM are at major risk for HCC.[34] On the efficacy of IFN therapy, it has been reported that T2DM reduces HCV eradication via combination IFN + ribavirin therapy.[26] Thus, it should be considered whether HCV-positive patients should be treated with antiviral drugs in the histological nonprogression stage and at a non-elderly age for prevention of T2DM onset. If

SVR obtained via antiviral therapy for HCV cannot only prevent progression to liver cirrhosis or HCC but also prevent the development of diabetes, the potential impact of IFN therapy is quite significant.

In conclusion, this retrospective study suggests that the annual incidence of T2DM among patients with HCV is 0.8% to 1.0%. Our results indicate that SVR causes a two-thirds reduction of T2DM development in HCV-positive patients treated with antiviral drugs.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C Patients with Genotype 1b and Low-virus Load

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Abstract

Objective The aim of this study was to evaluate the efficacy of combination therapy of peginterferon and ribavirin in patients infected with hepatitis C virus (HCV) genotype 1b and low virus load.

Methods Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of <100 KIU/mL at the initiation time of treatment. A total of 60 were enrolled in this retrospective cohort study. The treatment period of combination therapy was 39.8±16.1 weeks.

Results Of the 60 study patients, 47 had sustained virological response (SVR) by the intention to treat analysis. SVR occurred when serum HCV RNA was negative 8 weeks after the initiation of the treatment (p=0.004) and continuance of negative HCV RNA during treatment was ≥30 week (p=0.016). In rapid virological response, all of seven patients with continuance of negative HCV RNA 20 to 29 weeks during treatment had SVR. In early virological response nine of 10 patients with continuance of negative HCV RNA of 30 to 39 week during treatment had SVR.

Conclusion The duration of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA in patients with genotype 1b and low-virus load.

Key words: chronic hepatitis C, peginterferon, ribavirin, HCV genotype 1b, low virus load, duration of treatment

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Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-7). Hence, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C patients with high virus-load. Now, the selection of duration of treatment and optimum doses of combination therapy is an area of active investigation (8-16).

However, the dropout rates in patients treated with combination therapy was higher than those treated with IFN monotherapy (17, 18). On the other hand, some authors have reported that in half of the patients with a low virus load HCV RNA is eradicated by IFN monotherapy. Thus, for patients with a low virus load IFN monotherapy has been recommended as a first choice in Japan. However, there is also controversy over which patients should be treated with what agent and what regimen as a first choice for good prolonged prognosis in chronic hepatitis C patients with a low virus load. There is an ongoing need to refine treatment strategies in patients with a low virus load.

Thus, in the present study, we performed a retrospective study to examine the efficacy of combination therapy in pa-

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tients with genotype 1b and low virus load. Additionally, the relationship between attainment time of negativity of serum HCV RNA after the initiation of combination therapy and the continuance of negative HCV RNA in patients with genotype 1b and low HCV-RNA load of <100 KIU/mL were also evaluated.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of <100 KIU/mL before treatment; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 5) leukocytes >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; 6) follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions to each patient and informed consent was obtained from each patient.

From December 2004 to May 2007, 60 HCV patients were enrolled in this retrospective cohort study at the study hospital.

Patients were classified into three groups according to their response to combination therapy: rapid virological response (RVR), defined as undetectable HCV RNA at week 4 after the initiation of combination therapy; early virological response (EVR), defined as undetectable HCV RNA at week, 5 to 12 of combination therapy; and late virological response (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy. A SVR was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (19).

Next, predictors of SVR in patients with undetectable HCV RNA in serum during treatment were assessed by the multiple logistic regression analysis. Finally, SVR rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy were examined.

Combination therapy of pegylated-IFN and ribavirin

For the treatment regimen, the peginterferon (Peg-intron, Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol, Schering-Plough) were given at the dose described based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.4 μg/kg (range, 1.3-1.7 μg/kg) subcutaneously each week and oral ribavirin at a median dose of 12.0 mg/kg (range, 9.9-14.9 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60 μg for ≤40 kg, 80 μg for >40 kg and ≤60 kg, 100 μg for >60 kg and ≤80 kg, 120 μg for >80 kg and ≤100 kg, and 150 μg for >100 kg). The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1,000 mg for >80 kg). The regimen or treatment period was decided by the physician. A total of 39 patients were treated with a 48-week regimen and 16 patients were given combination therapy for a 24-week regimen. Treatment for the remaining five patients was discontinued because of treatment-related side effects within 26 weeks after the initiation of combination therapy.

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analyzed. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (20). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (21). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (19). Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

Liver histology before IFN therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with Hematoxylin and Eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were diagnosed according to the system of Desmet et al (22).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, body mass index, liver staging, a history of interferon therapy, a history of HCV load of ≥100 KIU/mL, HCV RNA level, biochemical factors (AST, ALT), platelet count, HCV RNA 4, 8, 12 week after the initiation of IFN therapy, continuous negative period of HCV RNA during IFN therapy and period of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. **Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients**

	Total	Response			
		RVR	EVR	LVR	p
Patients, n†	60	18	31	6	
Sex, male (%)†	42 (70%)	15 (83%)	23 (74%)	2 (33%)	0.063
Age (yrs)‡	51.9±10.1	50.8±9.3	52.1±10.8	53.9±10.9	0.713
BMI‡	21.9±3.1	23.2±3.6	21.2±2.9	21.9±2.3	0.177
A history of IFN†, (%)	28 (47%)	7 (39%)	13 (42%)	4 (67%)	0.085
History of maximum HCV RNA level of >100KIU/mL (+/-)†	43/17	13/5	21/10	4/2	0.498
HCV RNA(KIU/mL)§	52 (<5-99)	43(8-93)	58(<5-99)	72(21-90)	0.498
AST (IU/L)‡	58±32	61±47	56±24	51±18	0.480
ALT (IU/L)‡	73±52	80 ± 62	69 ± 37	82±59	0.456
FPG(mg/dL)‡	93.1±13.6	93.2±13.0	92.5±12.2	97.5±24.6	0.182
Triglyceride (mg/dL)‡	92.5±35.2	94.5±27.8	90.6±42.9	93.9±30.2	0.887
Platelet(10^4/mm^3)‡	18.7±6.3	20.9±4.7	19.6±5.9	13.7±5.6	0.106
Fibrosis staging† (Non-LC/LC)	54/6	18/0	26/5	5/1	0.067

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early virological response; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon; LC, liver cirrhosis; LVR, late virological response; RVR, rapid virological response

Normal reference ranges 6-50 IU/L for ALT, 11-38 IU/L for AST,

†Data expressed as number of patients (percentage)

‡Data expressed as mean ± standard deviation

§Data expressed as median (range)

Result

Clinical characteristics of the patients

A total of 60 patients were enrolled on present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =52 years, male/female =42/18, and median (range) HCV-RNA=52 (<5-99) KIU/mL. Two of the patients treated with 48-regimen and three out of five discontinued combination therapy due to side effects had positive HCV RNA during combination therapy. Patients with negativity of serum HCV RNA during combination therapy were classified into three groups according to the difference of response: RVR (n=18), EVR (n=31), and LVR (n=6). There were no significant differences in several factors in three groups as shown in Table 1.

Safety and tolerance of IFN

Of the 60 patients included in this study, five discontinued combination therapy because of IFN-related adverse events: one patient each with thrombocytopenia, general fatigue, psychiatric disorder, poor appetite, and cholecystitis. The onset of IFN-related side effects ranged from one to 11 weeks after initiation of IFN therapy. These side effects in five patients disappeared one month after cessation of IFN therapy.

Next, ten of the remaining 55 patients had dose reduction of interferon and/or ribavirin because of side effects: 5 cases of thrombocytopenia, 3 cases of general fatigue, and 2 cases of poor appetite. The onset of dose reduction due to IFN-related side effects ranged from 1 to 26 weeks after initiation of IFN therapy.

Efficacy of treatment

Out of 60 patients enrolled in the present study, 47 patients (78.3%) had SVR by the intention-to-treat analysis. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The SVR was significantly associated with the attainment time of negativity of serum HCV RNA and continuance period of negative HCV RNA. Multivariate analysis indicated that non-relapse occurred when serum HCV RNA at week 8 was negative (p=0.004) and continuance of negative HCV RNA during treatment was ≥30 weeks (p=0.016) (Table 3).

Table 2. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR (n=47)	Non-SVR (n=13)	p value
Age (years old) †	52.2 ± 10.1	53.4 ± 8.9	0.346
Sex (male/female) †	35/12	8/5	0.488
BMI	21.8 ± 3.2	22.2 ± 3.0	0.732
Liver staging (non-LC /LC)	42/5	12/1	1.00
a history of interferon (+/-)	22/25	6/7	1.00
a history of HCV load of ≥100KIU/mL (+/-)	31/16	7/6	0.520
HCV-load (KIU/mL) †	58 (<5-99)	46 (6-93)	0.375
AST (IU/L) †	49 ± 34	54 ± 22	0.102
ALT (IU/L) †	70 ± 55	83 ± 39	0.082
Platelet (10^4/mm^3) †	19.0 ± 6.5	17.6 ± 3.8	0.230
HCV RNA (-) 4W	17/46 (37%)	0/10 (0%)	0.023
HCV RNA (-) 8W	35/46 (76%)	1/10 (10%)	0.002
HCV RNA (-) 12W	44/46 (96%)	3/10 (30%)	<0.001
Continuous negative period (week)	34.9 ± 11.6	10.4 ± 12.1	<0.001
Period of IFN therapy (week)	41.6 ± 12.6	28.8 ± 19.6	<0.001

Data are number of patients, median (range) or mean±standard deviation. p value calculated by the Mann-Whitney U test

†ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virologic response

Table 3. Multivariate Analyses Identifying Predictors of SVR

Factor	Category	Odds ratio	95% Confidence interval	p value
HCV RNA week 8*	+ /-	1/69.1	4.0-1201.4	0.004
Continuance period of negative HCV RNA during treatment (week)	<30 / ≥30	1/34.5	1.9-500.0	0.016

HCV, hepatitis C virus

*HCV RNA at week 8 after the initiation of treatment

SVR based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA

All fifty-five patients with negativity of HCV RNA after the initiation of combination therapy had continuance of negative HCV RNA during combination therapy. SVR rate based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA during combination therapy are shown in Table 4. In the RVR group, all of seven patients with continuance of negative HCV RNA of 20 to 29 week during treatment had SVR. In the EVR group, patients with continuance of 30 to 39 week during treatment had SVR of ≥90%. In the LVR group, patients with continuance of 30 to 39 week during treatment had SVR of 50%.

Discussion

We have described the efficacy of combination therapy of peginterferon and ribavirin in patients infected with HCV genotype 1b and low virus load. The present study was limited to patients with genotype 1 and HCV-load of <100 KIU/mL. Another limitation is that the present study was not a randomized controlled study; thus, the treatment period was varied. Moreover, half of the patients had a history of IFN monotherapy and two-thirds of the patients had a history of maximum HCV RNA level of >100 KIU/mL. Clinical backgrounds of the enrolled patients were varied.

However, several findings from the present study have direct implications for combination therapy for chronic hepatitis C in the future. First, SVR was primarily associated with attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA. The period of combination

Table 4. SVR Based on the Attainment Time of Negative HCV RNA and Continuance Period of Negative HCV RNA during Combination Therapy

Response*	Continuance period of negative HCV RNA (week)					Total
	<10	10-19	20-29	30-39	40-49	
RVR	100% (1/1)	ND	100% (7/7)	ND	100% (10/10)	100% (18/18)
EVR	ND	63% (5/8)	ND	90% (9/10)	100% (13/13)	87% (27/31)
LVR	0% (0/2)	ND	ND	50% (2/4)	ND	33% (2/6)
Total	33% (1/3)	63% (5/8)	100% (7/7)	79% (11/14)	100% (23/23)	85% (47/55)

EVR, early virological response; HCV, hepatitis C virus; LVR, late virological response; ND, not done; RVR, rapid virological response

*Response of HCV RNA means attainment time of negativity of serum HCV RNA after the initiation of combination therapy

therapy is statistically significant by univariate analysis. However, multivariate analysis showed that early undetectable HCV RNA and prolonged negativity of serum HCV RNA during treatment were associated with the SVR. In the RVR group, all seven patients with continuance of negative HCV RNA for 20 to 29 week during treatment had SVR. This result suggests that a short course regimen of 24 or < 24 week in combination therapy may be suitable for patients who have genotype 1, low virus load, and RVR. Earlier studies have reported higher SVR rates in patients with undetectable HCV RNA at week 4 compared to those with detectable HCV RNA (7-9, 23). Jensen et al (8) has reported that patients with RVR should be treated for a short course regimen. On the contrary, it may be necessary to treat patients without RVR with a long course regimen. The present results coincided closely with these earlier results.

Secondly, in the EVR group, patients with continuance of negative HCV RNA of ≥30 weeks during treatment had SVR of ≥90%. However, one-third of the patients with continuance of negative HCV RNA of 10 to19 weeks relapsed after the termination of therapy. This result suggests that patient with EVR should be given combination therapy for a year. Third, in LVR group, half of the patients with continuance of negative HCV RNA of 30 to 39 weeks during treatment had SVR. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period of ≥ one year to obtain a high rate of SVR.

A previous study (24) indicates that the suitable treatment period of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA in patients with genotype 1b and a high virus load of ≥100 KIU/mL. Similarly, the present study suggests that in patients with genotype 1b and low-virus load, the period of combination therapy should be determined based on the attainment time of negativity of serum HCV RNA.

It is desirable to expose patients with chronic hepatitis C to the shortest duration of treatment possible to reduce the likelihood of adverse events and minimize costs. Long-term treatment can be associated with serious side effects and is costly. HCV treatment of combination therapy is expensive; a 24-week treatment course costs approximately 20,000 dollars. Thus, the results of this study underscore the importance of changing the duration of treatment based on the difference of attainment time of negative HCV RNA. To attain SVR rate of ≥90% in patients with undetectable HCV RNA and continuance of negative HCV RNA during treatment, it is desirable to give a short course regimen of ≤20-29 weeks in the RVR group, 30-39 week in the EVR group. Moreover, in LVR, prolonged combination therapy regimen of >48 weeks may be recommended.

In conclusion, the period of combination therapy for chronic hepatitis C should be determined based on attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA in patients with genotype 1b and low-virus load.

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Multivariate analysis of risk factors for the development of type 2 diabetes in nonalcoholic fatty liver disease

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Abstract
Purpose Diabetes is present in patients with nonalcoholic fatty liver disease (NAFLD). The aim of this retrospective cohort study was to assess the cumulative development of type 2 diabetes and predictive factors for its development in Japanese patients with NAFLD.
Methods A total of 6003 NAFLD patients diagnosed by ultrasonography were enrolled. The mean follow-up period was 4.9 years. An overnight (12 h) fasting blood sample or a casual blood sample was taken for routine analyses during follow up. The primary outcome was the development of type 2 diabetes. Evaluation was performed by using the Kaplan–Meier method and Cox proportional hazards analysis.
Results Of the 6003 NAFLD patients, 411 patients developed type 2 diabetes. The cumulative development rate of type 2 diabetes was 6.8% at the 5th year and 17.7% at the 10th year. Multivariate Cox proportional hazards analysis showed that type 2 diabetes development in patients with NAFLD occurred when patients had prediabetes status (hazard ratio 6.39; 95% confidence interval 5.00–8.18; $P < 0.001$), mean serum gamma-glutamyltransferase (GGT) level of more than 109 IU/l (hazard ratio 1.60; 95% confidence interval 1.22–2. 02; $P < 0.001$), mean serum triglyceride (TG) level of more than 150 mg/l (hazard ratio 1.28; 95% confidence interval 1.05–1.55; $P = 0.020$), and physical activity of less than 60 min per week (hazard ratio 1.60; 95% confidence interval 1.25–2.00; $P < 0.001$).
Conclusions The improvement of prediabetes status and physical activity, and the normalization of mean GGT and TG levels during follow up are important to prevent the development of T2DM in patients with NAFLD.

Keywords Nonalcoholic fatty liver disease · Type 2 diabetes mellitus · Cohort study

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in the western world [1–4]. Recently, it has developed rapidly in many Asian nations [5, 6]. NAFLD is considered to be the liver component of metabolic syndrome. It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and type 2 diabetes mellitus (T2DM) [4, 7–12] NAFLD often causes cardiovascular disease and stroke. Thus, NAFLD is emerging as a new significant health problem in many countries.

Although there is growing evidence to support the concept that NAFLD is a risk factor for developing T2DM, there have been few interventional studies to confirm this issue [13]. This issue needs to be confirmed with long-term follow up of patients with a high risk of developing diabetes. Thus, prospective studies including metabolic evaluations are clearly needed to clarify these issues.

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With this background in mind, a cohort study was initiated to investigate the cumulative incidence of and risk factors for T2DM after prolonged follow up in patients with NAFLD. The strengths of the current study lie in the large number of patients included and the long-term follow up of the patients.

Methods

Patients

The number of patients who were diagnosed with fatty liver by ultrasonography (US) [14] between January 1997 and December 2007 at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 10210. Of these, 6403 Japanese patients satisfied the following enrollment criteria; (1) no evidence of diabetes mellitus determined by plasma glucose and hemoglobin A1c (HbA1c), i.e., plasma glucose concentration of less than 126 mg per dl (6.9 mmol per l) in the fasting state, or less than 200 mg per dl (11.0 mmol per l) in the casual state and/or 2 h after a 75-g oral glucose load; HbA1c less than 5.8%; (2) current and past daily alcohol intake of less than 40 g/week; (3) negativity for hepatitis B surface antigen, hepatitis C virus (HCV) antibodies, antinuclear antibodies, and antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay, or spot hybridization; (4) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatoid arthritis; (5) no evidence of hepatocellular carcinoma nodules as shown by US and/or computed tomography (CT). Patients with any of the following criteria were excluded from the study: (1) those who were taking medicines known to alter glucose tolerance, (2) those who had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial, and (3) those who had findings suggestive of other chronic liver disease. Patients were classified as having normal glucose (normal glucose group) or prediabetes (prediabetes group) based on their fasting plasma glucose (FPG), casual plasma glucose, or 2-h plasma glucose, as follows. The normal glucose group had an FPG of less than 100 mg/dl, casual plasma glucose of less than 140 mg/dl, and/or 2-h plasma glucose of less than 140 mg/dl and the prediabetes group had an FPG of 100–125 mg/dl, casual plasma glucose of 140–200 mg/dl, and/or 2-h plasma glucose of 140–200 mg/dl [15].

Next, we assessed predictive factors for T2DM in patients with NAFLD. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by the Institutional Review Board of our hospital.

Medical evaluation

Diagnosis of fatty liver was based on the presence of an ultrasonographic pattern consistent with bright liver (brightness and posterior attenuation) with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins. US was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka, Tokyo, Japan; Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured with the patient in light clothing and without shoes, to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and the body mass index (BMI) was calculated as weight (in kg)/height (in m^2).

All the patients were interviewed at the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and health-related habits, including questions on alcohol intake and physical activity per week.

Follow up

The initiation of follow up was the day of the first diagnosis of NAFLD, determined by using abdominal US. After that, patients were followed up monthly to 6-monthly at the Toranomon hospital. Physical examination and biochemical tests were conducted at each examination, together with regular checkups, using abdominal CT or US imaging in each patient. An overnight (12 h) fasting blood sample or a casual blood sample was taken for routine analyses. These analyses included transaminase activity, gamma-glutamyltransferase (GGT), total cholesterol, and triglyceride (TG).

The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association [15]. That is, the criteria for the diagnosis of diabetes mellitus included: (a) casual plasma glucose 200 mg/dl or more; (b) FPG 126 mg/dl or more; (c) 2-h post-glucose (oral glucose tolerance test) 200 mg/dl or more. Five hundred and two patients were lost to follow up. Because the appearance of T2DM was not identified in these 502 patients, they were considered as censored data in the statistical analysis [16]. Patients treated with anti-insulin resistance agents were regarded as withdrawals at the time of starting the anti-insulin resistance treatment.

Statistical analysis

The cumulative incidence rate of T2DM was calculated from the first time NAFLD was confirmed by US to the appearance of T2DM, using the Kaplan–Meier method.

Table 1 Characteristics of subjects enrolled

Characteristic	
N	6003
Sex (male/female)	5298/705
Age (years)	48.8 ± 8.6
Height (cm)	167.8 ± 7.3
Body weight (kg)	70.6 ± 9.7
BMI	25.1 ± 2.6
Albumin (g/dl)	4.2 ± 0.2
Blood glucose level (normal/prediabetes)	3517/2486
FPG (mg/dl)	98.9 ± 9.3
Triglyceride (mg/dl)	160.8 ± 105.4
Total cholesterol (mg/dl)	210.3 ± 32.2
HDL cholesterol (mg/dl)	47.7 ± 11.9
AST (IU/L)	28.7 ± 14.5
ALT (IU/L)	36.4 ± 25.1
GGT (IU/L)	73.5 ± 79.7
Hemoglobin (g/dl)	15.0 ± 1.1
Platelet count ($\times 10^4/mm^3$)	23.0 ± 4.8
Follow-up period (years)	4.9 ± 3.0

Data are numbers of patients or mean ± SD

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *FPG* fasting plasma glucose, *GGT* gamma-glutamyltransferase

Differences in the development of T2DM were tested using the log-rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following 11 variables were analyzed as potential covariates for the incidence of T2DM: age, sex, glucose level (normal or prediabetes), BMI, albumin level, alanine aminotransferase (ALT) level, GGT level, TG level, and total cholesterol level at the initiation of follow up at our hospital; and physical activity and mean serum levels of ALT, GGT, and TG during follow up. A P value of less than 0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL, USA).

Results

Patients' characteristics

Table 1 shows the characteristics of the 6003 patients diagnosed with NAFLD in the present study. The mean age was 48.8 years, and most patients were male (88.3%). The prediabetes rate at the starting time of follow up was 41.4% (2486/6003). The rates of elevated mean GGT and TG during follow up were 17.4% (1046/6003) and 42.7% (2564/6003), respectively. The mean follow-up period was 4.9 years.

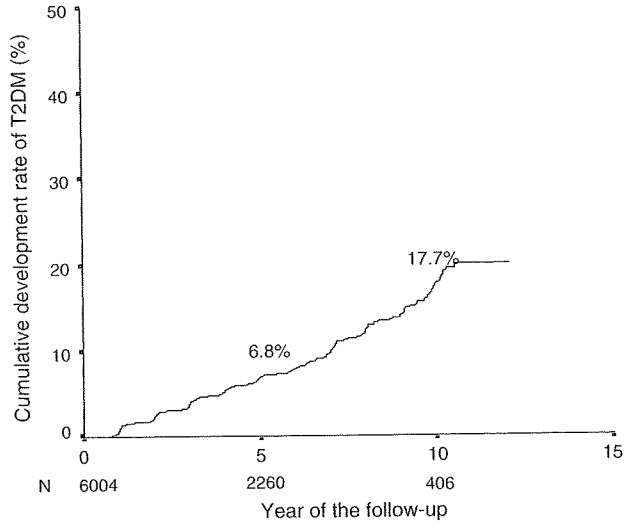

Fig. 1 Cumulative development rate of type 2 diabetes mellitus (*T2DM*) in 6003 patients with nonalcoholic fatty liver disease (NAFLD)

Incidence of T2DM in patients with NAFLD

Of the 6003 NAFLD patients, 411 patients developed T2DM. The cumulative development rates of T2DM in the 6003 patients with NAFLD were 6.8% at the 5th year and 17.7% at the 10th year, determined by the Kaplan–Meier method (Fig. 1). The factors associated with the incidence of T2DM are shown in Table 2. Multivariate Cox proportional hazards analysis showed that the development of T2DM in patients with NAFLD occurred when the patient had prediabetes (hazard ratio 6.39; 95% confidence interval 5.00–8.18; $P < 0.001$), mean serum GGT level of more than 109 IU/l (hazard ratio 1.60; 95% confidence interval 1.22–2. 02; $P < 0.001$), mean serum TG level of more than 150 mg/l (hazard ratio 1.28; 95% confidence interval 1.05–1.55; $P = 0.020$), and physical activity of less than 60 min per week (hazard ratio 1.60; 95% confidence interval 1.25–2.00; $P < 0.001$).

Prediabetes enhanced the development of T2DM by about nine point five times compared to the normal glucose level. In addition to prediabetes, the three factors of physical activity of less than 60 min per week, and elevated mean GGT and/or TG levels during follow up were high risk factors for developing diabetes. The cumulative development rates of T2DM based on differences of glucose levels at the initiation of follow up and differences in mean GGT and mean TG between levels during follow up, as well as such differences of physical activity, are shown in Fig. 2. Prediabetes was the strongest predictor compared to physical activity, mean GGT, and mean TG.

Table 2 Predictive factors for T2DM development

Variables	Univariate analysis		Cox regression	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age[a] (years, ≥50/<50)	1.21 (0.99–1.48)	0.063		
Gender[a] (F/M)	0.77 (0.54–1.09)	0.144		
BMI[a] (≥25/<25)	1.24 (1.02–1.50)	0.030		
ALT[a] (IU/L, ≥36/<36)	1.22 (1.00–1.49)	0.048		
GGT[a] (IU/L, ≥109/<109)	1.42 (1.13–1.80)	0.003		
Glucose level[a] (prediabetes/normal)	9.97 (7.55–13.17)	<0.001	6.39 (5.00–8.18)	<0.001
Triglyceride[a] (mg/dl, ≥150/<150)	1.19 (0.97–1.47)	0.095		
Total cholesterol[a] (mg/dl, ≥220/<220)	0.99 (0.81–1.21)	0.890		
Albumin[a] (g/dl, <3.9/≥3.9)	1.12 (0.85–1.46)	0.428		
Mean ALT[b] (IU/L, ≥36/<36)	1.62 (1.30–2.02)	<0.001		
Mean GGT[b] (IU/L, ≥109/<109)	2.05 (1.65–2.52)	<0.001	1.60 (1.22–2.02)	<0.001
Mean triglyceride[b] (mg/dl, ≥150/<150)	1.52 (1.25–1.84)	<0.001	1.28 (1.05–1.55)	0.020
Physical activity[c] (±)	1.95 (1.53–2.48)	<0.001	1.60 (1.25–2.00)	<0.001

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *HR* hazard ratio, *GGT* gamma-glutamyltransferase

[a] Data at the initiation of follow-up

[b] Data during follow up

[c] −, Physical activity of less than 60 min per week during follow up; +, physical activity of 60 min or more per week during follow up

Incidence of T2DM in NAFLD patients with and without prediabetes

As noted above, prediabetes was an important factor in enhancing the development of T2DM. Next, we assessed whether the three factors of physical activity, mean GGT, and mean TG during follow up were important in reducing the development of T2DM in NAFLD patients with prediabetes. We classified all the patients into three risk groups based on the combination of the three factors of physical activity, mean GGT, and mean TG during follow up. The low-risk group was defined as patients with physical activity of 60 min or more per week; normal mean GGT, at 109 IU/l or less; and normal mean TG, at less than 150 mg/dl during follow up. The high-risk group was defined as patients with physical activity of less than 60 min per week; abnormal mean GGT, at more than 109 IU/l; and abnormal mean TG, at 150 mg/dl or more during follow up. The intermediate-risk group was defined as patients excluded from the low- and high-risk groups. In the patients with prediabetes, the low-risk group showed a significant reduction in the development of T2DM compared with the high-risk and intermediate-risk group (Fig. 3a). In the patients with normal glucose levels, the development rate of T2DM was significantly different among the three groups (Fig. 3b).

Discussion

We have described the incidence of the development of diabetes in NAFLD patients in the present study. Our present study indicated that the annual incidence of T2DM during prolonged follow up in NAFLD patients was about 1.7%. The present study was limited by being a retrospective cohort trial. Another limitation of the study was that patients were treated with different types of exercise and diet. Moreover, although NAFLD can be categorized into simple steatosis and steatohepatitis, in the present study the condition was evaluated without histological differentiation between simple steatosis and steatohepatitis. This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are that it was a long-term follow up with large numbers of patients included.

The present study showed several findings with regard to the development of T2DM in NAFLD patients. First, patients with NAFLD were at high risk of developing of T2DM compared with the risk in patients with HCV infection. Our previous study showed that the annual incidence of T2DM among patients with HCV was 0.8–1.0% [17]. On the other hand, the annual incidence of T2DM among patients with NAFLD was about 1.7% in the present study. Several reports have shown that nonalcoholic steatohepatitis (NASH) exerts more severe insulin resistance, which closely correlates with T2DM, than simple steatosis [18–22]. In the present study, NAFLD patients were evaluated without discriminating between NASH and simple steatosis by histological examination. However, if the disease in NAFLD patients could be discriminated by histological examination, we predict that patients with NASH would have a high annual incidence compared to those with simple steatosis.

Second, prediabetes was the most important factor that enhanced the development of T2DM in patients with NAFLD. Prediabetes enhanced the development of T2DM by about 6.4 times compared to that in patients with a normal glucose level. This result shows that NAFLD

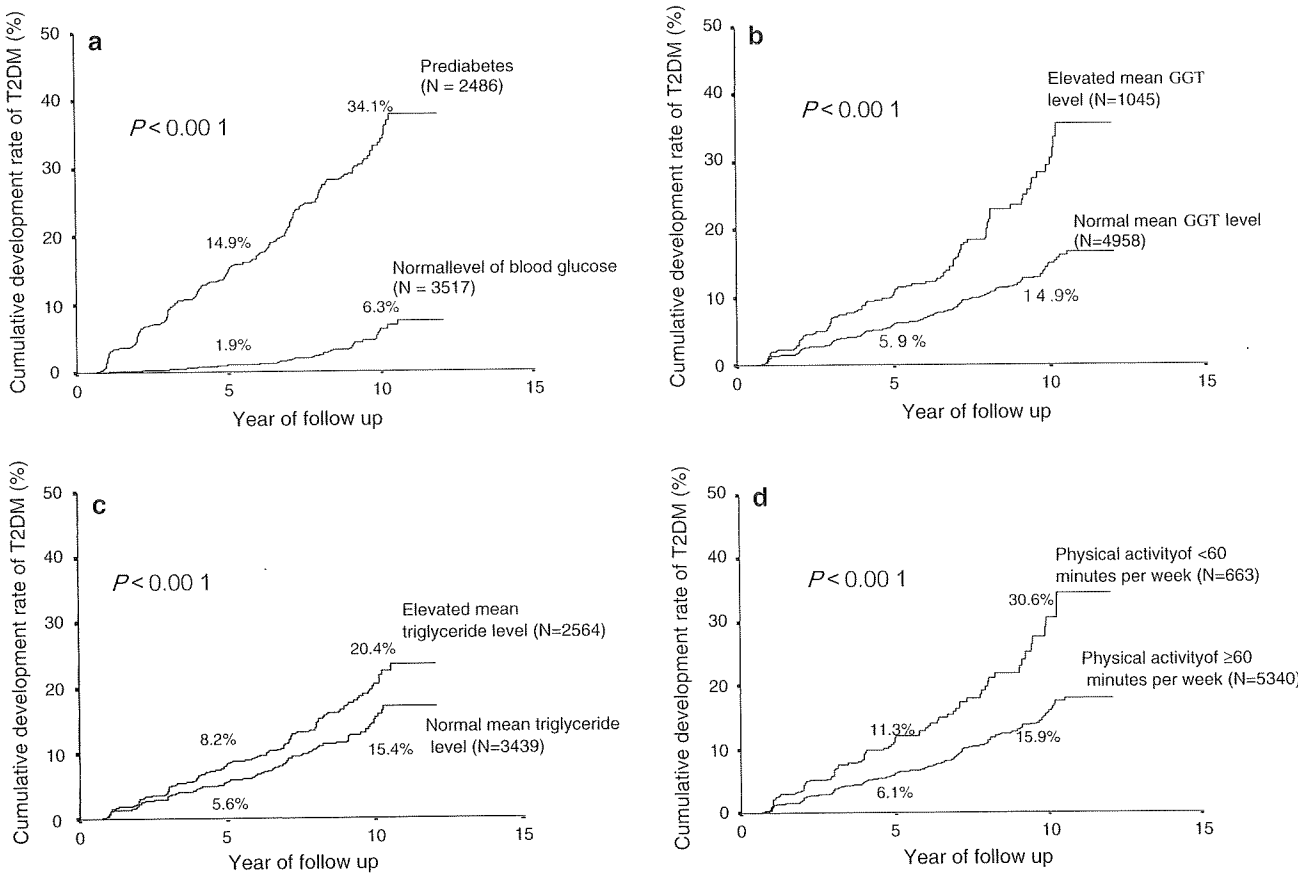

Fig. 2 Cumulative development rate of T2DM in NAFLD patients. **a** Cumulative development rate of T2DM based on differences between glucose levels at the initiation of follow up and during follow up. **b** Cumulative development rate of T2DM based on the differences between mean gamma-glutamyltransferase (*GGT*) levels at the initiation of follow up and during follow up. **c** Cumulative development rate of T2DM based on the differences between mean triglyceride levels at the initiation of follow up and during follow up **d** Cumulative development rate of T2DM based on the differences between physical activity at the initiation of follow up and during follow up

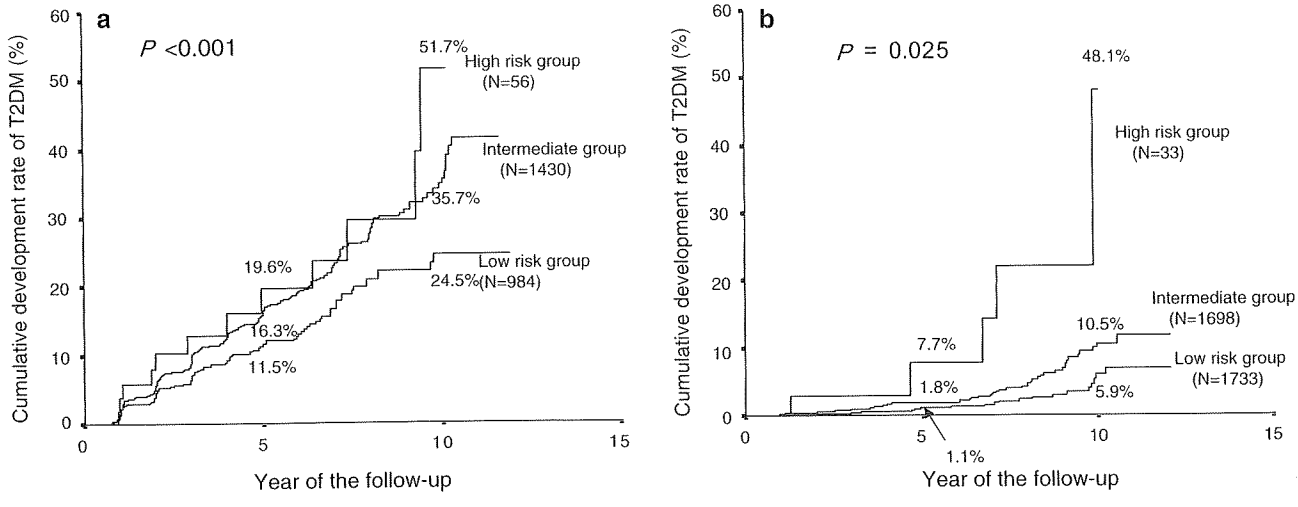

Fig. 3 **a** Cumulative development rate of T2DM in NAFLD patients with prediabetes based on risk stratification according to differences in physical activity, mean levels of GGT, and mean levels of triglyceride during follow up. **b** Cumulative development rate of T2DM in NAFLD patients with normal glucose level based on risk stratification according to differences in physical activity, mean levels of GGT, and mean levels of triglyceride during follow up

patients with prediabetes should be carefully followed to reduce the development of T2DM. The next problem is that the prediabetes rate in patients with NAFLD was high. The present study showed that the prediabetes rate in NAFLD patients without T2DM was about 40% at the time of the initiation of follow up.

Third, in addition to prediabetes, physical activity of less than 60 min per week, and elevation of mean GGT and TG during follow up enhanced the development of T2DM in patients with NAFLD. The hazard ratio for these factors was weaker than that for prediabetes status. However, physical activity of 60 min or more per week, and normalization of mean GGT and TG during follow up reduced the development of T2DM even in NAFLD patients with prediabetes. The finding that physical activity reduced the development of T2DM is in accordance with the data reported by the Diabetes Prevention Program Research Group [22]. About the GGT level, Fraser et al. [23] have shown that GGT is associated with T2DM and/or insulin resistance by a metaanalysis. Normalization of mean GGT and TG during follow up is speculated to relieve the degree of steatosis. Thus, regarding the daily management of patients with NAFLD, physicians should pay attention to the onset and early diagnosis of T2DM. When NAFLD occurs, improvement of prediabetes status and physical activity, and normalization of mean GGT and TG during follow up is important to prevent the onset of T2DM.

There was not a significant difference between male and female patients in the development of T2DM in the present study. Serum ALT and GGT levels are usually higher in males than in females. In the present study, the serum ALT at the initiation of follow up was 37.6 ± 25.1 IU/l in males and 27.3 ± 33.7 IU/l in females. The serum GGT at the initiation of follow up was 78.4 ± 82.8 IU/l in males and 36.2 ± 30 IU/l in females. However, age at the initiation of follow up was 48.3 ± 8.4 years in males and 53.1 ± 8.7 years in females. The results show that the development of T2DM in males was the same as that in females due to their young age, in spite of the elevation of serum ALT and GGT.

The prevalence of T2DM is increasing dramatically in the United States, and increases in many newly developed and developing countries in Asia, including Japan, have been ever greater over the past decades [24]. Now, approximately 8–10% of adults in Japan have T2DM. T2DM is a serious, costly disease. Treatment of T2DM may prevent some of its devastating complications, but does not usually restore normoglycemia or eliminate all the adverse consequences [25]. In general, T2DM is associated with a genetic predisposition, but it is also strongly influenced by lifestyle-related factors, such as eating habits and/or physical activity [22, 24, 25]. The risk factors associated with T2DM include family history, age, gender, obesity, smoking, HCV infection, visceral fat, and physical activity. The present study shows that the four factors of glucose level, physical activity, mean GGT, and mean TG are associated with the development of T2DM in patients with NAFLD.

In conclusion, our retrospective study suggests that the annual incidence of T2DM among patients with NAFLD was about 1.7%. The improvement of prediabetes status and physical activity, and the normalization of mean GGT and TG during follow up are important to prevent the development of T2DM in patients with NAFLD.

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Conflict of interest statement The authors declare that there is no conflict of interest associated with this study.

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CLINICAL RESEARCH STUDY

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Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C

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ABSTRACT

BACKGROUND: The significance of antiviral therapy for elderly patients with chronic hepatitis C virus (HCV) infection has not been elucidated.

PATIENTS AND METHODS: Among 5645 patients with HCV-related chronic liver disease, the prognosis of 1917 elderly patients aged 60 years or more was analyzed. A total of 454 patients underwent interferon (IFN) therapy. By using multivariate analysis, carcinogenesis and survival were analyzed according to initial findings.

RESULTS: At 10 and 15 years, cumulative survivals in untreated elderly patients were 90.7% and 72.7% in the high platelet ($\geq$150,000/mm^3) group, 78.6% and 47.8% in the intermediate (100,000-149,000/mm^3) group, and 52.5% and 25.0% in the low platelet group (<100,000/mm^3), respectively. At 5 and 10 years, hepatocarcinogenesis rates in the intermediate and low platelet groups were 10.9% and 21.6% in the IFN group (N = 217) and 19.5% and 43.0% in the untreated group (N = 459), respectively ($P = .0005$). IFN independently decreased carcinogenesis risk with a hazard ratio of 0.56 ($P = .035$). In the high platelet group, 5- and 10-year carcinogenesis rates were 3.7% and 8.3% in the IFN-treated group (N = 228) and 5.1% and 14.0% in the untreated group (N = 585), respectively ($P = .69$). IFN treatment significantly increased cumulative survivals in the lower platelet subgroup ($P = .0001$) but did not affect the higher platelet subgroup ($P = .08$). IFN was independently associated with a longer survival in the lower platelet subgroup (hazard ratio 2.33, $P = .005$).

CONCLUSION: In elderly patients with chronic HCV, IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival.

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KEYWORDS: Chronic hepatitis C virus; Elderly; Hepatocellular carcinogenesis; Interferon; Survival

Hepatitis C virus (HCV) is one of the principal causes of hepatocellular carcinoma and often causes high morbidity and mortality in many countries.[1-5] Because interferon (IFN) has antiviral, antifibrotic, and anti-inflammatory actions, it is still a main arm in the treatment of chronic HCV.[6,7] Many authors have demonstrated that IFN prevents hepatocarcinogenesis and eventually prolongs the survival period of patients.[8-13] Radical eradication of HCV by IFN depends on viral load, HCV subtype, certain mutations of hepatitis virus gene, liver histology, modes of IFN administration, and various host factors, including a patient's age.[14-16] When a significant side effect occurs during IFN therapy, cessation or early withdrawal of the therapy often failed to attain a successful result. Early withdrawal and treatment failure are likely more common in elderly patients and patients with an advanced stage of liver disease.

The number and rate of elderly patients with HCV-positive chronic hepatitis are currently increasing in the United States and Japan[17-19] because of a significant decrease of new blood-borne HCV infections and an aging

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