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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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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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.

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.

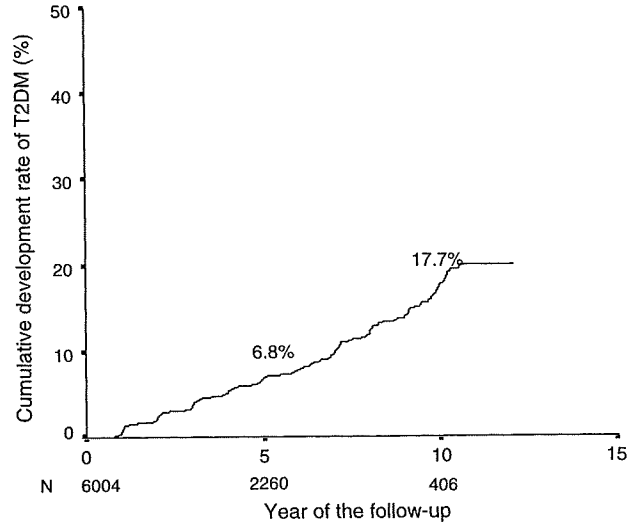

Fig. 1 Cumulative development rate of type 2 diabetes mellitus (*T2DM*) in 6003 patients with nonalcoholic fatty liver disease (NAFLD)

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

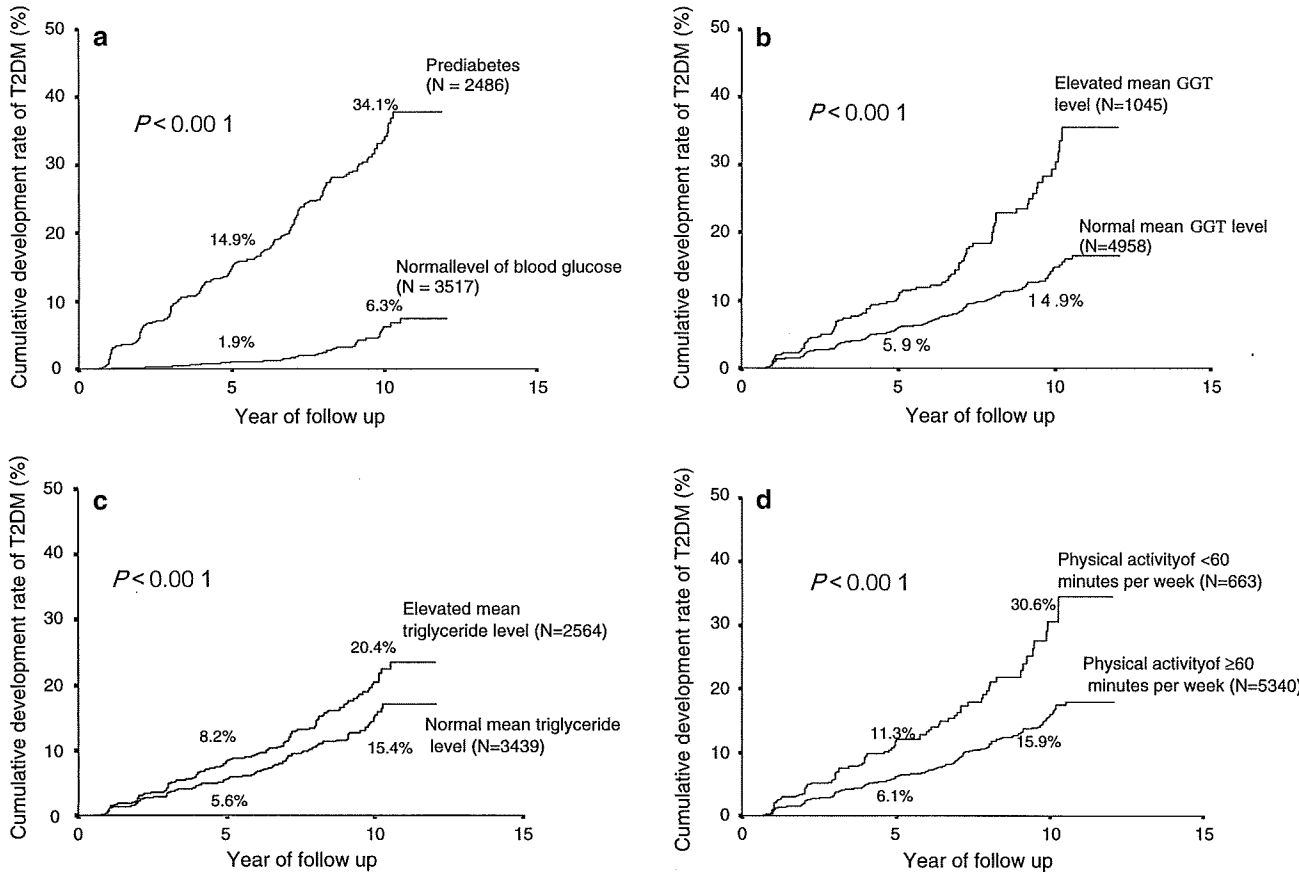

Fig. 2 Cumulative development rate of T2DM in NAFLD patients. **a** Cumulative development rate of T2DM based on differences between glucose levels at the initiation of follow up and during follow up. **b** Cumulative development rate of T2DM based on the differences between mean gamma-glutamyltransferase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

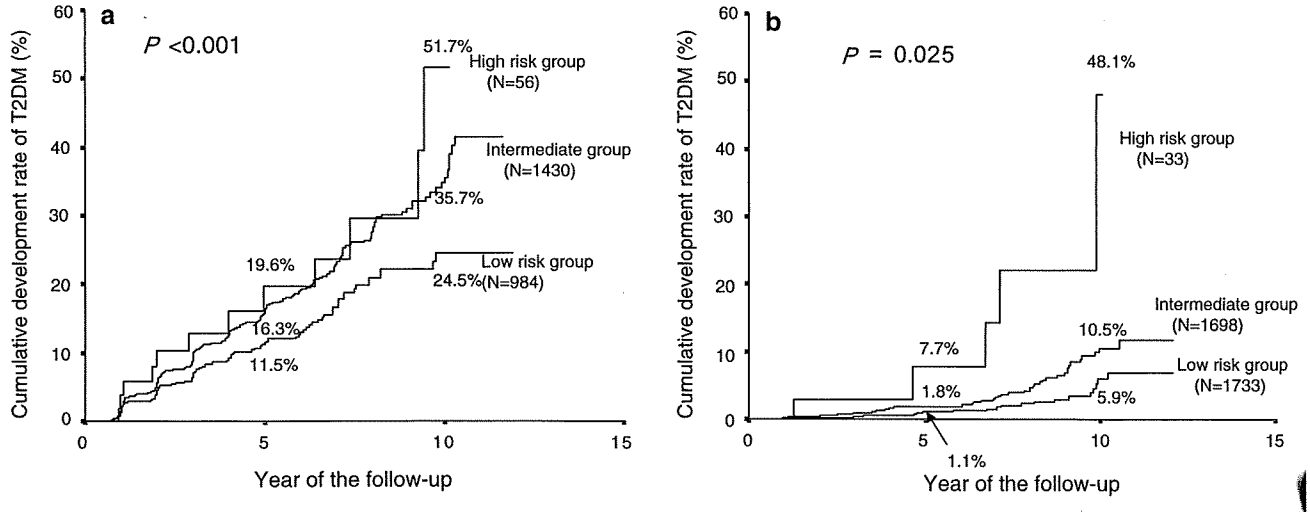

Fig. 3 **a** Cumulative development rate of T2DM in NAFLD patients with prediabetes based on risk stratification according to differences in physical activity, mean levels of GGT, and mean levels of triglyceride during follow up. **b** Cumulative development rate of T2DM in NAFLD patients with normal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

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 × 1000/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.

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/mm^3, $P < .001$) 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.

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 thrombocytopenia. 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	P[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 (×1000/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.
[a]Expressed by median (range).
[b]Expressed by median (25th percentile, 75th percentile).
[c]Mann–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 (≥150,000/mm^3), intermediate (100,000-149,000/mm^3), and low (<100,000/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%,

and 86.8% at 5 years, 90.7%, 78.6%, and 52.5% at 10 years, and 72.7%, 47.8%, and 25.0% at 15 years, respectively (Figure 2). A significant difference was observed among the 3 subgroups ($P < .0001$).

Adverse Effects and Effect of Interferon in the Elderly

Thirty-nine patients discontinued IFN therapy because of adverse effects: severe fatigue or anorexia in 10 patients (25.6%), depression in 10 patients (25.6%), hematologic disorder in 6 patients (15.4%), ophthalmic disorders in 4 patients (10.3%), and other side effects in 9 patients (23.1%). Duration of the therapy ranged from 2 weeks to 8.1 years, with a median of 24 weeks.

Among 393 patients with available judgment of IFN effect, 140 (35.6%) had a sustained virologic response, 80 (20.4%) had a biochemical response, and 173 (44.0%) had no response.

Hepatocarcinogenesis Rates in Elderly Patients with or without Interferon

During observation, hepatocellular carcinoma developed in 334 (17.4%) of 1917 patients: 285 (19.5%) in the untreated group and 49 (10.8%) in the IFN group.

Hepatocarcinogenesis rates in the untreated and IFN groups were 13.1% and 7.0% at 5 years, 29.9% and 13.9% at 10 years, and 45.5% and 33.4% at 15 years, respectively. The carcinogenesis rate in the IFN-treated group was significantly lower than in the untreated group (log-rank test, $P < .0001$).

Carcinogenesis rates also were evaluated in the subgroups with sustained virologic response (N = 140), biochemical response (N = 80), and no response (N = 173). Cumulative carcinogenesis rates were 2.5%, 1.3%, and 9.1% at 5 years, 2.5%, 11.0%, and 18.1% at 10 years, and 2.5%, 39.6%, and 41.2% at 15 years, respectively. A significant difference was found among the 4 groups, including the untreated patient group ($P < .0001$).

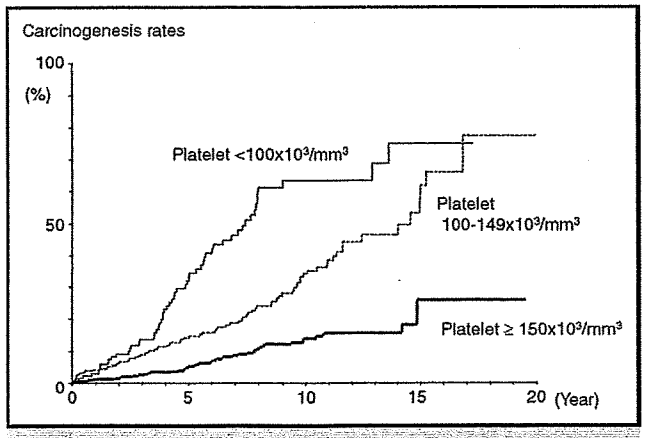

Figure 1 Hepatocarcinogenesis rates in patients without IFN therapy, according to initial platelet count. The lower the initial platelet count was, the higher the hepatocellular carcinogenesis was in the untreated cohort ($P < .0001$).

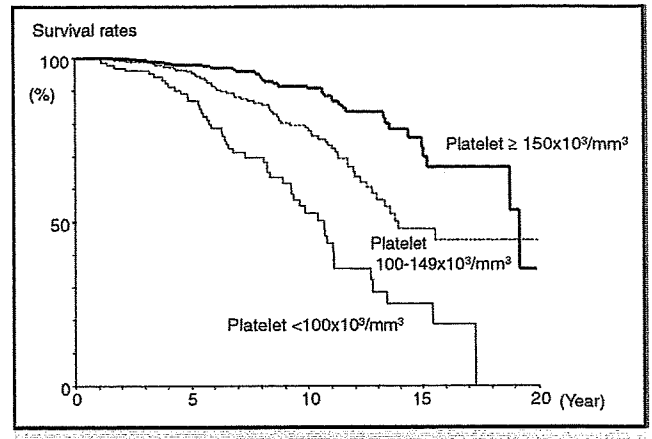

Figure 2 Cumulative survival in patients without IFN therapy, according to initial platelet count. Survival of patients with high platelet count was significantly higher than those with a low or intermediate platelet count ($P < .0001$).

Carcinogenesis rates were compared between those with or without IFN treatment in a subgroup with a high platelet count of 150,000/mm^3 or more. Cumulative carcinogenesis rates in the untreated (N = 585) and treated groups (N = 228) were 5.1% and 3.7% at 5 years, 14.0% and 13.1% at 10 years, and 26.1% and 25.9% at 15 years, respectively. The carcinogenesis rate in the IFN therapy group was slightly lower than in the untreated group, but no statistical significance was found in the high platelet subgroup ($P = .69$). Next, carcinogenesis rates were analyzed between those with or without IFN in a combined subgroup with low and intermediate platelet counts of less than 150,000 mm^3. Carcinogenesis rates in untreated (N = 459) and treated (N = 217) groups were 19.5% and 10.9% at 5 years, 43.0% and 21.6% at 10 years, and 65.3% and 39.4% at 15 years, respectively (Figure 3). The carcinogenesis rate in the group with IFN therapy was significantly lower in the untreated group ($P = .0005$).

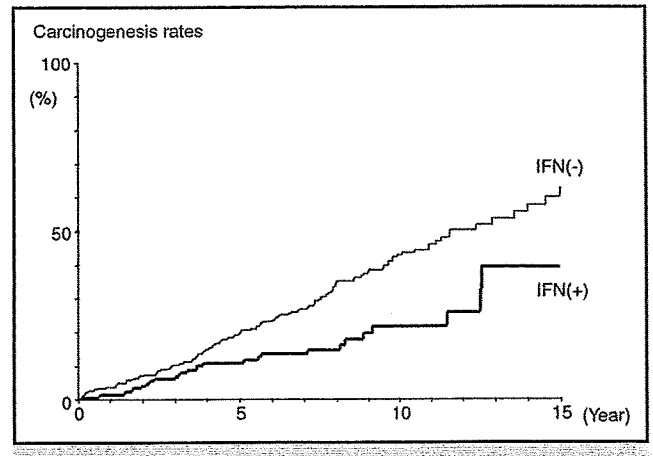

Figure 3 Hepatocarcinogenesis rates in patients with a low or intermediate platelet count. Carcinogenesis rate of patients with IFN therapy was significantly lower than those without therapy ($P = .0005$). IFN = Interferon.

Table 2 Independent Factors Associated with Hepatocellular Carcinogenesis in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

Factors	(Category)	Hazard Ratio (95% CI)	P
Platelet count	1: ≥150,000/mm^3	1	
	2: 100,000-149,000/mm^3	2.42 (1.71-3.40)	<.001
	3: <100,000/mm^3	5.64 (3.88-8.22)	<.001
Alanine aminotransferase	1: <75 IU/L	1	
	2: ≥75IU/L	2.02 (1.48-2.77)	<.001
Gender	1: Female	1	
	2: Male	1.79 (1.35-2.37)	<.001
IFN	1: No therapy	1	
	2: No response	0.74 (0.44-1.25)	.26
	3: Biochemical response	0.52 (0.17-1.65)	.27
	4: Sustained virologic response	0.063 (0.009-0.449)	.006

CI = confidence interval; IFN = interferon.

Factors Affecting Hepatocellular Carcinogenesis

In the first proportional hazard analysis using IFN therapy factor as a time-dependent covariate, factors associated with carcinogenesis were explored in the entire elderly cohort. Hepatocarcinogenesis is independently associated with low platelet count ($P < .001$), high alanine aminotransferase value ($P < .001$), male sex ($P < .001$), and IFN therapy (hazard ratio = 0.67, $P = .045$).

Next, multivariate analysis was performed using factors of each IFN effect: sustained virologic response, biochemical response, no response, and no IFN therapy. Carcinogenesis was significantly associated with platelet count, male sex, alanine aminotransferase value, and sustained virologic response after IFN therapy (Table 2). Patients with low and intermediate platelet counts showed high hazard ratios and high alanine aminotransferase value; male gender showed high hazard ratios. Sustained virologic response significantly decreased the hazard ratio to 0.063 ($P = .006$).

The role of IFN treatment factor was not significant (hazard ratio 0.87, $P = .67$) in the high platelet group (≥150,000/mm^3), but it was significant (hazard ratio 0.56, $P = .035$) in the low or intermediate platelet group (<150,000/mm^3).

Survival of Elderly Patients

A total of 276 patients (14.4%) died during observation: 255 (17.4%) in the untreated group and 21 (4.6%) in the treated group. Crude survivals in the untreated and IFN groups were 92.9% and 98.7% at 5 years, 76.6% and 92.6% at 10 years, and 54.3% and 70.4% at 15 years, respectively. Survival in the IFN-treated group was significantly higher ($P < .0001$).

When a subgroup with high platelet counts (≥150,000/mm^3) was analyzed, survivals in the untreated and IFN groups were 97.9% and 99.6% at 5 years, 90.7% and 94.5% at 10 years, and 72.7% and 76.9% at 15 years, respectively. Survival was not significantly different ($P = .08$). Survival also was analyzed in a subgroup with low or intermediate platelet count (<150,000/mm^3). Cumulative survivals in the untreated and treated groups were 93.2% and 97.5% at 5 years, 70.8% and 89.9% at 10 years, and 41.2% and 64.9% at 15 years, respectively (Figure 4). Survival in the IFN therapy group was significantly higher than in the untreated group ($P = .0001$).

Factors Affecting Survival in the Elderly

Independent factors associated with survival were explored in all the elderly patients. Multivariate hazard analysis disclosed that survival is independently associated with low platelet count ($P < .001$), male sex ($P < .001$), older age ($P < .001$), and IFN therapy (hazard ratio = 0.56, $P = .041$).

In the high platelet group (≥150,000/mm^3), only gender and age were independently associated with survival. The factor of IFN therapy only showed a hazard ratio for death of 0.70 in the multivariate analysis. In the low or intermediate platelet group (<150,000/mm^3), platelet count, age,

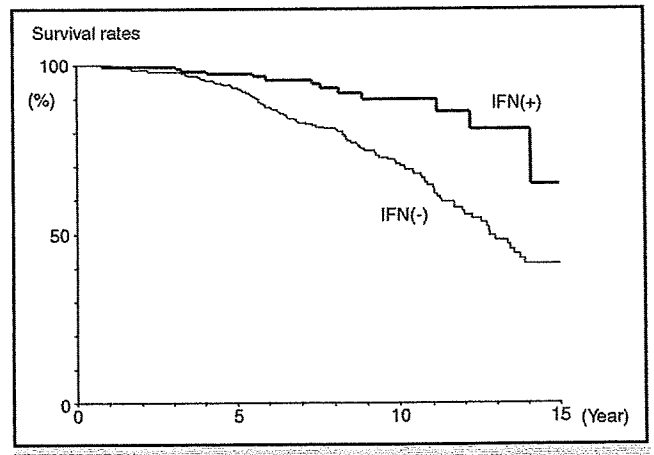

Figure 4 Cumulative survival in patients with a low or intermediate platelet count. Survival of patients with IFN therapy was significantly higher than those without therapy ($P = .0001$). IFN = Interferon.

Table 3 Independent Factors Associated with Survival Period in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

Factors	(Category)	Hazard Ratio (95% CI)	*P*
Subgroup with High Platelet Count ($\geq$150,000/mm^3)			
Gender	1: Female	1	
	2: Male	2.81 (1.46-5.41)	.002
Age	by 1 y	1.11 (1.04-1.18)	.002
IFN	1: No	1	
	2: Yes	0.70 (0.32-1.18)	.39 (NS)
Subgroup with Low or Intermediate Platelet Count (<150,000/mm^3)			
Platelet count	1: 100,000-149,000/mm^3	1	
	2: <100,000/mm^3	3.14 (2.19-4.50)	<.001
Age	by 1 y	1.09 (1.05-1.13)	<.001
IFN	1: No	1	
	2: Yes	0.43 (0.24-0.77)	.005
Gender	1: Female	1	
	2: Male	1.56 (1.09-2.22)	.015

CI = confidence interval; IFN = interferon; NS = not significant.

IFN therapy, and sex were independently associated with hepatocellular carcinogenesis. IFN significantly decreased the hazard of death by 0.43 in the subgroup of low or intermediate platelet count ($P = .005$) (Table 3).

DISCUSSION

This retrospective study was undertaken to evaluate whether IFN therapy could decrease hepatocellular carcinogenesis and increase survival in HCV-positive elderly patients aged 60 years or more at the initial hospital visit. Because it seemed to require at least 5 years to obtain a statistical difference in carcinogenesis rates and survival between IFN-treated and untreated groups, a prospective randomized trial with untreated control patients is difficult to perform from both ethical and medical viewpoints. We therefore attempted to carry out this retrospective study to show an impact of IFN treatment with a statistical adjustment and stratification using a large number of patients under a long-term observation period.

There were significant differences in carcinogenesis and survival among patients with HCV, according to initial platelet count. Because this study dealt with all patients with HCV-related hepatitis who visited Toranomon Hospital irrespective of IFN treatment, evaluation of liver histology was performed in approximately two thirds of the patients. Platelet count has been considered a simple indicator for the progression of hepatitis, and the patients without liver biopsy were well stratified by the initial platelet count in our study. From statistics of the nationwide census for the longevity of each age group in 2003, the life expectation was 21.9 and 27.5 years for 60-year-old Japanese men and women, respectively, and 18.0 and 23.07 years for 65-year-old Japanese men and women, respectively. In view of the median age (65 years) of the untreated cohort with HCV infection, the survival of patients with high platelet counts was almost the same as that of the general population in Japan (Figure 2). Physicians should consider the longevity without IFN therapy and the cost, side effects, and risks caused by IFN for more stratified age groups of the elderly.

Although several authors have shown that effects of both IFN monotherapy[20,26,27] and IFN plus ribavirin combination therapy[28,29] were not different between elderly and younger patients with chronic HCV in regard to viral elimination and normalization of transaminase, recent reports[16,21] have shown lower virologic response rates. A possible low response rate in the elderly was closely associated with a high rate of adverse reactions,[16,20,21] and hematologic side effects seemed significant in the elderly group.[22] The low discontinuation rate (43/454, 9.5%) in the current study was partly attributable to the low rate of IFN plus ribavirin combination therapy. Horiike et al,[27] Floreani et al,[16] and Koyama et al[21] recommended IFN therapy for select patient groups with a low HCV RNA titer, non-genotype 1, or relatively young age of less than 65 years.

We previously reported a high carcinogenesis rate in elderly patients with chronic HCV who underwent IFN therapy.[30] When crude hepatocarcinogenesis rates were compared between untreated and IFN-treated groups in the current study, IFN significantly decreased the carcinogenesis rate in the elderly patients with varied severity of liver disease. As was found in the general results of patients, including the younger age group,[13] carcinogenesis in patients with sustained virologic response was significantly lower than that of patients with no response or without IFN therapy. The carcinogenesis rate was low for several years after cessation of IFN administration and increased gradually after 8 years in the group with a biochemical response (Figure 3). The cancer appearance curve of the biochemical response group implied that the normal and stable hepatitis

state in the early years contributed to suppress the process of carcinogenesis, and that reactivation of hepatitis induced the progression of hepatic oncogenesis in the later years.

Among patients with a high platelet count and mild liver disease, IFN did not decrease the rate of hepatocarcinogenesis. IFN significantly decreased the carcinogenesis rate in patients with a low or intermediate platelet count. In view of the less effective rate and high adverse reaction rate by IFN in elderly patients, IFN therapy should be considered primarily for those with a low platelet count of 150,000/mm^3 or less. Because low platelet count was closely associated with advanced disease and high risk for carcinogenesis, treatment efficacy appeared prominent in the subgroup with low and intermediate platelet counts. The best candidates for IFN therapy were those with a low platelet count, also in regard to cost-effectiveness. Because a low platelet count is closely associated with advanced stages of liver disease, IFN therapy should be avoided for elderly patients with decompensated cirrhosis or severely decreased platelet count of less than 50,000/mm^3. A sustained virologic response improves clinical symptoms in decompensated cirrhosis,[31] but IFN often induces severe complications even in young patients with decompensated cirrhosis.[32] An elderly patient with hepatitis without decompensation can be a candidate for IFN therapy if careful, close hematologic monitoring is performed. Low-dose, intermittent, long-term IFN therapy also should be considered for these patients to obtain a sustained biochemical response without creating profound and irreversible side effects. Because elderly patients generally showed some difficulties with IFN treatment, our current study demonstrated practical information about carcinogenesis and the life expectancy of elderly patients with HCV and the order of priority in management of IFN for these patients. IFN administration is preferably considered and initiated at the age of 60 years or less to reduce side effects.

CONCLUSIONS

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

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Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study

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SUMMARY. An impact of serum hepatitis B virus (HBV) DNA on hepatocarcinogenesis has not been investigated in a cohort of patients with non-B, non-C cirrhosis. Eighty-two consecutive Japanese patients with cirrhosis, who showed negative hepatitis B surface antigen and negative anti-hepatitis C virus, were observed for a median of 5.8 years. Hepatitis B virus core (HBc) region and HBx region were assayed with nested polymerase chain reaction. Both of HBc and HBx DNA were positive in 9 patients (11.0%) and both were negative in 73. Carcinogenesis rates in the whole patients were 13.5% at the end of the 5th year and 24.6% at the 10th year. The carcinogenesis rates in the patients with positive DNA group and negative DNA group were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively ($P = 0.0078$). Multivariate analysis showed that men ($P = 0.04$), presence of HBc and HBx DNA (hazard ratio: 8.25, $P = 0.003$), less total alcohol intake ($P = 0.010$), older age ($P = 0.010$), and association of diabetes ($P = 0.005$) were independently associated with hepatocellular carcinogenesis. Existence of serum HBV DNA predicted a high hepatocellular carcinogenesis rate in a cohort of patients with non-B, non-C cirrhosis.

Keywords: hepatitis B virus, hepatocellular carcinogenesis, liver cirrhosis, occult hepatitis B virus infection, proportional hazard model.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia [1,2]. It is also one of the most common neoplasms in Japan [3]. Hepatitis B virus (HBV) infection is the primary cause of cirrhosis and HCC and one of the major causes of death globally [4]. Needless to say, a cohort of patients with HBV-related chronic hepatitis and cirrhosis has a significantly high risk for HCC development [5–7]. In our retrospective cohort studies concerning HBV-related disease, cumulative hepatocellular carcinogenesis rates in chronic hepatitis ($n = 610$) and cirrhosis ($n = 180$) were 2.1% and 7.2% at the end of the 5th year, and 4.9% and 27.2% at the 10th year, respectively [5,7]. Abundant epidemiological and molecular biological evidence shows that HBV is an important factor in the development of HCC [8–10], but the precise role of HBV in the oncogenesis is still unknown.

HBV infection is usually diagnosed when the circulating hepatitis B surface antigen (HBsAg) is detected. However, the availability of highly sensitive molecular biology techniques has allowed the identification of HBV infection in HBsAg-negative individuals with or without circulating antibodies to HBsAg and/or hepatitis B core antigen (anti-HBc) [11–16]. Much evidence suggests that this so-called occult HBV infection is highly prevalent in a number of patient subgroups including those with HCV infection [16,17], cryptogenic advanced liver fibrosis [18] and HCC [17,19–27]. Although Marusawa *et al.* [28] and Uetake *et al.* [29] described the relationship between anti-HBc and HCC appearance rate in each study, impact of occult HBV infection on carcinogenesis cannot be evaluated because of lack of HBV DNA assay. As all the previous studies were performed as a pilot study or a case-controlled one, actual risk ratio of occult HBV infection for hepatocellular carcinogenesis has not been reported in a cohort study until now.

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartic transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCR, polymerase chain reaction.

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We, therefore, analysed a retrospective cohort of consecutive patients with cirrhosis for a long period, in order to elucidate the influence of occult HBV infection on the carcinogenesis rate from non-B, non-C cirrhosis.

PATIENTS AND METHODS

Patients

Among 103 consecutive patients diagnosed as having non-B, non-C cirrhosis by peritoneoscopic liver biopsy at Toranomon Hospital, Tokyo, Japan in the period from 1976 to 1998, initial frozen sera at the time of the diagnosis of cirrhosis were available for the assay of HBV DNA in 82 patients (79.6%). The cohort of 82 patients was retrospectively observed for a long period. All the patients showed negative HBsAg, negative anti-hepatitis C virus (HCV) and negative HCV RNA. Patients with a possible association of HCC at the time of the diagnosis of cirrhosis were strictly excluded from this study. No patient received interferon or other antiviral therapy after the diagnosis of cirrhosis.

Background and laboratory data of the patients

There were 67 men and 15 women aged 34–80 with a median age of 58 years. A total of 47 patients (57.3%) had a history of alcohol intake of more than 500 kg until the diagnosis of liver cirrhosis. Fifteen patients (18.3%) had decompensated cirrhosis with ascites, a history of encephalopathy, or both. The median value of indocyanine green retention rate at 15 min (ICG R15) was 33% (range, 7–75%), and total bilirubin concentration was 1.3 mg/dL (range 0.4–20.9 mg/dL).

Measurement of hepatitis virus markers

Hepatitis virus markers were assayed using frozen sera at −80 °C. All sera were tested for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan), anti-HCV (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot), and HCV RNA with reverse transcription-nested polymerase chain reaction (PCR).

HBV DNA was analysed for the region of HBc and HBx by sensitive nested PCR according to Yotsuyanagi *et al.* [30]. Fifty microlitres of STE solution [100 mmol/L Tris–HCl (pH 8.0), 100 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid (pH 8.0), and 0.2% sodium dodecyl sulphate] with 20 μg of proteinase K (Boehringer, Mannheim, Germany) were added to serum samples. Mixed samples were then incubated for 2 h at 55 °C. DNA was extracted twice with phenol/chloroform, once with chloroform, and precipitated with ethanol. The DNA pellet was dissolved in 25 μL of TE buffer [10 mmol/L Tris–HCl (pH 8.0) and 1 mmol/L ethylenediaminetetraacetic acid (pH 8.0)].

Prepared DNA was subjected to amplification using nested PCR technique. HBV DNA was amplified using two independent pairs of primers, with each primer complementary to sequences in the X or core region of the HBV genome [30]. Amplification was performed using a thermal cycler for a total of 40 cycles, with each cycle consisting of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, in 100 μL of reaction mixture containing 200 mmol/L of each dNTP, 1X PCR buffer [50 mmol/L KCl, 10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$ and 0.001% (w/v) gelatine], and 2 units of Ampli-Taq polymerase (Perkin Elmer Cetus Corp., Norwalk, CT, USA). The PCR products were separated in a 2% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Dassel, Germany). The membrane was then probed with digoxigenin-labelled oligonucleotides, which hybridize specifically with the core or X gene. Results were considered valid only if the same results were obtained in at least two separate experiments.

We considered the cases with positivity in at least two different viral genomic regions as HBV DNA positive. Appropriate negative controls were included in each PCR. The limit of sensitivity of our nested PCR methods ranged from 10 to 1 genome equivalents/mL.

Follow-up of patients

Follow-up of the patients was made on a monthly or bimonthly basis after diagnosis of cirrhosis by monitoring alpha-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was made at least once a year for each patient with CT or US. After 1988, in order to detect HCC earlier, imagings were done three or more times per year in a majority of patients.

No patient underwent interferon therapy after the diagnosis of cirrhosis, but some of the patients received an oral or intravenous administration of medicinal herbs during the follow-up period.

All patients were finally evaluated in November 2004. The cases lost to follow-up were 13 (15.9%). The median observation period of the total patients was 5.8 years with a range of 0.1–34.8 years.

Statistical analysis

Differences of background features and laboratory data between the patients with and without HBV DNA were analysed by chi-square test, Fisher's exact test and Mann–Whitney's *U*-test. The time between diagnosis of cirrhosis and appearance of HCC was analysed using the Kaplan–Meier technique [31] and differences in curves were tested using log-rank test [32]. Those patients who had been lost to follow-up were regarded as censored data at the time of missing in the statistics. Independent risk factors associated with the appearance rate of HCC were studied using the stepwise Cox regression analysis [33]. Potential risk factors

assessed for hepatocellular carcinogenesis included the following 18 variables: age, sex, association of diabetes mellitus, total alcohol intake, history of cigarette smoking, family history of liver disease, history of blood transfusion, state of cirrhosis (presence of ascites and/or a history of encephalopathy), HBc DNA, HBx DNA, aspartic transaminase (AST), alanine transaminase (ALT), albumin, bilirubin, globulin, AFP, platelet, and ICG R15. A probability less than 0.05 was considered as significant. Data analysis was performed using computer program SPSS version 11 [34].

RESULTS

HCC appearance rate in all the patients

During the observation period, HCC appeared in 16 patients (19.5%). Median interval between the diagnosis of cirrhosis and HCC was 5.6 years (range 0.7–15.6 years) in the patients with HCC development. The cumulative HCC appearance rate in the 82 patients was 13.5% at the end of the fifth year after the diagnosis of cirrhosis, 24.6% at the end of tenth year, 33.3% at the 15th year, and 41.6% at the end of 20th year.

HCC appearance rates according to serum HBV DNA

Among the 82 patients, 9 patients (11.0%) showed positive serum HBV DNA and 73 (89.0%) negative HBV DNA. The former 9 patients had both HBc DNA and HBx DNA, and the latter 73 had neither of them. Table 1 summarizes the profiles and laboratory data of each group. There was no demographic difference between the two groups. There was also no statistically significant difference between them except for ALT value, which was lower in the patient group with positive HBV DNA (P = 0.028).

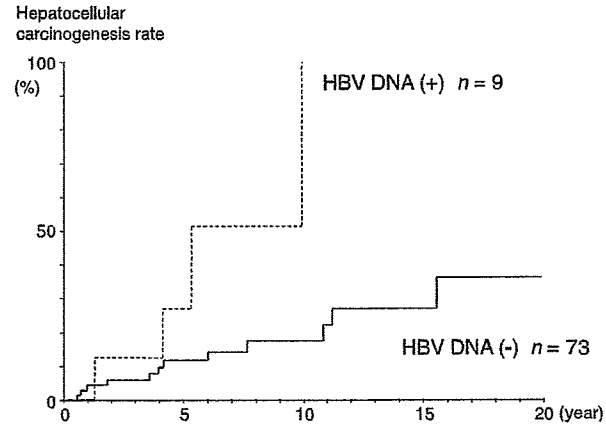

Fig. 1 Hepatocellular carcinogenesis curves of the patients with and without serum hepatitis B virus DNA. Carcinogenesis rates were 12.5% and 6.0% at the end of the third year, 27.0% and 11.8% at the fifth year, and 100% and 17.6% at the tenth year, respectively.

Figure 1 shows the curves of crude HCC appearance rate in the two patients group with and without serum HBV DNA. The third-year HCC appearance rates in the patients with and without DNA were 12.5% and 6.0%, the 5th-yr rates 27.0%, 11.8%, the tenth-yr rates 100% and 17.6%, respectively. The HCC appearance rate of the patient group

Table 1 Demography and laboratory data of patients with and without serum hepatitis B virus DNA

	HBV DNA*		
	Positive (n = 9)	Negative (n = 73)	P
Demographic and background features			
Sex – men/women	8/1	59/14	0.55
Age (median, range)	51 (45–68)	58 (34–80)	0.44
History of transfusion	1 (11.1%)	14 (19.4%)	0.55
Alcohol intake of 500 kg or more	5 (55.6%)	42 (58.3%)	0.87
Diabetes mellitus	3 (33.3%)	15 (20.8%)	0.40
Observation period (years)	5.7 (1.0–21.0)	6.1 (0.1–34.8)	0.92
Laboratory data (median, range)			
ICG R15 (%)	34 (12–51)	32.5 (7–75)	0.78
AST (IU/L)	32 (17–86)	40.5 (14–184)	0.26
ALT (IU/L)	16 (9–43)	28.5 (4–160)	0.028
Albumin (g/dL)	3.8 (2.6–4.5)	3.6 (1.7–5.2)	0.20
Bilirubin (mg/dL)	0.9 (0.5–2.8)	1.3 (0.4–20.9)	0.14
Platelet (×1000/mm^3)	142 (67–232)	104 (27–647)	0.18
AFP (ng/mL)	5 (3–9)	6 (1–98)	0.38

ICG R15, indocyanine green retention rate at 15 min; AST, aspartic transaminase; ALT, alanine transaminase; AFP, alpha-fetoprotein. *HBV DNA was assessed for HBc and HBx DNA using polymerase chain reaction