

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- α 2a, 304 cases; recombinant IFN- α 2b, 235 cases; natural IFN- α , 1,355 cases; natural IFN- β , 522 cases; total dose of IFN = 598 ± 170 MU.

†Outbreak of combination therapy: recombinant IFN- α 2b + ribavirin, 175 cases; total dose of IFN = 537 ± 196 MU; total dose of ribavirin = 182 ± 69 g; pegylated IFN- α 2b + 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

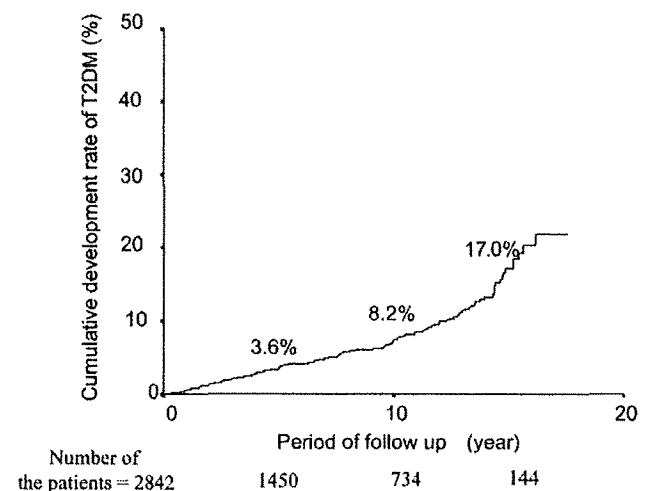


Fig. 1. Cumulative development rate of T2DM in patients treated with IFN.

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, $\geq 1,000$ / $< 1,000$) | 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.

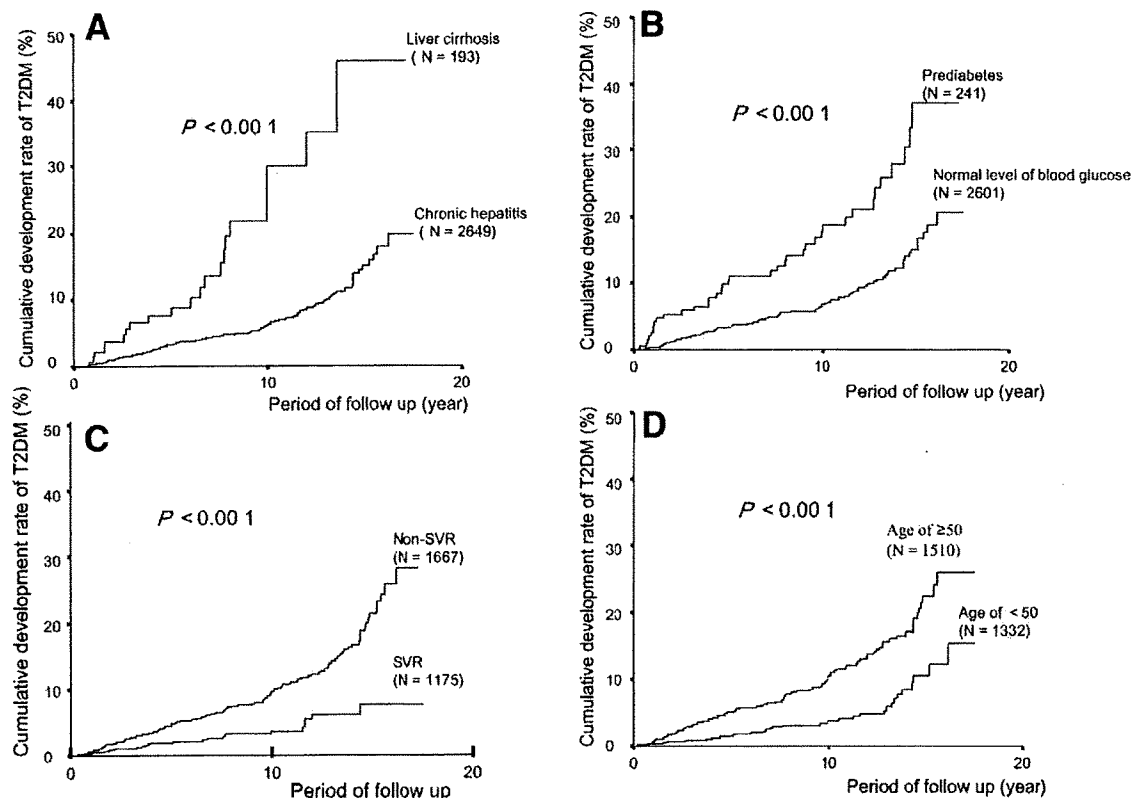


Fig. 2. Cumulative development rate of T2DM in patients treated with IFN. (A) Cumulative development rate of T2DM based on difference of hepatic fibrosis. (B) Cumulative development rate of T2DM based on the difference of glucose level. (C) Cumulative development rate of T2DM based on the difference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

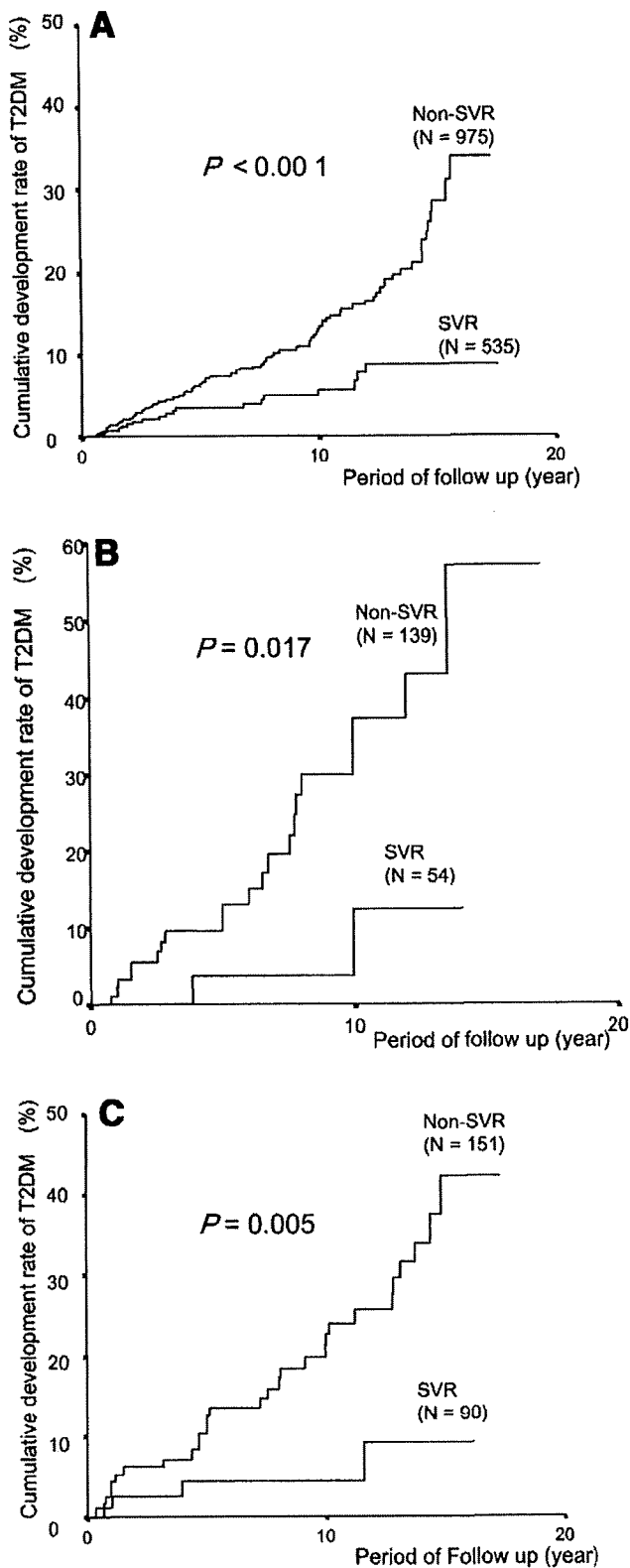


Fig. 3. Cumulative development rate of T2DM in patients with SVR or without SVR after IFN therapy. (A) Cumulative development rate of T2DM based on SVR or non-SVR in patients with age ≥ 50 years. (B) Cumulative development rate of T2DM based on SVR or non-SVR in patients with liver cirrhosis. (C) Cumulative 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 antiviral 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 resistance²⁴; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance and T2DM development²⁵; 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.

Acknowledgment: The authors acknowledge the editorial assistance of Thomas Hughes.

References

- Kiyosawa K, Furuta S. Review of hepatitis C in Japan. *J Gastroenterol Hepatol* 1991;6:383-391.
- Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, et al. The natural history of community acquired hepatitis C in the United States. *N Engl J Med* 1992;327:1899-1905.
- van Rossum TG, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. *Aliment Pharmacol Ther* 1998;12:199-205.
- Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989;2:1006-1008.
- Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, et al. Hepatitis C-associated hepatocellular carcinoma. *HEPATOLOGY* 1990;12:589-591.
- Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. *Lancet* 1990;335:873-874.
- Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993;328:1797-1801.
- Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *HEPATOLOGY* 1993;18:47-53.
- Gumber SC, Chopra S. Hepatitis C: a multifaceted disease- review of extrahepatic manifestations. *Ann Intern Med* 1995;123:615-620.
- Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med* 1993;328:465-470.
- Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, André C, et al. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 1995;122:169-173.
- Boadas J, Rodriguez-Espinosa J, Enriquez J, Miralles F, Martínez-Cerezo FJ, González P, et al. Prevalence of thyroid autoantibodies is not increased in blood donors with hepatitis C virus infection. *J Hepatol* 1995;22:611-615.
- Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992;327:1490-1495.
- Imazeki F, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. *Liver Int* 2008;28:355-362.
- Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, et al. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003;38:355-360.
- Genuth S, Alberti KG, Bennett P, Buse J, DeFronzo R, Kahn R, et al. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003;26:3160-3167.
- Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *HEPATOLOGY* 1994;19:1513-1520.
- Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. *Control Clin Trials* 1984;5:348-361.
- Fabris P, Betterle C, Greggio NA, Zanchetta R, Bosi E, Biasin MR, et al. Insulin-dependent diabetes mellitus during alpha-interferon therapy for chronic viral hepatitis. *J Hepatol* 1998;28:514-517.
- di Cesare E, Previti M, Russo F, Brancatelli S, Ingemi MC, Scoglio R, et al. Interferon-alpha therapy may induce insulin autoantibody development in patients with chronic viral hepatitis. *Dig Dis Sci* 1996;41:1672-1677.
- Fajans S. Classification and diagnosis of diabetes. In: Rifkin H, Porte D, eds. *Diabetes Mellitus: Theory and Practice*. 4th ed. New York: Elsevier, 1990:346-356.
- Simó R, Lecube A, Genescà J, Esteban JI, Hernández C. Sustained virological response correlates with reduction in the incidence of glucose abnormalities in patients with chronic hepatitis C virus infection. *Diabetes Care* 2006;29:2462-2466.
- Romero-Gómez M, Fernández-Rodríguez CM, Andrade RJ, Diago M, Alonso S, Planas R, et al. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. *J Hepatol* 2008;48:721-727.
- Romero-Gómez M. Insulin resistance and hepatitis C. *World J Gastroenterol* 2006;12:7075-7080.
- Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004;126:840-848.
- Romero-Gómez M, Del Mar Vilorio M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005;128:636-641.
- Waki K, Noda M, Sasaki S, Matsumura Y, Takahashi Y, Isogawa A, et al. Alcohol consumption and other risk factors for self-reported diabetes among middle-aged Japanese: a population-based prospective study in the JPHC study cohort I. *Diabet Med* 2005;22:323-331.
- Thuluvath PJ, John PR. Association between hepatitis C, diabetes mellitus, and race. a case-control study. *Am J Gastroenterol* 2003;98:438-441.
- Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, et al. Hepatitis C virus infection and incident type 2 diabetes. *HEPATOLOGY* 2003;38:50-56.
- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047-1053.
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352:837-853.
- UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 1998;352:854-865.
- Elbein SC. The search for genes for type 2 diabetes in the post-genome era. *Endocrinology* 2002;143:2012-2018.
- Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *HEPATOLOGY* 2008;47:1856-1862.

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

Yasuji Arase¹, Fumitaka Suzuki¹, Norio Akuta¹, Hitomi Sezaki¹, Yoshiyuki Suzuki¹, Yusuke Kawamura¹, Masahiro Kobayashi¹, Tetsuya Hosaka¹, Hiromi Yatsuji¹, Miharu Hirakawa¹, Satoshi Saito¹, Kenji Ikeda¹, Mariko Kobayashi² and Hiromitsu Kumada¹

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

(Inter Med 48: 253-258, 2009)

(DOI: 10.2169/internalmedicine.48.1629)

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 combi-

nation 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-

¹Department of Hepatology, Toranomon Hospital, Tokyo and ²Hepatic Research Unit, Toranomon Hospital, Tokyo

Received for publication August 25, 2008; Accepted for publication October 21, 2008

Correspondence to Dr. Yasuji Arase, es9y-ars@asahi-net.or.jp

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/mm³, platelet count >80,000/mm³, 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°C 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 | | | p |
|---|------------|-----------|-----------|-----------|-------|
| | | RVR | EVR | LVR | |
| 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 fa-

tigue, 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 ≥ 100 KIU/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 ($10^3/\text{mm}^3$) † | 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/- | 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 $\geq 90\%$. 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 $\geq 90\%$. However, one-third of the patients with continuance of negative HCV RNA of 10 to 19 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 \geq 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 $\geq 90\%$ 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.

Acknowledgement

The present work was supported in part by grants-in-aid from Okinaka Memorial Institute for Medical Research and the Japanese Ministry of Health, Labour and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

References

- Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358: 958-965, 2001.
- Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347: 975-982, 2002.
- Hadziyannis SJ, Sette H, Morgan TR, et al; PEGASYS Interna-

- tional Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* **140**: 346-355, 2004.
4. McHutchison JG, Manns M, Patel K, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* **123**: 1061-1069, 2002.
 5. Shiffman ML, Di Bisceglie AM, Lindsay KL, et al; Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial Group. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* **126**: 1015-1023, 2004.
 6. Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* **132**: 103-112, 2007.
 7. Schalm SW, Weiland O, Hansen BE, et al. Interferon-ribavirin for chronic hepatitis C with and without cirrhosis: analysis of individual patient data of six controlled trials. Eurohep Study Group for Viral Hepatitis. *Gastroenterology* **117**: 408-413, 1999.
 8. Jensen DM, Morgan TR, Marcellin P, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* **43**: 954-960, 2006 (Erratum in *Hepatology* **43**: 1410, 2006).
 9. Bronowicki JP, Ouzan D, Asselah T, et al. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* **131**: 1040-1048, 2006.
 10. Dalgard O, Bjørø K, Hellum KB, et al. Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* **40**: 1260-1265, 2004.
 11. Mangia A, Santoro R, Minerva N, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* **352**: 2609-2617, 2005.
 12. Bruno S, Cammà C, Di Marco V, et al. Peginterferon alfa-2b plus ribavirin for naïve patients with genotype 1 chronic hepatitis C: a randomized controlled trial. *J Hepatol* **41**: 474-481, 2004.
 13. Lindahl K, Stahle L, Bruchfeld A, et al. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* **41**: 275-279, 2005.
 14. von Wagner M, Huber M, Berg T, et al. Peginterferon-alpha-2a (40 KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* **129**: 522-527, 2005.
 15. Krawitt EL, Gordon SR, Grace ND, et al; for the New York New England Study Team. A study of low dose peginterferon alpha-2b with ribavirin for the initial treatment of chronic hepatitis C. *Am J Gastroenterol* **101**: 1268-1273, 2006.
 16. Ferenci P, Fried MW, Shiffman ML, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* **43**: 425-433, 2005.
 17. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* **43**: 54-63, 2006.
 18. Arase Y, Suzuki F, Suzuki Y, et al. Side effects of combination therapy of peginterferon and ribavirin for chronic hepatitis-C. *Intern Med* **46**: 1827-1832, 2007.
 19. Doglio A, Laffont C, Caroli-Bosc FX, et al. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. *J Clin Microbiol* **37**: 1567-1569, 1999.
 20. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* **36**: 862-865, 1998.
 21. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* **19**: 13-18, 1994.
 22. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **19**: 1513-1520, 1994.
 23. Davis GL, Wong JB, McHutchison JG, et al. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* **38**: 645-652, 2003.
 24. Arase Y, Suzuki F, Sezaki H, et al. Suitable treatment period in patients with virological response during combination therapy of peginterferon and ribavirin for chronic hepatitis C. *Intern Med* **47**: 1301-1307, 2008.

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

Yasuji Arase · Fumitaka Suzuki · Kenji Ikeda ·
Hiromitsu Kumada · Hiroshi Tsuji ·
Tetsuro Kobayashi

Received: 28 March 2009 / Accepted: 24 May 2009 / Published online: 17 June 2009
© Springer 2009

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-glutamyl-transferase (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.

Y. Arase (✉) · F. Suzuki · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital,
2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan
e-mail: es9y-ars@asahi-net.or.jp

Y. Arase · H. Tsuji
Department of Health Management,
Toranomon Hospital, Tokyo, Japan

T. Kobayashi
Third Department of Internal Medicine (Metabolism),
University of Yamanashi, Yamanashi, Japan

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 erythematosus 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 m²).

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 | |
|---|---------------|
| <i>N</i> | 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 (×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%

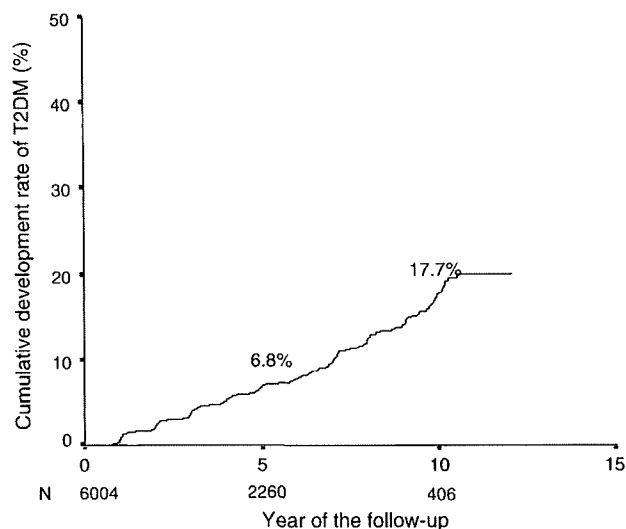


Fig. 1 Cumulative development rate of type 2 diabetes mellitus (T2DM) in 6003 patients with nonalcoholic fatty liver disease (NAFLD)

(2564/6003), respectively. The mean follow-up period was 4.9 years.

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 (\pm) | 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

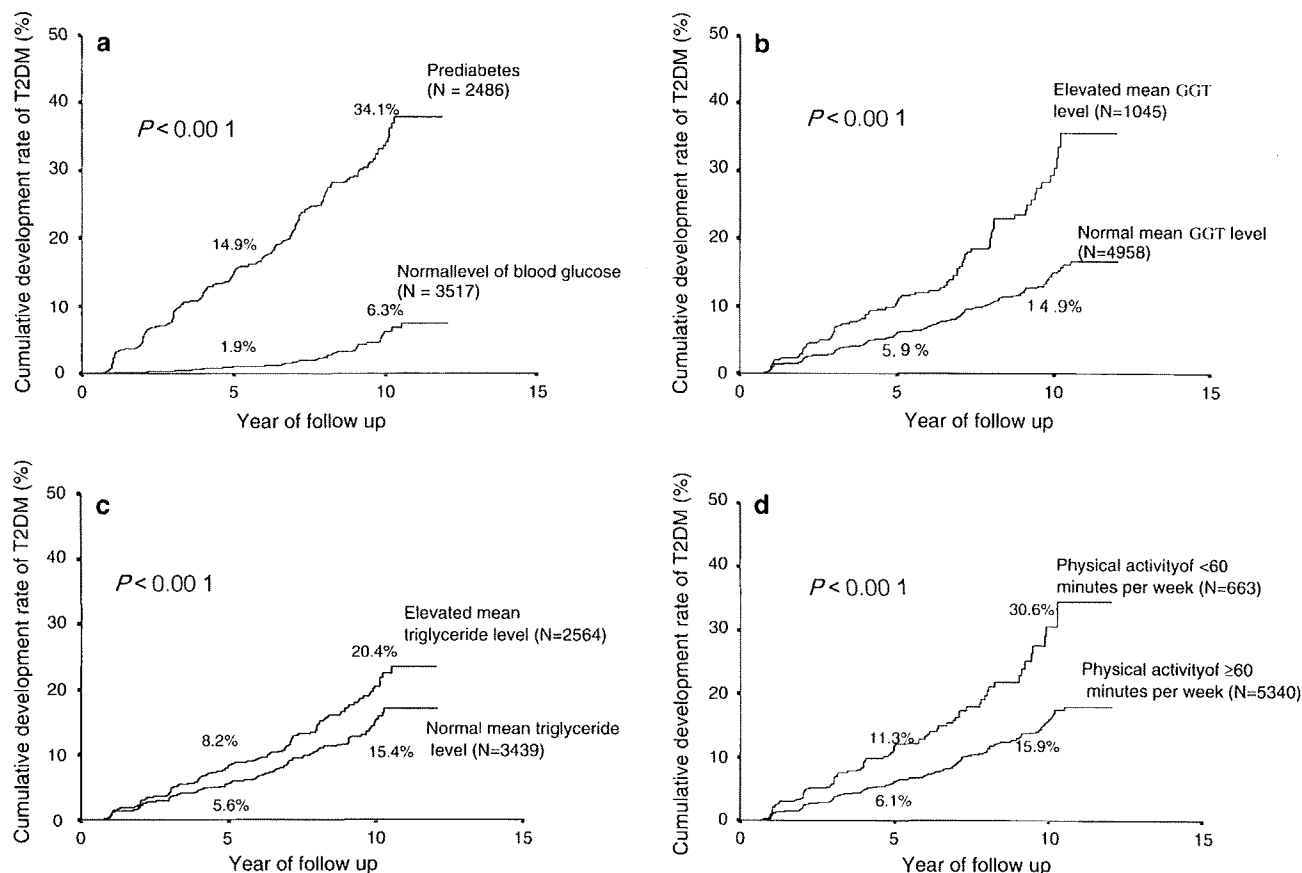


Fig. 2 Cumulative development rate of T2DM in NAFLD patients. **a** Cumulative development rate of T2DM based on differences between glucose levels at the initiation of follow up and during follow up. **b** Cumulative development rate of T2DM based on the differences between mean gamma-glutamyltransferase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

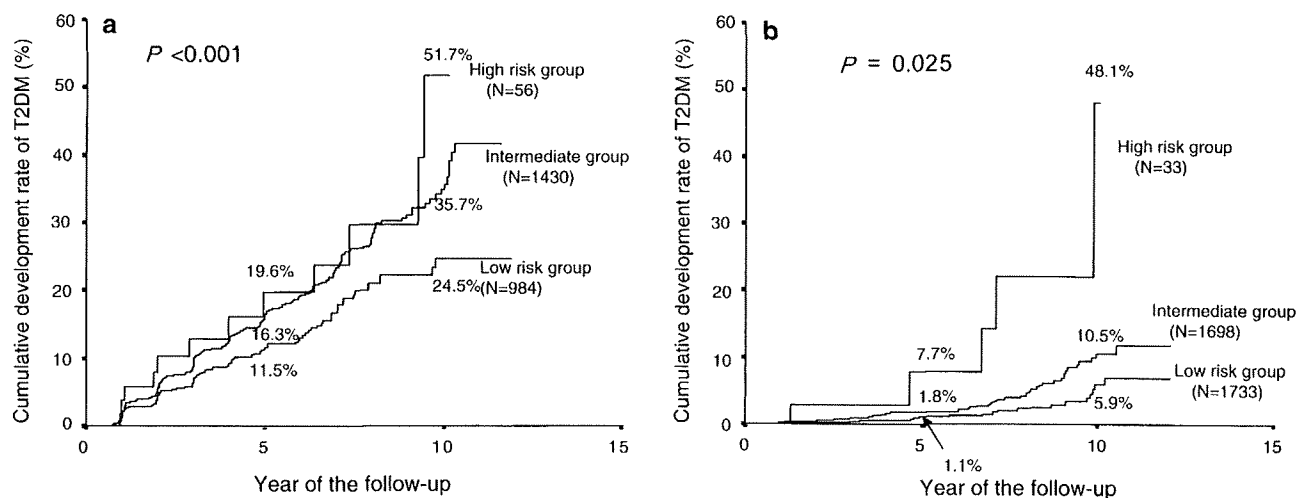


Fig. 3 a Cumulative development rate of T2DM in NAFLD patients with prediabetes based on risk stratification according to differences in physical activity, mean levels of GGT, and mean levels of triglyceride during follow up. **b** Cumulative development rate of

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.

Acknowledgments The present work was supported in part by Grants-in-Aid from the Okinaka Memorial Institute for Medical Research and the Japanese Ministry of Health, Labour and Welfare. Moreover, the authors greatly acknowledge the editorial assistance of Thomas Hughes.

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

References

1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346:1221–31.
2. Williams R. Global changes in liver disease. *Hepatology*. 2006;44:521–6.
3. Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology*. 2008;134:1682–98.
4. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology*. 2009;49:306–17.
5. Fan JC, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol*. 2009;50:204–10.
6. Watanabe S, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. *J Gastroenterol*. 2008;43:509–18.
7. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology*. 1999;30:1356–62.
8. Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes*. 2005;54:333–9.
9. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab*. 2008;294:E15–26.
10. Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology*. 2004;39:909–14.
11. Tanné F, Gagnadoux F, Chazouillères O, Fleury B, Wendum D, Lasnier E, et al. Chronic liver injury during obstructive sleep apnea. *Hepatology*. 2005;41:1290–6.
12. Kheirandish-Gozal L, Sans Capdevila O, Kheirandish E, Gozal D. Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. *Chest*. 2008;133:92–9.
13. Fan JG, Li F, Cai XB, Peng YD, Ao QH, Gao Y. Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. *J Gastroenterol Hepatol*. 2007;22:1086–91.
14. Lonardo A, Bellini M, Tartoni P, Tondelli E. The bright liver syndrome. Prevalence and determinants of a “bright” liver echopattern. *Ital J Gastroenterol Hepatol*. 1997;29:351–6.

15. Genuth S, Alberti KG, Bennett P, Buse J, DeFronzo R, Kahn R, et al. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care*. 2003;26:3160–7.
16. Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. *Control Clin Trials*. 1984;5:348–61.
17. Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology*. 2009;49:739–44.
18. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Man CD, et al. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci USA*. 2006;103:18273–7.
19. Perseghin G, Bonfanti R, Magni S, Lattuada G, De Cobelli F, Canu T, et al. Insulin resistance and whole body energy homeostasis in obese adolescents with fatty liver disease. *Am J Physiol Endocrinol Metab*. 2006;291:E697–703.
20. Vega GL, Chandalia M, Szczepaniak LS, Grundy SM. Metabolic correlates of nonalcoholic fatty liver in women and men. *Hepatology*. 2007;46:716–22.
21. van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, et al. Visceral fat a key mediator of steatohepatitis in metabolic liver disease. *Hepatology*. 2008;48:449–57.
22. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346:393–403.
23. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. *Diabetes Care*. 2009;32:741–50.
24. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047–53.
25. Waki K, Noda M, Sasaki S, Matsumura Y, Takahashi Y, Isogawa A, et al. Alcohol consumption and other risk factors for self-reported diabetes among middle-aged Japanese: a population-based prospective study in the JPHC study cohort I. *Diabet Med*. 2005;22:323–31.

Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C

Kenji Ikeda, MD, PhD,^{a,b} Yasuji Arase, MD,^{a,b} Yusuke Kawamura, MD,^{a,b} Hiromi Yatsuji, MD,^{a,b} Hitomi Sezaki, MD,^{a,b} Tetsuya Hosaka, MD,^{a,b} Norio Akuta, MD, PhD,^{a,b} Masahiro Kobayashi, MD,^{a,b} Satoshi Saitoh, MD,^{a,b} Fumitaka Suzuki, MD, PhD,^{a,b} Yoshiyuki Suzuki, MD, PhD,^{a,b} Hiromitsu Kumada, MD, PhD^{a,b}

^aDepartment of Hepatology, Toranomon Hospital, Tokyo, Japan; and ^bOkinaka Memorial Institute for Medical Research, Tokyo.

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 ($\geq 150,000/\text{mm}^3$) group, 78.6% and 47.8% in the intermediate ($100,000\text{-}149,000/\text{mm}^3$) group, and 52.5% and 25.0% in the low platelet group ($< 100,000/\text{mm}^3$), 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.

© 2009 Elsevier Inc. All rights reserved. • *The American Journal of Medicine* (2009) 122, 479-486

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 Japan¹⁷⁻¹⁹ because of a significant decrease of new blood-borne HCV infections and an aging

Funding: This work was partly supported by a grant of Ministry of Health, Labor, and Welfare, Japan.

Conflict of Interest: None of the authors have any conflicts of interest associated with the work presented in this manuscript.

Authorship: All authors had access to the data and played a role in writing this manuscript.

Requests for reprints should be addressed to Kenji Ikeda, MD, PhD, Department of Hepatology, Toranomon Hospital, Toranomon 2-2-2, Minato-ku, Tokyo, 105-8470, Japan.

E-mail address: ikedakenji@tora.email.ne.jp

society, such as in Japan. In elderly patients with chronic hepatitis or cirrhosis type C, adverse effects of IFN are more prevalently found and hematologic disorders often disturb the completion of the therapy. As a result, IFN administration is considered less effective in elderly patients.^{16,20-22} Because the fibrotic stage of liver disease is often correlated with a patient's age, an elderly patient naturally has a high risk of carcinogenesis and mortality. IFN is effective in reducing hepatocarcinogenesis and improving the survival of patients with HCV-related chronic hepatitis, but the clinical influence of IFN is considered less advantageous in elderly patients because of the short life expectancy. There has been little information on the prognosis of elderly patients with HCV-related chronic liver disease and the significance of antiviral therapy for elderly patients.

To clarify whether IFN had similar advantages between young and elderly patients, we analyzed a large cohort of HCV-positive elderly patients in regard to hepatocellular carcinogenesis and survival at a single institution. We also attempted to elucidate favorable indications and the best candidates for IFN therapy among elderly patients, if any.

PATIENTS AND METHODS

Entire Population and Analyzed Cohorts

A total of 7235 patients were diagnosed with HCV-positive chronic liver disease with positive anti-HCV antibody and detectable HCV-RNA (nested polymerase chain reaction) and negative hepatitis B surface antigen from 1974 to 2004 at the Department of Hepatology, Toranomon Hospital, Tokyo. Anti-HCV and HCV-RNA were assayed using stored frozen sera. There were 4121 men and 3114 women, with a median age of 54 years (range, 1-92 years). We excluded 1144 patients with acute hepatitis, overt alcoholic liver disease or fatty liver, association of other types of liver disease (eg, primary biliary cirrhosis, autoimmune hepatitis), or association with hepatocellular carcinoma or other. We also excluded 446 patients with a short observation period (<6 months).

There were 3728 patients aged less than 60 years and 1917 patients aged 60 years or more. The diagnosis was established by peritoneoscopy or biopsy in 636 patients and by clinical data in 1281 patients. The ratio of women was higher (36.9% vs 54.4%, $P < .001$) and history of IFN

therapy was lower (60.3% vs 23.7%, $P < .001$) in elderly patients. Median albumin value was lower (4.3 vs 4.1 g/dL, $P < .001$) and platelet count was lower (181,000 vs 155,000/mm³, $P < .001$) in elderly patients. This study analyzed 1917 elderly patients with HCV: 454 patients (23.7%) with IFN therapy and 1463 patients (76.3%) without IFN therapy.

CLINICAL SIGNIFICANCE

- Significant differences in hepatocarcinogenesis and survival exist among patients with HCV, according to initial platelet count.
- IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.
- Asymptomatic elderly patients with HCV should be observed carefully as to hepatocarcinogenesis by using ultrasonography when the platelet count is $150 \times 1000/\text{mm}^3$ or less.
- IFN therapy should be considered in elderly patients when they have intermediate and low platelet counts.
- In view of the side effects in elderly patients, treatment should be initiated as soon as possible after diagnosis of chronic HCV.

Interferon Treatment and Judgment of Effect

Among 454 patients with IFN therapy, 413 received IFN monotherapy and 41 received IFN plus ribavirin combination therapy as an initial antiviral therapy. Of 413 patients with IFN monotherapy, 272 patients received IFN every day for the first 2 to 8 weeks and then 2 to 3 times per week for the following 16 to 96 weeks (median, 24 weeks), 108 patients received IFN 3 times per week for 24 to 104 weeks, and 33 patients received IFN for 4 to 8 weeks. Among 346 patients without viral elimination after initial IFN therapy, 186 patients underwent repeated IFN therapy including IFN plus ribavirin combination therapy. The age at the time of initiation of therapy ranged from 60 to 84 years, with a median of 64 years.

Most patients ($N = 451$) with IFN therapy showed varied degrees of influenza-like symptoms, leukocytopenia, and thrombocy-

topenia. Forty-three patients discontinued IFN therapy because of significant adverse reactions: depression in 10 patients, marked anorexia in 9 patients; psychosis, epilepsy, or loss of consciousness in 8 patients; ophthalmic diseases in 3 patients; severe cytopenia in 3 patients; interstitial pneumonia in 2 patients; and other conditions in 8 patients. No patients had decompensated liver disease with ascites, encephalopathy, jaundice, or variceal bleeding.

Judgment of IFN effect was classified according to elimination of HCV RNA and alanine aminotransferase for 6 months after the end of treatment. Sustained virologic response was defined as persistent disappearance of HCV RNA after therapy, biochemical response was defined as normal alanine aminotransferase values without elimination of HCV RNA for at least 6 months after therapy, and no response was defined as persistently abnormal or only transient normalization of alanine aminotransferase for less than 6 months. Because 12 patients (2.6%) were lost to follow-up and 49 patients (10.8%) were still in the course of IFN therapy, the judgment was made in 393 (86.6%) of 454 patients.

Table 1 Profiles and Laboratory Data of 1917 Elderly Patients at the Initial Visit to Toranomon Hospital

| | No Therapy N = 1463 | IFN Therapy N = 454 | <i>P</i> ^c |
|--|------------------------|------------------------|-----------------------|
| Demography | | | |
| Sex (M/F) | 660/803 | 214/240 | .45 |
| Age (y) ^a | 65 (60-88) | 62 (60-80) | <.001 |
| Observation period (y) ^a | 5.91 (0.5-27.6) | 6.23 (0.5-17.6) | .23 |
| Lost to follow-up (y) | 165 (11.3%) | 12 (2.6%) | <.001 |
| Laboratory Data^b | | | |
| Albumin (g/dL) | 4.1 (3.8-4.3) | 4.1 (3.9-4.3) | .11 |
| Bilirubin (mg/dL) | 0.6 (0.5-0.9) | 0.7 (0.5-0.8) | .14 |
| Aspartic aminotransferase (IU/L) | 51 (33-83) | 70 (46-106) | <.001 |
| Alanine aminotransferase (IU/L) | 56 (32-97) | 90 (56-148) | <.001 |
| Hemoglobin (g/dL) | 13.8 (12.9-14.7) | 14.2 (13.3-15.1) | <.001 |
| Platelet count ($\times 1000/\text{mm}^3$) | 157 (120-198) | 150 (122-195) | 0.12 |
| Alpha-fetoprotein (ng/mL) | 4 (3-6) | 4 (3-6) | .80 |
| HCV | | | |
| subtype 1 (1a/1b) | 714 (79.2%) | 154 (58.8%) | <.001 |
| subtype 2 (2a/2b) | 150 (16.6%) | 102 (38.9%) | |
| others | 38 (4.2%) | 6 (2.3%) | |

IFN = interferon; HCV = hepatitis C virus.

^aExpressed by median (range).

^bExpressed by median (25th percentile, 75th percentile).

^cMann-Whitney or chi-square test.

Follow-up of and Diagnosis of Hepatocellular Carcinoma

Follow-up of patients was made on a monthly to trimonthly basis after the initial visit. Imaging diagnosis was made 1 or more times per year with ultrasonography, computed tomography, or magnetic resonance imaging.

Statistical Analysis

Obtained clinical data were analyzed on an intention-to-treat basis. Nonparametric procedures were used for the analysis of background characteristics of the patients, including the Mann-Whitney *U*, Kruskal-Wallis, and chi-square tests.

Hepatocellular carcinogenesis and survival were calculated using the Kaplan-Meier test. The differences in carcinogenesis curves were tested using the log-rank test.²³ Independent factors associated with the appearance rate of hepatocellular carcinoma were studied using time-dependent Cox regression analysis.²⁴ The following 16 variables were analyzed for potential covariates for liver carcinogenesis at the initial hospital visit: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, association of diabetes, aspartic aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, albumin, bilirubin, hemoglobin, platelet count, serologic grouping of HCV, IFN administration, and effect of IFN treatment (time-dependent variable). A *P* value of less than .05 was considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.²⁵

RESULTS

Demographics of Elderly Patients with or without Interferon Therapy

Table 1 summarizes the profiles and data of the 1917 elderly patients with or without IFN therapy during clinical course. The median age of the patients with IFN was younger by 3 years. Although aminotransferases were significantly higher in the treated group, albumin, bilirubin, and platelet count were not different between the 2 groups.

Hepatocarcinogenesis and Survival without Interferon Therapy

Liver cancer developed in 285 (19.5%) of 1463 elderly patients without IFN therapy. Hepatocarcinogenesis rates were 13.1% at the end of 5 years, 29.9% at 10 years, 45.5% at 15 years, and 55.1% at 20 years. Carcinogenesis rates were calculated in subgroups according to initial platelet count: high ($\geq 150,000/\text{mm}^3$), intermediate (100,000-149,000/ mm^3), and low ($< 100,000/\text{mm}^3$). Cumulative carcinogenesis rates in the subgroups of high, intermediate, and low platelet counts were 5.1%, 14.2%, and 32.1% at 5 years, 14.0%, 34.2%, and 63.4% at 10 years, and 26.1%, 57.5%, and 74.9% at 15 years, respectively (Figure 1). The carcinogenesis rate was significantly different among the 3 subgroups ($P < .0001$).

Survival in the elderly patients without IFN therapy was 92.9% at 5 years, 76.6% at 10 years, 54.3% at 15 years, and 37.2% at 20 years. Survivals in the subgroups with high, intermediate, and low platelet counts were 97.9%, 95.9%,