PEG-IFN/RBV therapy. These results indicate the importance of immunological status and immunological response to treatment in patients difficult to treat with PEG-IFN/RBV therapy for CHC.

The present univariate analyses revealed that there were many factors relating to RVR, cEVR, and SVR including LDL-C, HOMA-IR, fatty change in liver tissue, and hyaluronic acid, however some of these factors had not been examined in some participating institutes. We consider that we must perform a prospective mass study using many factors including immunological aspects, viral factors, disease status, and therapeutic aspects to elucidate the reason that older female patients are resistant to a combination of PEG-IFN and RBV therapy in CHC with a high viral load genotype 1b.

In conclusion, our results demonstrated that wild type core aa 70, two or more aa mutations in the ISDR, low viral load, high PLT counts, and male gender are useful markers for predicting SVR.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Etiology of liver cirrhosis in Japan: a nationwide survey

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Abstract
Background Little is understood about worldwide changes in the epidemiological distribution of the etiology of liver cirrhosis (LC). The present study examines the etiology of liver cirrhosis in Japan using a nationwide survey.
Methods We analyzed data from 33,379 patients with LC at 58 hospitals and presented the findings in a poster symposium regarding the etiology and clinical features of LC in Japan that was included in the program of the 44th Annual Meeting of the Japan Society of Hepatology. We identified the distribution of the etiology of LC and compared the present with previous Japanese findings to estimate the future of etiological changes in LC.
Results The etiological agents were as follows: hepatitis B virus (HBV) 13.9%, hepatitis C virus (HCV) 60.9%, alcohol 13.6%, primary biliary cirrhosis (PBC) 2.4% and autoimmune hepatitis (AIH) 1.9%. Cirrhosis was considered to be related to nonalcoholic steatohepatitis (NASH) in 2.1% of the patients. The ratio of HCV-related LC was significantly higher among patients with hepatocellular carcinoma (HCC) ($P < 0.0001$) compared to those without, whereas the ratios of alcohol, PBC, AIH were lower. HCC was evident in 31.5% of NASH-related LC.
Conclusions The major etiology of liver cirrhosis in Japan remains HCV. Our survey revealed the prevalence of NASH-related LC in Japan and the frequency of HCC. Future changes in etiology must be considered in establishing preventive or educational strategies, as well as in developing new treatment strategies.

Keywords Carcinogenesis · Hepatitis B virus · Hepatitis C virus · Hepatocellular carcinoma · Nonalcoholic steatohepatitis

Abbreviations

AIH	Autoimmune hepatitis
ANA	Anti-nuclear antibody
BMI	Body mass index
DM	Diabetes mellitus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LC	Liver cirrhosis
MetS	Metabolic syndrome

Participating investigators of The Japan Etiology of Liver Cirrhosis Study Group are listed in the Appendix.

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NAFLD Nonalcoholic fatty liver disease
NASH Nonalcoholic steatohepatitis
PBC Primary biliary cirrhosis
PSC Primary sclerosing cholangitis

Introduction

Liver cirrhosis (LC) is a life-threatening, major worldwide health problem that is defined as regenerative nodule development after chronic liver diseases. A considerable ratio of patients with LC can progress to liver failure, hepatocellular carcinoma (HCC) and portal hypertension. Chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and alcohol consumption are the major global causes of liver cirrhosis, but the epidemiology and etiology are not well described. Alcohol and HCV are common causes of LC in European, North American and other developed countries, whereas HBV is the major cause in many Asian and African countries [1, 2]. Information about the exact ratios of LC etiology in individual areas or countries is minimal, and few Japanese reports describe analyses of large patient cohorts or nationwide surveys. Globally, about 57% of cirrhosis was attributable in 2007 to either HBV (30%) or HCV (27%) [2]. Reports indicate that alcohol is the leading cause of LC, followed by HCV in the United States of America (USA) and the United Kingdom (UK), whereas HCV is the major cause in Italy [1–5]. On the other hand, HBV is the major cause of LC in endemic areas. Liver cirrhosis of unknown causes has been referred to as cryptogenic cirrhosis, and nonalcoholic steatohepatitis (NASH) is recognized as an important cause of cryptogenic LC and/or HCC [6–9]. However, the exact prevalence of NASH-related LC is unknown.

Based on this background we analyzed the etiology and clinical features of 33,379 patients with LC from 58 hospitals nationwide and then determined accurate etiological ratios for liver cirrhosis in Japan in July 2008.

Patients and methods

Patients

A group of 58 hospitals throughout Japan responded to mailed questionnaires regarding the etiology of liver cirrhosis. The data from 33,379 patients with liver cirrhosis were presented in a poster symposium regarding the etiology and clinical features of LC at the 44th Annual Meeting of the Japan Society of Hepatology during June 2008. We included all university hospitals and other major hospitals in Japan that contribute to the care of liver diseases. The appendix lists the cooperating institutions. The ethics committees of the appropriate institutional review boards approved this study in accordance with the Declaration of Helsinki 2000.

Criteria and questionnaire

Table 1 lists the criteria for LC and the definition of etiology applied in this study. We enrolled patients who were

Table 1 Criteria for diagnosis of liver cirrhosis and classification of etiology

I. Criteria for diagnosis of liver cirrhosis

Autopsy, laparoscopy or abdominal imaging (left lobe hypertrophy with splenomegaly, nodular changes in liver surface) and laboratory findings (low platelet count, albumin, and/or prolonged prothrombin time) compatible with liver cirrhosis. Also clinically diagnosed in patients with clinical findings of esophageal varices, ascites, or hepatic encephalopathy. Patients diagnosed solely based on histological liver biopsy findings are excluded.

II. Criteria for classification of etiology

1. Hepatitis B virus (HBV): positive for HBsAg and/or anti-HBc with high titer
2. Hepatitis C virus (HCV): positive for anti-HCV and HCV-RNA
3. HBV + HCV
4. Alcohol: criteria proposed by the Japanese Study Group of Alcoholic Liver Disease
5. Primary biliary cirrhosis
6. Other biliary cirrhosis (primary sclerosing cholangitis, etc.)
7. Autoimmune hepatitis
8. Metabolic diseases (Wilson disease, hemochromatosis, etc.)
9. Congestive disease
10. Parasites
11. Other known etiology
12. Nonalcoholic steatohepatitis (NASH): fulfillment of criteria described below and not meeting any criteria for above known etiologies.
13. Unknown etiology

Table 2 Criteria for NASH-related cirrhosis

I. Clinically supposed NASH-related cirrhosis

Fulfillment of the following criteria but without liver biopsy.

1. Alcohol consumption: less than 20 g/day
2. No other etiology for liver disease
3. Combined with diseases or states that could cause fatty liver diseases such as obesity (body mass index >25), diabetes mellitus and metabolic syndrome.

II. Histologically diagnosed NASH-related cirrhosis

Fulfillment of Criteria I, and histological liver biopsy findings are suitable with NASH (micronodular cirrhosis, perisinusoidal fibrosis, fatty change).

histologically and clinically diagnosed with LC, and with LC complicated by HCC. Since consensus has not been reached regarding criteria for liver cirrhosis caused by NASH, we tentatively established the criteria shown in Table 2. Final diagnosis of LC and diagnosis of etiology was determined in each institution. We also collected clinical information (age, gender, body mass index, complicating diseases and laboratory data) about patients with LC related to NASH or of unknown etiology. Alcoholic LC was diagnosed according to the criteria proposed by Takada et al. [10]. The time of diagnosis was not restricted in this retrospective study.

Statistical analyses

Data were statistically analyzed by the χ^2 test and by Student's t test using SPSS version 13.0J software (SPSS, Inc., Tokyo, Japan). All statistical tests were two-sided. P values below 0.05 were considered significant.

Results

Etiology of liver cirrhosis

Of the 33,379 patients with LC included in this study, 20,817 (62.4%) were male, 12,562 (37.6%) were female and 16,117 overall (48.3%) had HCC at the time of diagnosis with LC.

Figure 1 compares the etiology with previous Japanese data. The present study found the following causes of LC: HCV 60.9%, HBV 13.9%, alcohol 13.6%, PBC 2.4%, NASH-related 2.1% and AIH 1.9%. Among the remaining 4.0% of patients, other known and unknown etiologies were identified in 1.0 and 3.0%, respectively. Other known etiologies comprised: other biliary cirrhosis 0.3%, congestive cirrhosis 0.3% and parasites 0.1%. Other biliary cirrhosis ($n = 104$ patients) comprised primary sclerosing cholangitis (PSC; $n = 66$), congenital biliary atresia ($n = 16$), and others ($n = 22$). Metabolic diseases ($n = 91$) comprised Wilson's disease ($n = 55$), hemochromatosis ($n = 24$), glycogen storage disease ($n = 5$), citrunemia ($n = 3$), porphyria ($n = 3$) and amyloidosis ($n = 1$).

The results show that hepatitis virus, particularly HCV, remains a major cause of liver cirrhosis in Japan. On the other hand, the incidences of HBV and of alcohol-induced LC are decreasing. We focused on NASH-related LC for this analysis, and speculated that NASH represents a major unrecognized etiology. Data from 1998 show total values for LC excluding HBV, HCV and alcohol, of 8.8%, compared with the current value of 9.9%. Thus, NASH seemed to have historically been categorized as liver cirrhosis of unknown etiology.

Geographic differences in Japan

Figure 2 shows the geographic distribution of the etiology of liver cirrhosis in Japan. The most prevalent source in almost all areas in Japan except Okinawa was HCV. Alcohol was the most prevalent in Okinawa, followed by HCV and HBV. The prevalence of HBV was relatively higher in Hokkaido, Kyushu and in some western areas, and NASH was also more frequent (10%; fivefold higher than in other areas) in Japan.

Differences between males and females

Figure 3a shows differences in the etiology of LC between males and females. The ratio of alcohol was higher among males (19.2 vs. 4.3%; $P < 0.0001$, χ^2 test), whereas the ratios of PBC (0.6 vs. 5.3%), AIH (0.4 vs. 4.3%) and NASH (1.4 vs. 3.4%) were higher among females ($P < 0.0001$ for all). More female patients had LC of unknown etiology (2.3 vs. 4.0%, $P < 0.0001$). The numbers of males and females in the group with other known etiology were PSC, 44 and 22, Wilson's disease, 31 and 24 and hemochromatosis, 18 and 6, respectively.

Difference in etiology with or without HCC

The patients were categorized based on the presence of HCC, and then the etiology was analyzed (Fig. 3b). The

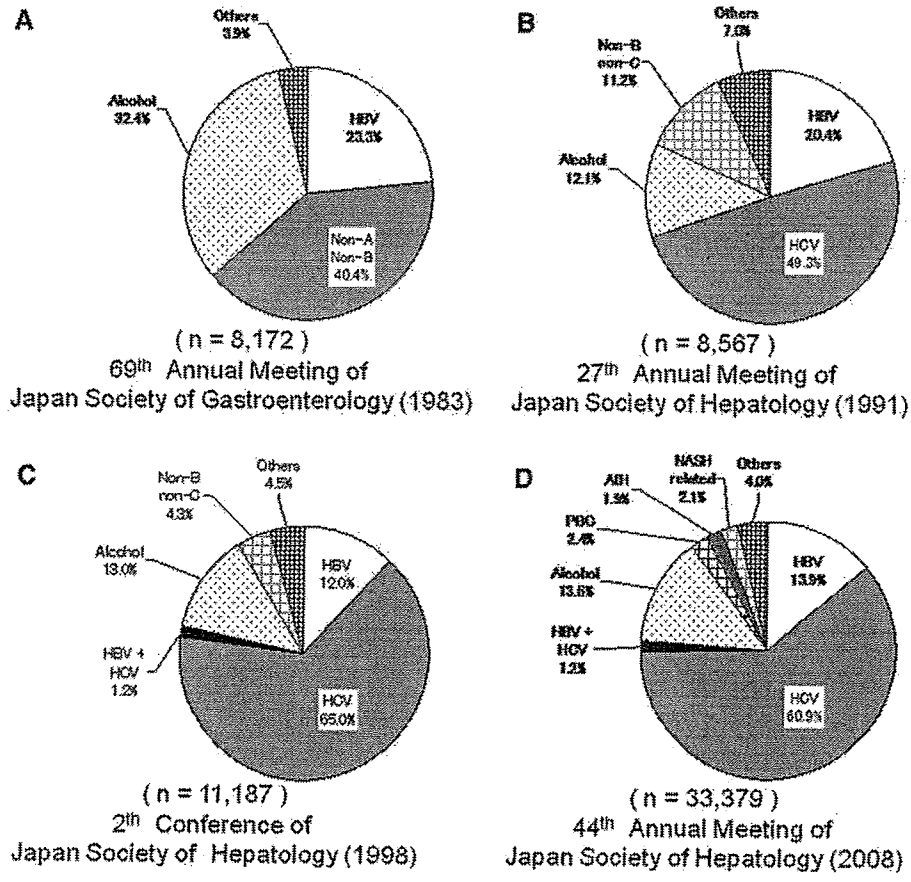

Fig. 1 Etiology of liver cirrhosis in Japan. Data presented at the 69th Annual Meeting of Japan Society of Gastroenterology in 1983 (edited by Dr. Sukeo Yamamoto) (**a**), 27th Annual Meeting of Japan Society of Hepatology in 1991 (edited by Dr. Yasuyuki Ohta) (**b**), 2nd Conference of Japan Society of Hepatology in 1998 (edited by Dr. Kennichi Kobayashi) (**c**), and 44th Annual Meeting of Japan Society of Hepatology (present study) (**d**)

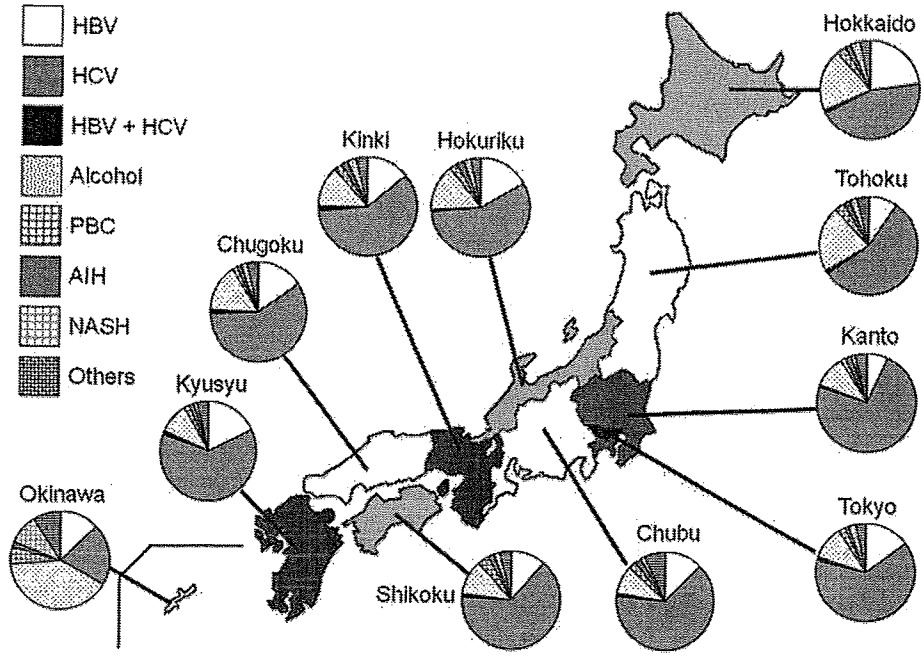

Fig. 2 Geographic distribution of etiology of liver cirrhosis in Japan

ratios among patients without HCC were HCV 49.4%, alcohol 20.5%, HBV 13.7%, PBC 4.0% and AIH 3.1%. On the other hand, the ratio of HCV was significantly higher (73.1%; $P < 0.0001$; χ^2 test), whereas those of alcohol, PBC, AIH were significantly lower (6.3, 0.6, 0.6%, $P < 0.0001$, respectively), and the findings were similar for HBV (14.1%). Among the metabolic diseases, HCC was complicated with Wilson's disease, hemochromatosis and

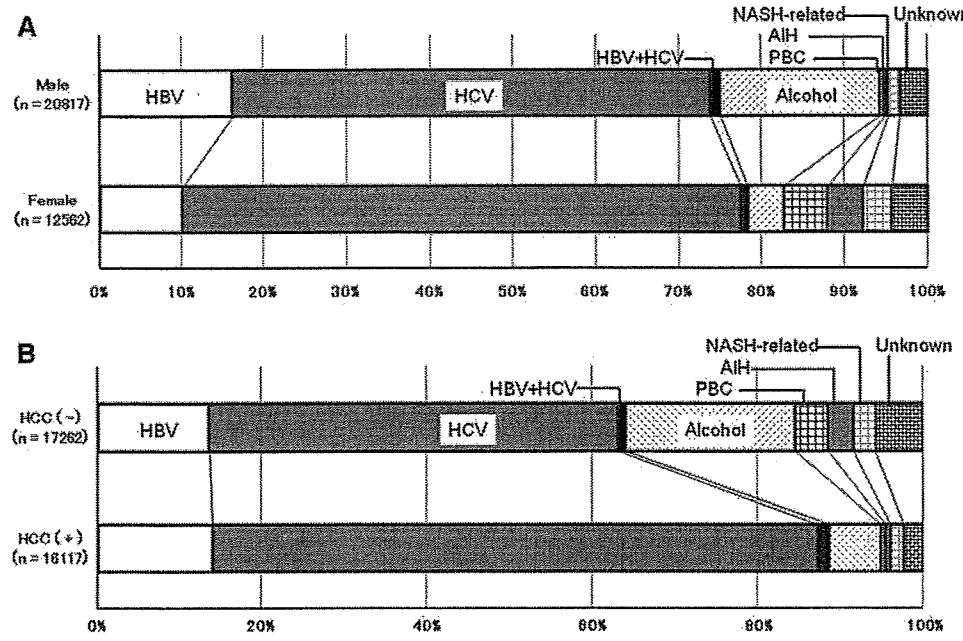

Fig. 3 Etiology of liver cirrhosis classified by gender and hepatocellular carcinoma. Data groups are separated based on gender (**a**) and hepatocellular carcinoma (**b**)

Table 3 Clinical features of NASH-related liver cirrhosis

	Total	Male (M)	Female (F)	*P* value (M vs. F)
Patients (%)	647	261 (40.3)	386 (59.7)	
Age (years)	66.6 ± 10.9	64.2 ± 12.2	68.2 ± 9.5	<0.001*
BMI (kg/m^2)	27.6 ± 4.5	26.9 ± 4.1	28.0 ± 4.8	<0.005*
Total cholesterol (mg/dl)	168.7 ± 49.1	170.1 ± 49.0	167.7 ± 49.2	NS
Triglyceride (mg/dl)	114.6 ± 114.6	124.5 ± 93.7	107.9 ± 68.0	<0.05*
Fasting plasma glucose (mg/dl)	138.6 ± 56.8	140.6 ± 58.5	137.2 ± 55.6	NS
Fasting insulin (μU/ml)	22.5 ± 25.2	20.1 ± 14.8	24.1 ± 30.3	NS
HOMA-IR	7.74 ± 9.24	7.51 ± 9.05	7.90 ± 9.41	NS
Hypertension (%)	316 (50.2)	110 (43.1)	206 (54.9)	<0.001*
Diabetes Mellitus (%)	424 (66.6)	175 (67.8)	249 (65.7)	NS
Hepatocellular carcinoma (%)	199 (31.5)	109 (42.2)	90 (24.1)	<0.05*

NS not significant

* *P* value determined by Student's *t* test

glycogen storage disease in 2 of 55, 4 of 24 and 2 of 5 patients, respectively.

NASH-related cirrhosis

Nonalcoholic steatohepatitis was associated with 2.1% of all LC; that is, in 2.7 and 1.6% of the groups without and with HCC complications, respectively. Table 3 shows the clinical background including laboratory data, complications and features of NASH-related LC in 647 patients. Mean age and body mass index (BMI) were 66.6 ± 10.9 and 27.6 ± 4.5, respectively. The women were older and had a higher BMI ($P < 0.001$ and <0.005, respectively) than the men. Hypertension and diabetes mellitus were complications in 50.2 and 66.6%, respectively, of those with NASH-related LC. HCC was frequently complicated with NASH-related LC (31.5%), especially among males (males vs. females: 42.2 vs. 24.1%, $P < 0.005$). Moreover, 10% of NASH-related LC was complicated with HCC during our 10-year study period. However, precise data about the study period of each followed-up patient was unavailable, so the accurate occurrence rate of HCC among patients with NASH could not be determined from this study. The prevalence of hypertension was higher in women, whereas that of HCC was higher among men. Anti-nuclear antibody (ANA) was found in 36.7% of all patients (males vs. females: 31.5 vs. 37.2%). One-third of patients with NASH were also positive for ANA, and the

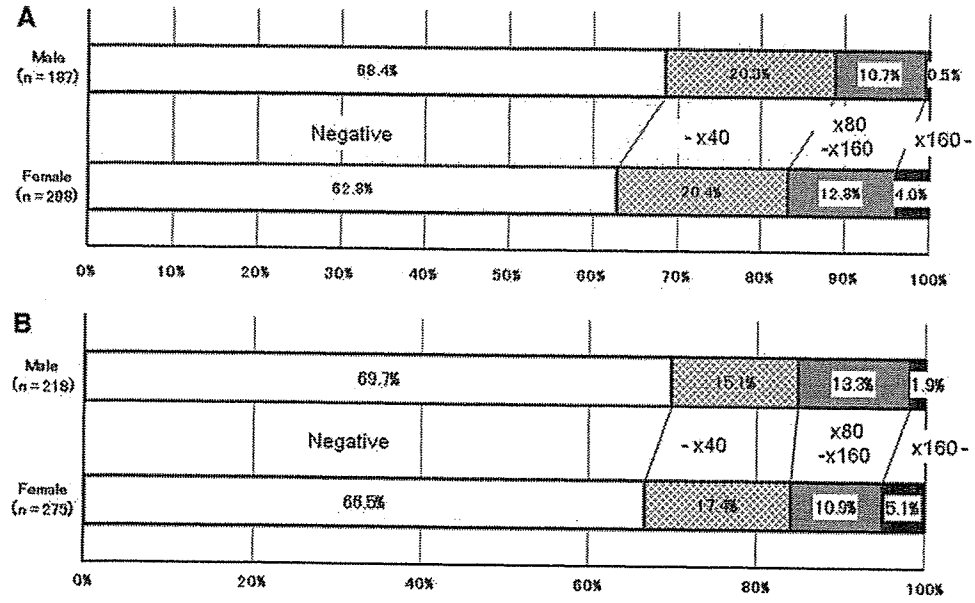

Fig. 4 Prevalence of anti-nucleic antibody (ANA) in liver cirrhosis that is NASH-related and of unknown etiology. Data show prevalence of ANA in LC related to NASH (**a**), and in LC of unknown etiology classified according to gender (**b**)

Table 4 Clinical features of liver cirrhosis of unknown etiology

	Total	Male (M)	Female (F)	*P* value (M vs. F)
Case (%)	801	385 (48.1)	416 (51.9)	
Age (years)	68.6 ± 11.1	66.8 ± 11.4	70.3 ± 10.5	<0.001*
BMI (kg/m^2)	23.9 ± 4.3	23.6 ± 3.8	24.2 ± 4.7	<0.001*
Total cholesterol (mg/dl)	152.4 ± 46.8	155.3 ± 51.1	149.7 ± 42.5	NS
Triglyceride (mg/dl)	88.3 ± 53.0	94.7 ± 58.6	82.4 ± 46.6	<0.01*
Fasting plasma glucose (mg/dl)	118.1 ± 46.6	118.5 ± 42.2	117.8 ± 50.3	NS
Fasting insulin (μU/ml)	14.0 ± 12.2	14.4 ± 14.4	13.6 ± 10.2	NS
HOMA-IR	4.0 ± 3.8	3.9 ± 2.8	4.2 ± 4.5	NS
Hypertension (%)	230 (30.7)	97 (27.6)	133 (33.5)	NS
Diabetes mellitus (%)	229 (30.3)	120 (33.8)	109 (27.3)	<0.001*
Hepatocellular carcinoma (%)	248 (32.6)	157 (43.0)	91 (23.0)	<0.001*

NS not significant

* *P* value determined by Student's *t* test

prevalence of ANA positivity was higher among females than males ($P < 0.005$; Fig. 4a).

Table 2 shows that we diagnosed NASH-related LC in one group that had been histologically diagnosed with NASH (39.4%; 255/647), and in another with clinically supposed NASH (60.6%; 392/647) without a histological examination. The frequency of HCC did not differ between histologically proven and supposed NASH (28.2 vs. 33.6%). Moreover, the positive rates of HBc antibodies did not differ between NASH-related LC with and without HCC (22.2 vs. 23.0%). We could not identify any other significant differences in the backgrounds of the patients between these groups. The positive ratio for ANA also did not differ between histologically diagnosed and supposed NASH (33.0 vs. 36.8%, respectively).

Cirrhosis of unknown etiology

We found LC of unknown etiology in 3.0% of the patients (3.8 and 1.8% with and without HCC complications, respectively). Table 4 shows the clinical data of 801 patients (mean age 68.6 ± 11.1 years). Age, BMI, triglyceride levels and HCC occurrence were significantly higher among females than males, findings similar to those of NASH-related LC (Table 3). Complication with diabetes mellitus was more prevalent among males than females among the patients with LC of unknown etiology. On the other hand, hypertension was more evident in females than in males among the patients with NASH-related LC. ANA was found in 33.9% of all patients (males vs. females: 30.3 vs. 33.5%; Fig. 4b). The positivity rate in females was

higher than that in males ($P < 0.005$), but that of ANA did not differ between total LC of unknown etiology and NASH-related LC (33.9 vs. 36.7%, respectively).

Discussion

The present study confirmed that HCV infection is the most prevalent cause of LC in Japan, accounting for about 60% of all LC. The induction of LC by HBV and alcohol was similarly prevalent, and these comprised the second and third most prevalent etiological factors. Causes related to NASH accounted for only 2.1% of LC in Japan. Among other types of metabolic cirrhosis, Wilson's disease was the leading cause, followed by hemochromatosis. A comparison with previous Japanese data showed that HCV remains the major etiology of liver cirrhosis in Japan, while the ratio of HBV has decreased over the past 17 years (Fig. 1b). Patients in Japan become infected with HCV at peak ages that are about 10–20 years older than those in the USA and European countries [11]. The present etiological features of HCV-induced LC in Japan are thus likely to be reflected in the USA and in European countries 10–20 years later.

The distribution of the etiology of liver cirrhosis in each area was similar in all geographic areas of Japan except Okinawa (Fig. 2), which was under American occupation for 27 years after World War II. Compared with other parts of Japan, Okinawa has more American cultural influences including food (such as fast-foods that have higher fat content and more calories). By the year 2000 (60 years after World War II), the mean lifespan in Okinawa had decreased from being the longest in Japan, while the prevalence of obesity had increased to being the highest in Japan [12, 13]. Such influences will be related to the increased ratio of NASH compared with other areas of Japan. Moreover, the prevalence of alcohol-related cirrhosis is higher and the ratio of HCV is lower in Okinawa than in other areas of Japan. These reasons will be clarified in future studies.

We analyzed the data based on gender and HCC (Fig. 3). The results indicated that alcohol-induced LC is more predominant among males, whereas HCV, autoimmune liver diseases (AIH or PBC) and NASH are more predominant among females. AIH occurs more frequently among females, and males consume more alcohol than females in Japan. Annual health screening has revealed that more males are HBs-antigen positive [14], whereas the frequency of HCV-antibody positivity is similar in both males and females [15]. More females than males had LC of unknown etiology. We suspect that this group included some patients with autoimmune or NASH-related cirrhosis that was not classified by the present criteria.

The ratios of etiologies among patients with HCC in the present study were comparable with those of other national surveys of HCC in Japan [16]. Additionally, our analyses clarified differences in etiological ratios between LC with and without HCC (Fig. 3). The ratio of HCV was higher among LC patients with HCC (67.2 vs. 57.5%, $P < 0.0001$), indicating that HCV itself has the potential to evoke hepatocarcinogenesis in patients with LC. On the other hand, HBV, alcohol, AIH and NASH would have less potential to evoke HCC than HCV. Although a prospective study is required to confirm this notion, these data are nevertheless sufficient to suggest that HCV infection contributes to hepatocarcinogenesis. Notably, many patients with HCC also had LC caused by alcohol, PBC, AIH and that related to NASH. Therefore, cirrhotic patients with not only viral hepatitis but also with these non-viral etiologies should be screened for HCC.

Recently, NASH has become recognized as an important cause of LC. Diagnostic criteria for nonalcoholic fatty liver disease (NAFLD) or NASH have been discussed elsewhere [17, 18]. Although the gold standard for a diagnosis of NASH is a liver biopsy, a risk of sampling error persists [17]. Furthermore, to obtain liver biopsies from patients with advanced cirrhosis is hazardous. Therefore, the actual prevalence of NASH-related LC has been difficult to define. Cryptogenic cirrhosis is thought to include NASH-related LC, and metabolic syndrome (MetS) is often a complication of NASH [18]. However, the importance of NASH in the etiology of liver cirrhosis remains ambiguous.

We considered that clinical etiologic criteria without a liver biopsy are needed to determine the accurate ratios of NASH-related cirrhosis due to the above reasons. The etiological criteria for clinically supposed NASH-related cirrhosis satisfied all of the factors listed in Table 2. We included obesity, diabetes mellitus (DM) and MetS among these criteria. The backgrounds of the patients diagnosed histologically and non-histologically with NASH-related LC did not differ. Under this classification, 0.9 and 1.2% of the 2.1% of patients with NASH-related LC were diagnosed histologically and non-histologically, respectively. Bell et al. reported that NAFLD accounted for 14.7% of LC in USA [4]. Thus, the frequency of NASH (NAFLD)-related LC is lower in Japan than in the USA, as is the frequency of MetS [19, 20]. The data show that the present frequency of NASH-related LC in Japan is quite low. However, the frequency of NASH-related LC will increase in Japan due to alterations in lifestyles (such as increased food consumption, type of food, stress and sedentary lifestyles), whereas that of HCV or HBV-related LC will decrease [21], as shown in Okinawa.

The frequency of HCC combined with NASH-related LC was high, especially among males (Table 3). The total

frequency of HCC in NASH-related LC herein was higher than in previous reports [22]. One reason for this is that our criteria for NASH-related LC included patients with higher-risk HCC compared with other studies. However, the frequencies of HCC in histologically defined and non-histologically supposed NASH did not differ. Another explanation is that occult HBV could be related to hepatocarcinogenesis in NASH-related HCC. However, the positivity rates of HBc antibody did not differ between NASH-related LC with and without HCC. Thus, the above factors probably did not influence our results. The reported incidence of HCC is higher in males with type 2 DM than in females [23, 24]. This evidence might be related to our findings, but further studies are required to reach a conclusion.

According to our criteria, cryptogenic LC (or LC of unknown etiology) of patients who do not consume alcohol but who were obese or complicated with DM or MetS were classified as having NASH-related LC. On the other hand, some patients with NASH-related cirrhosis without obesity, DM and MetS might have been included in the group with LC of unknown etiology. This group accounted for 3% of the studied patients, which was lower than that previously reported [1, 3, 4]. This group included LC due to viruses other than HBV and HCV, undiagnosed congenital diseases, undiagnosed AIH and patients with NASH who did not fulfill the study criteria. Patients who had been HBV carriers but who had become negative for HBsAg, or patients with occult HBV might have also been included [25]. However, the backgrounds of the male and female patients with NASH-related LC and LC of unknown etiology were quite similar (Tables 3, 4), as were the positivity and distribution of the ANA titers in both groups (Fig. 4). Further studies should clarify the real cause of LC with unknown etiology. Nevertheless, the present results suggest that some patients with NASH-related LC were included in the group with unknown etiology, rather than patients with undiagnosed AIH who are positive for ANA. If so, the estimated frequency of NASH-related LC will be 5–6% of all LC.

Our nationwide survey determined the etiology of liver cirrhosis in Japan. Infection with HCV remains a major cause of LC, and the ratio has persisted at around 60% for 10 years. Liver cirrhosis associated with HCV accounted for significantly more patients with LC with HCC than without, suggesting that HCV has carcinogenic potential. NASH-related LC accounted for 2.1% of the total LC in Japan and this might increase in the future. The present epidemic status in Japan might reflect the status of LC in the USA and European countries 10–20 years later.

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Appendix

The Japan Etiology of Liver Cirrhosis Study Group consists of the following members: Takashi Goto (Akita University), Mamiko Takeuchi (Anjo Kosei Hospital), Shigeki Miyoshi (Asahikawa Medical College), Yutaka Yonemitsu (Chiba University), Ikuta Tanaka (Date Red Cross Hospital), Toshimitsu Murohisa (Dokkyo Medical University School), Yoshio Tokumoto (Ehime University Graduate School of Medicine), Yoshinori Horie (Eiju Sogo Hospital), Atsushi Takahashi (Fukushima Medical University School of Medicine), Makoto Shiraki (Gifu University Graduate School of Medicine), Hiroko Yamada (Gunma University Graduate School of Medicine), Ryoichi Okamoto (Hiroshima City Hospital), Takahiro Asakami (Hiroshima University), Shuhei Hige (Hokkaido University Hospital), Yoshiaki Inui (Hyogo Prefectural Nishinomiya Hospital), Kazuto Fukuda (Ikeda Municipal Hospital), Yuko Nagaoki (International Medical Center of Japan), Hidekatsu Kuroda (Iwate Medical University), Takuya Nagano (Kagawa Prefectural Central Hospital), Akihiro Deguchi (Kagawa University School of Medicine), Masayoshi Yamada (Kanazawa Medical University), Akito Sakai (Kanazawa University Graduate School of Medical Science), Nobuyuki Toshikuni (Kawasaki Hospital, Kawasaki Medical School), Keisuke Ojiro (Keio University School of Medicine), Chitomi Hasebe (Keiyukai Yoshida Hospital), Yoko Kudo (Kumamoto University of Medicine), Kazuhisa Nakamura (Kyorin University School of Medicine), Kanji Yamaguchi (Kyoto Prefectural University of Medicine), Eiji Takeshita (Matsuyama Red Cross Hospital), Satoshi Nakayama (Mishuku Hospital), Yuka Takahashi (Musashino Red-Cross Hospital), Shunsuke Nojiri (Nagoya City University Graduate School of Medical Sciences), Masao Fujimoto (Nara Medical University), Naota Taura (NHO Nagasaki Medical Center), Hiroshi Matsumura (Nihon University School of Medicine), Minoru Nomoto (Niigata University), Shinichi Fujioka (Okayama Saiseikai General Hospital), Bon Shoji (Okayama University Graduate School of Medicine), Hiroyasu Morikawa (Osaka City University School of Medicine), Ryoichi Ebara (Osaka Police Hospital), Mie Inao (Saitama Medical University), Ayana Endo (Sapporo City General Hospital), Hideyuki Nomura (Shin-Kokura Hospital), Satoru Jyoshita (Shinshu University School of Medicine), Yoshihiko Morisawa (Teikyo University School of Medicine), Takeshi Matsui (Teine Keijinkai Hospital), Masanori Ito (Tokyo Medical University Kasumigaura Hospital), Naoaki Hashimoto (Tokyo Teishin Hospital), Maki Tobari (Tokyo Women's Medical University), Miharu Hirakawa (Toranomon Hospital), Kenji Oyama (Tottori University), Shingo Arakaki (University of Ryukyus), Makoto Kadokura (University of Yamanashi), Masanori Matsuda

(University of Yamanashi), Tsuyoshi Matsumoto (Uwajima City Hospital), Satoshi Ugajin (Yamagata University), Makoto Segawa (Yamaguchi University Graduate School of Medicine) and Toshiya Ihii (Yokohama-city Seibu Hospital, St. Marianna University School of Medicine).

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CLINICAL STUDIES

Diabetes pattern on the 75 g oral glucose tolerance test is a risk factor for hepatocellular carcinoma in patients with hepatitis C virus

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diabetes mellitus – glucose tolerance test – hepatitis C virus – hepatocellular carcinoma – oxidative stress – tumor necrosis factor-alpha

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Abstract

Background: Patients with hepatitis C virus (HCV) frequently show glucose intolerance. Diabetes mellitus (DM) has been proposed to be a risk factor for hepatocellular carcinoma (HCC). *Aims:* The aim of this study is to clarify the influence of glucose intolerance as evaluated by the 75 g oral glucose tolerance test (OGTT) on hepatocarcinogenesis in patients with HCV. *Methods:* This study was carried out in a cohort of 197 patients with HCV who had not been previously diagnosed as having DM. All patients underwent the 75 g OGTT at entry. They were also screened for HCC and, thereafter, the rate of hepatocarcinogenesis was compared between the patients with and without glucose intolerance. *Results:* Based on the results of the 75 g OGTT, 125 (63%) had normal glucose tolerance (NGT), 49 (25%) had impaired glucose tolerance (IGT) and 23 (12%) had the DM pattern. HCC occurred more frequently in patients with the DM pattern than in patients with either NGT or IGT. Even in patients without advanced liver fibrosis, HCC was more frequently observed in patients with DM than in patients with NGT. A multiple logistic regression analysis showed advanced liver fibrosis, the DM pattern on the 75 g OGTT, an older age and γ-glutamyltransferase to all be independent risk factors related to hepatocarcinogenesis. *Conclusions:* A DM pattern on the 75 g OGTT was thus found to be associated with hepatocarcinogenesis and the 75 g OGTT is considered to be useful for identifying this risk factor for HCC in patients with HCV.

There is a high frequency of chronic hepatitis C (CH-C) worldwide and this condition progresses to cirrhosis and hepatocellular carcinoma (HCC) over a period of 20–30 years (1–3). Interferon (IFN) is often administered to treat CH-C and it can induce viral clearance and yield biochemical and histological improvement. Diabetes mellitus (DM) reduces the therapeutic effectiveness of IFN-α-2b plus ribavirin in patients with hepatitis C virus (HCV) (4). DM is often found in patients with HCV, especially in those with liver cirrhosis (5, 6). HCV infection itself may be associated with glucose intolerance. HCV core transgenic mouse demonstrates that glucose intolerance is usually due to insulin resistance induced by the HCV core protein (7). Tumour necrosis factor (TNF)-α has a crucial role in insulin resistance in this mouse model.

There are several risk factors for hepatocarcinogenesis associated with HCV, such as age, gender, total alcohol intake, cirrhosis, hepatic steatosis, HCV genotype and oxidative stress (8–12). DM is also a risk factor of HCC (13, 14). DM has a negative impact on patients with HCV because it reduces the efficacy of anti-HCV treatment and contributes to the development of HCC.

An accurate diagnosis of DM is sometimes difficult in patients with CH-C. A 75 g oral glucose tolerance test (OGTT) is frequently used to assess glucose tolerance. It identifies patients at the early stage of glucose intolerance, such as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). Previous reports of hepatocarcinogenesis in patients with HCV have not assessed the early stage of glucose intolerance by OGTT. Therefore, to clarify the association of DM with hepatocarcinogenesis, hepatocarcinogenesis was investigated in the patients with HCV evaluated with the 75 g OGTT. The results showed that DM or the early stage of glucose intolerance contributes to HCC in patients with HCV.

Patients and methods

Patients

This study retrospectively enrolled 197 patients with HCV who were admitted to the Ehime University Hospital from April 1992 to June 2006. Their glucose intolerance was evaluated with the 75 g OGTT and they underwent a liver biopsy. In this study, we excluded any patients with IFG because there were only two patients

with IFG during the study period. All patients were administered antiviral therapy by IFN during observations. They were positive for both anti-HCV antibody and serum HCV-RNA detected by the polymerase chain reaction (PCR). Hepatitis B virus (HBV) infection or autoimmune liver diseases were excluded. This study protocol was approved by the Institutional Review Board of Ehime University Hospital.

Evaluation of glucose intolerance

No patient had been treated by medical agents for diabetes before enrollment in this study. Their glucose intolerance was evaluated based on a 75 g OGTT according to the World Health Organization criteria: normal glucose tolerance (NGT), fasting plasma glucose (FPG) < 110 mg/dl and 2-h plasma glucose (PG) < 140 mg/dl; IGT, FPG < 110 mg/dl and 2-h PG between 140 and 200 mg/dl and DM, FPG ≥ 126 mg/dl or a 2-h PG level ≥ 200 mg/dl (15). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as reported previously (16).

Liver histopathological examination

Among the liver biopsy specimens obtained from all patients for histological examination, 72, 55, 53 and 17 were from patients with mild, moderate and severe fibrosis and liver cirrhosis respectively. In addition, 99, 95 and three were from patients with mild, moderate and severe histological activity respectively. The specimens were histologically classified according to the criteria of the International Hepatitis Group (17).

The degree of steatosis was determined based on the Brunt grading system (18) by the pathologist who reviewed the biopsy specimens without any information concerning the clinical characteristics. The steatosis grades were as follows: grade 0, no steatosis; grade 1, up to 33%; grade 2, up to 65% and grade 3, more than 66%.

Estimation of hepatitis B virus and hepatitis C virus markers and laboratory investigations

The presence of hepatitis B surface antigen and anti-HCV antibody was determined using an enzyme immunoassay kit (Dainabot, Tokyo, Japan; Kokusai-Shiyaku, Kobe, Japan). The HCV-RNA titres immediately before IFN therapy were determined using either an Amplicor-Monitor (Roche Diagnostics, Branchburg, NJ, USA) and expressed as kcopies/ml or kilo-international units/ml (KIU/ml) or the branched DNA probe assay (Chiron, Emeryville, CA, USA) and expressed as 10^6 genomic equivalents/ml (mEq/ml). The patients were separated according to a viral load of more (high viral load) or less (low viral load) than 8×10^6 mEq/ml by a branched DNA probe assay or 800 kcopies/ml (800 KIU/ml) by the Amplicor-Monitor assay. The serotype of HCV was determined using an enzyme immunoassay (Ohtsuka Laboratories Co. Ltd, Tokushima, Japan).

Treatment schedule of interferon therapy

All the 197 patients were treated with antiviral therapy at the Ehime University Hospital. Twenty-eight patients were treated with a natural IFN-β injection intravenously every day for 6–12 weeks at an average dose of 414 million international units (MIU). One hundred and fourteen patients were treated with IFN-α alone. IFN-α was administered intramuscularly every day for the first 14 days and then three times each week for 12–22 weeks, at an average dose of 653 MIU. Seven patients were treated with intravenous injections of IFN-β and IFN-α daily for 2–4 weeks and intramuscular injections of IFN-α three times each week for 16–22 weeks, at an average dose of 505 MIU. Twenty-six patients were treated with IFN-α-2b intramuscularly every day for the first 2 weeks and then three times a week for the following 22 weeks, at an average dose of 672 MIU. Ribavirin was combined with IFN-α-2b at a daily dose of 600 or 800 mg, depending on the body weight (< 60 or ≥ 60 kg respectively). Five patients were treated with pegylated IFN-α-2a once a week for 24–48 weeks. Seventeen patients were treated with pegylated IFN-α-2b once a week for 48 weeks in combination with ribavirin at a daily dose of 600 or 800 mg, depending on body weight (< 60 or 60–80 respectively).

Criteria for interferon effectiveness

All patients were followed-up for 24 weeks after antiviral therapy. HCV-RNA was assayed periodically during this period. Ninety-one patients (46%) were virologically sustained responders, who had no detectable HCV-RNA according to PCR assays during the follow-up period, and 106 (54%) were non-responders, who remained positive for HCV-RNA after antiviral therapy, irrespective of the HCV-RNA levels or a relapse during the follow-up period.

Diagnosis of hepatocellular carcinoma

All patients were examined for HCC by either abdominal ultrasonography or contrast-enhanced computed tomography every 3–6 months. If a mass lesion was detected, magnetic resonance imaging, abdominal angiography and a tumour biopsy guided by ultrasonography were performed to confirm the diagnosis of HCC. The mean follow-up period was 78 ± 45 months.

Measurement of protein carbonyls and tumour necrosis factor-α

In order to evaluate the levels of oxidative stress and TNF-α of the patients, separate serum aliquots were frozen at -20 °C and stored until the measurement. A patient was selected from each group according to the 75 g OGTT, whose sex and liver fibrosis stage were matched. Twenty-four, 23 and 24 patients from NGT, IGT and DM were eligible. The serum protein carbonyl levels were measured using a commercially available ELISA kit (BioCell

Corporation Limited, Auckland, New Zealand) involving a reaction of the protein with dinitrophenylhydrazine and detection with an antidinitrophenylhydrazine antibody according to the manufacturer's instructions. The total protein concentrations were measured for reference using the DC protein assay (Bio-Rad Laboratories, Richmond, CA, USA). The level of TNF-α was measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The detection limit was 0.5 pg/ml.

Statistical analysis

All data are expressed as the mean ± standard deviation. Student's *t*-test was used to evaluate the continuous variables. Multiple comparisons were analysed using ANOVA and Tukey's HSD test. The difference in proportions was evaluated using the χ^2 test or Fisher's exact test. The cumulative hepatocarcinogenesis was calculated using the Kaplan–Meier method and the differences between the survival curves were tested using the log-rank test. Variables were assessed to determine independent predictive factors that relate to hepatocarcinogenesis using the Cox regression analysis. At first, we selected variables with a *P*-value of < 0.15, which is known to be related with hepatocarcinogenesis. Next, we used those variables in a Cox regression analysis.

The model was simplified in a stepwise fashion by removing variables with $P > 0.05$. A value of $P < 0.05$ was considered to be significant. Calculations were performed using SPSS for Windows, Release 15.0J (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics according to the results of the 75 g oral glucose tolerance test

The characteristics of patients classified as glucose intolerance are compared in Table 1. Based on the results of the 75 g OGTT, 125 (63%) were NGT, 49 (25%) were IGT and 23 (12%) were DM pattern. The DM pattern was seen in 4% of the patients with mild fibrosis (F1), 15% of the patients with moderate fibrosis (F2), 11% of the patients with severe fibrosis (F3) and 35% of the patients with liver cirrhosis (F4; Fig. 1). The rate of males was significantly higher in IGT than in NGT ($P < 0.05$). The patients with the DM pattern seemed to have more progressive liver fibrosis but there was no difference in the state of liver fibrosis (F1, F2 vs. F3, F4) between the patients with DM and NGT. The steatosis grade of liver biopsy specimens was not significantly different according to glucose intolerance. The patients with the DM pattern tended to consume more alcohol than the patients with NGT ($P < 0.05$). The body mass index (BMI), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in the patients with DM than in those with NGT ($P < 0.05$, $P < 0.01$, $P < 0.01$ respectively). HOMA-IR was significantly higher in patients with DM than in those with NGT and IGT ($P < 0.05$ and $P < 0.01$ respectively). Furthermore, the average of HOMA-IR even in the patients with NGT was over 2, which is considered as indicative of insulin resistance. The platelet count and insulinogenic index (ΔIRI/ΔBS) were significantly lower in patients with DM than in those with NGT ($P < 0.05$ and $P < 0.01$ respectively) and ΔIRI/ΔBS were also significantly lower in the patients with IGT than in those with NGT ($P < 0.01$). Triglyceride was significantly higher in patients with DM and IGT than in those with NGT ($P < 0.05$ and $P < 0.05$ respectively). No difference was found in either the virological state or the response to IFN therapy among these groups.

Evaluation of protein carbonyl and tumour necrosis factor-α levels among the patients with or without glucose intolerance

Serum protein carbonyl levels were compared among the patients with NGT, IGT and DM (Fig. 2A). The levels of protein carbonyl in the patients with NGT, IGT and DM were 5.9 ± 2.1, 6.3 ± 1.9 and 6.3 ± 1.7 respectively. No significant differences were observed in the protein carbonyl levels. Furthermore, the protein carbonyl levels were evaluated between the patients with and without HCC (Fig. 2B). No significant difference was found between these two groups. The levels of TNF-α in the patients with NGT, IGT and DM were 36.0 ± 56.1, 42.1 ± 82.4 and 14.8 ± 22.3 respectively. The range of each value was wide and no significant differences of TNF-α were identified between the patients with and without HCC as well as in the patients with and without glucose intolerance.

Cumulative hepatocarcinogenesis rates according to the patterns of 75 g oral glucose tolerance test

During the follow-up period, 18 patients (9.1%) developed HCC. In the patients with NGT, IGT and DM pattern, the cumulative hepatocarcinogenesis rates were 7.1%, 0% and 10.3% at the end of 5 years and 9.2%, 8.4% and 60.7% at the end of 10 years respectively. Hepatocarcinogenesis was significantly higher in patients with the DM pattern than in patients with NGT and IGT ($P < 0.01$ and < 0.01 respectively; Fig. 3). Next, the cumulative hepatocarcinogenesis rates in the patients with mild liver fibrosis (F1, F2) were evaluated to determine the impact of glucose intolerance, especially in patients with an early stage of fibrosis. The cumulative hepatocarcinogenesis rates were significantly higher in patients with the DM pattern than in patients with NGT ($P < 0.01$; Fig. 4).

Multivariate analysis of factors associated with hepatocarcinogenesis

The data were analysed to determine the variables that independently influence hepatocarcinogenesis in patients with HCV. A univariate analysis showed eight

Table 1. Clinical and virological characteristics of the 197 patients with hepatitis C virus according to the 75 g oral glucose tolerance test

	NGT ($n=125$)	IGT ($n=49$)	DM ($n=23$)
Sex (M/F)†	70/55	38/11	18/5
Age (years)	51 ± 12	52 ± 11	55 ± 9
Body mass index (kg/m^2)*	23.5 ± 3.4	23.8 ± 2.7	25.6 ± 3.6
Alcohol consumption* (< 500 kg/≥ 500 kg)	111/14	37/12	15/8
Total protein (g/dl)	7.4 ± 0.6	7.5 ± 0.7	7.7 ± 0.6
Serum albumin (g/dl)	4.2 ± 0.4	4.2 ± 0.5	4.2 ± 0.4
AST (IU/L)**	64 ± 39	72 ± 45	94 ± 60
ALT (IU/L)**	96 ± 70	112 ± 76	149 ± 120
γ-GTP (IU/L)	66 ± 81	81 ± 62	95 ± 73
α-fetoprotein (ng/ml)	12.6 ± 23.6	14.0 ± 19.5	18.7 ± 18.9
Platelet count (× 10^4/μl)*	17.8 ± 6.5	16.8 ± 5.4	14.1 ± 6.4
Total cholesterol (mg/dl)	174 ± 32	173 ± 22	173 ± 39
Triglyceride (mg/dl)*,†	102 ± 47	140 ± 123	147 ± 89
IRI-AUC180	280.6 ± 138.4	309.1 ± 164.9	316.9 ± 151.5
HOMA-IR**,§	2.6 ± 2.2	2.7 ± 1.3	6.8 ± 12.3
ΔIRI/ΔBS**,‡	1.58 ± 1.09	1.00 ± 0.81	0.49 ± 0.40
HCV serotype			
1/2/1+2	79/42/4	32/15/2	13/10/0
HCV-RNA titre			
High/low	17/108	37/12	7/16
Histological fibrosis			
Mild	57	12	3
Moderate	31	16	8
Severe	30	17	6
Cirrhosis	7	4	6
Histological activity*			
Mild	66	25	8
Moderate	58	23	14
Severe	1	1	1
Steatosis grade			
Grade 1	82	30	13
Grade 2	31	13	8
Grade 3	10	5	2
Grade 4	2	1	0
Response to interferon (SVR/non-SVR)	61/64	18/31	12/11

Data are expressed as mean ± SD. The homeostasis model for assessment of insulin resistance (HOMA-IR) was evaluated only in patients with fasting plasma glucose < 140 mg/dl. The steatosis grade was determined by the Brunt grading system.

*$P < 0.05$ compared between DM and NGT.

**$P < 0.01$ compared between DM and NGT.

†$P < 0.05$ compared between NGT and IGT.

‡$P < 0.01$ compared between NGT and IGT.

§$P < 0.01$ compared between DM and IGT.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; ΔIRI/ΔBS, insulinogenic index; DM, diabetes mellitus; F, female; γ-GTP, γ-glutamyltransferase; HCV, hepatitis C virus; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; M, male; NGT, normal glucose tolerance; SD, standard deviation; SVR, sustained viral response.

parameters that significantly influenced hepatocarcinogenesis: advanced liver fibrosis (F3, F4) ($P < 0.01$), DM pattern on 75 g OGTT ($P < 0.01$), an older age ($P < 0.01$), platelet counts ($P < 0.01$), AST ($P < 0.01$), ALT ($P = 0.013$), prothrombin time ($P = 0.010$) and the cumulative alcohol intake ($P = 0.043$) and a marginally significant association was observed in the patients without a sustained viral response by antiviral therapy ($P = 0.070$), serum albumin ($P = 0.091$), α-foetoprotein ($P = 0.109$) and γ-glutamyltransferase (GTP) ($P = 0.144$). Next, these results were analysed to identify the independent risk factors of hepatocarcinogenesis. A multiple logistic regression analysis showed advanced liver fibrosis (F3, F4), a DM pattern on the 75 g OGTT, an older age and γ-GTP to all be independent risk factors associated with hepatocarcinogenesis [odds ratio 8.135 (1.677–12.766), $P = 0.005$; 4.627 (1.677–12.766), $P = 0.003$; 1.094 (1.021–1.172), $P = 0.011$; 1.007 (1.002–1.011), $P = 0.002$ respectively; Table 2].

Discussion

The present study shows evidence that DM evaluated by the 75 g OGTT is a risk factor of hepatocarcinogenesis in

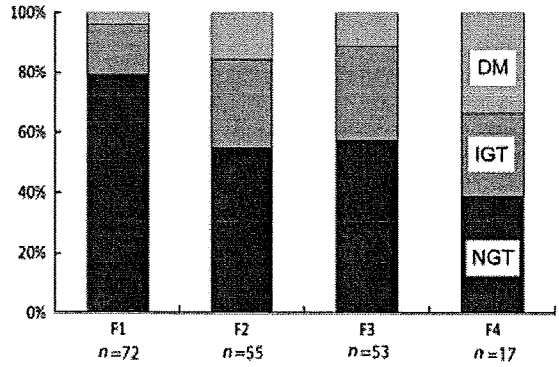

Fig. 1. Results of the 75 g oral glucose tolerance test according to the degree of liver fibrosis. DM, diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

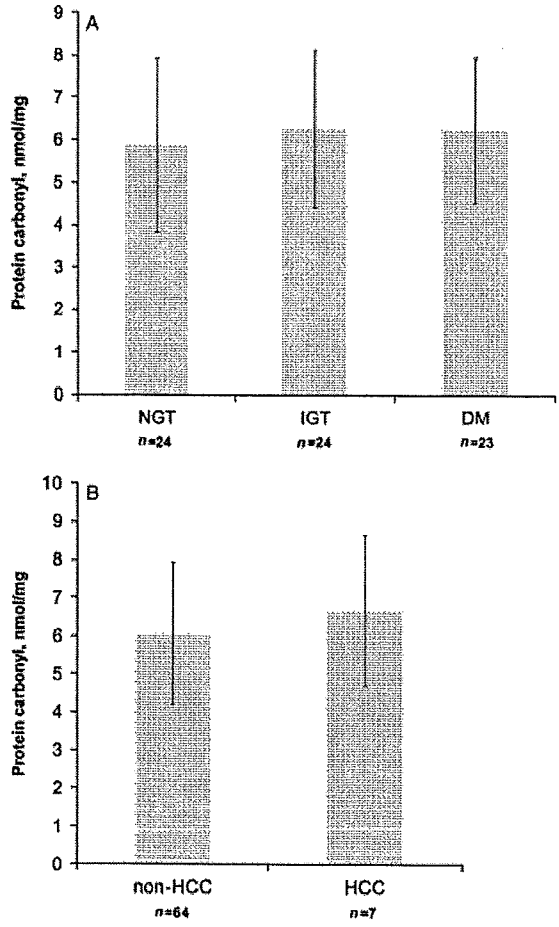

Fig. 2. The serum carbonyl levels were compared between patients (A) with or without glucose intolerance and (B) between patients with and without HCC. DM, diabetes mellitus; HCC, hepatocellular carcinoma; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

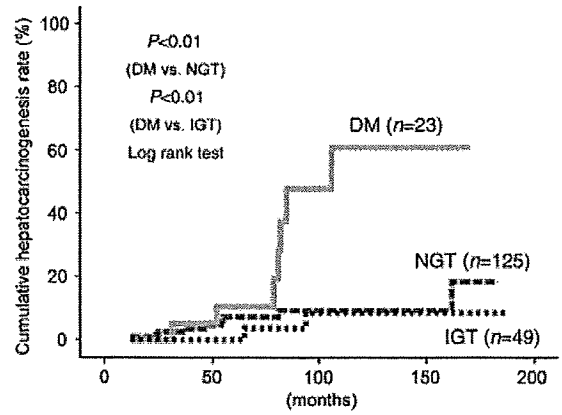

Fig. 3. Cumulative hepatocarcinogenesis rates based on the 75 g oral glucose tolerance test. The rate is significantly higher in patients with a DM pattern than in those with NGT and IGT. DM, diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

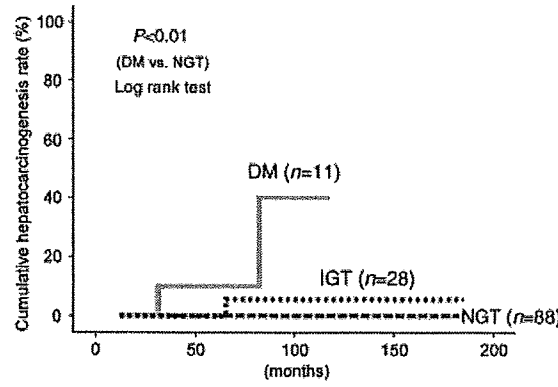

Fig. 4. The cumulative hepatocarcinogenesis rate based on the 75 g oral glucose tolerance test was significantly higher in patients with DM in comparison with those with NGT, even in those patients without advanced liver fibrosis. DM, diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

Table 2. Multiple logistic regression analysis of the variables on hepatocarcinogenesis in the patients with hepatitis C virus

Variable	Category	*P*-value	Hazard ratio (95% CI)*
Hepatic fibrosis stage	1: F1, F2 2: F3, F4	0.005	8.135 (1.870–35.394)
Result of 75 g OGTT	1: NGT, IGT 2: DM pattern	0.003	4.627 (1.677–12.766)
Age (years)		0.011	1.094 (1.021–1.172)
γ-GTP		0.002	1.007 (1.002–1.011)

*Values are the hazard ratio of hepatocarcinogenesis.

CI, confidence interval; DM, diabetes mellitus; γ-GTP, γ-glutamyltransferase; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

patients with HCV. Several reports have been published regarding the relationship between DM and hepatocarcinogenesis in patients with HCV. One described DM to be a risk factor of hepatocarcinogenesis in patients with HCV (19). Another showed DM to be a risk factor in patients with HCV who have advanced liver fibrosis (20). A study in Taiwan investigated the risk of HCC associated with HBV/HCV infections and metabolic status, such as obesity and DM (21). It showed that diabetes and obesity increased the risk of HCC, especially in patients with HCV. These reports addressed hepatocarcinogenesis in patients with HCV complicated with previously diagnosed and/or treated DM. The current study showed that patients with HCV with glucose intolerance based on the 75 g OGTT had a malignant potential even if they had not been diagnosed with DM.

The patients with IGT progressed to DM if their hepatitis continued and progressed to advanced liver fibrosis over several years and, therefore, IGT is considered to be a possible risk factor for carcinogenesis by identifying a condition that could progress to DM.

In patients without advanced liver fibrosis, hepatocarcinogenesis was found more frequently in patients with DM. Because there were few occurrences of HCC, the malignant potential of DM could not be defined in the patients without advanced liver fibrosis. However, these results indicate that a strict evaluation of glucose intolerance is needed in patients with HCV even if they do not progress to eventually develop advanced liver fibrosis.

Hepatocellular carcinoma can be prevented in many ways for patients with HCV. Especially, the eradication of HCV by anti-HCV therapy with IFN has a high impact in preventing the occurrence of HCC (22). Even in patients with HCV who failed to clear HCV-RNA, HCC was observed to be prevented (23). In our subjects, all patients were treated with IFN. However, the effect of antiviral therapy in regard to the occurrence of HCC was not marginally significant. In a multivariate analysis, the eradication of HCV did not significantly correlate with the occurrence of HCC in our current study.

Steatosis has been reported to be a risk factor of HCC for patients with HCV (24). We evaluated the degree of the hepatic steatosis and examined the association with the hepatocarcinogenesis. However, no relationship between steatosis and hepatocarcinogenesis was observed. Our patients mainly had the HCV genotype 1 or 2, but not genotype 3, and their BMI was generally not too high. Therefore, the steatosis grade was relatively low and it is therefore not considered to have affected hepatocarcinogenesis.

It is unclear how glucose intolerance influences hepatocarcinogenesis. Oxidative stress could be an important factor in hepatocarcinogenesis (25). Recently, protein carbonyl was proposed as a good marker of oxidative protein in blood samples (26). The serum protein carbonyl levels were measured in some patients at the time of enrolment. However, no differences were observed in the protein carbonyl levels among the patients with or without glucose intolerance. The accumulation of DNA damage due to oxidative stress should be important for hepatocarcinogenesis. However, no evidence of the mechanism by which glucose could contribute to hepatocarcinogenesis through oxidative stress was seen.

Hyperglycaemia increases the cancer risk (27) and postprandial hyperinsulinaemia is associated with accelerated HCC growth (28). Insulin may therefore be a key molecule associated with hepatocarcinogenesis. Insulin acts as a growth factor via the activation of mitogen-activated protein kinases (29, 30). Therefore, hyperinsulinaemia may be one reason that hepatocarcinogenesis is associated with hyperglycaemia. Furthermore, HOMA-IR was significantly higher in patients with the DM pattern than in those with NGT and IGT. These findings indicate that insulin resistance is important for a progression to DM in the patients with HCV. Insulin resistance, which leads to hyperinsulinaemia, is partially caused by increased production levels of TNF-α. In fact, the production of TNF-α has been reported to increase in chronic liver injury (31) and TNF-α is one of the causes of DM in patients with CH-C (32, 33). The study using a mouse model transgenic for the HCV core gene revealed that HCV causes insulin resistance and that a high level of TNF-α contributes to insulin resistance in these transgenic mice (7). Therefore, hyperinsulinaemia might be induced by TNF-α and other inflammatory cytokines. In the current cohort, TNF-α did not significantly increase in the patients with glucose intolerance. Other mechanisms may therefore be associated with hyperinsulinaemia and insulin resistance. HOMA-IR was significantly higher in patients with the DM pattern than in those with NGT and IGT. In addition, obesity is a risk factor for HCC in patients with decompensated cirrhosis (34). The BMI of the patients with the DM pattern was found to be higher than that of NGT. These results support the higher incidence of HCC in patients with a DM pattern.

It is unknown whether the good control of blood glucose levels prevents hepatocarcinogenesis. There is no evidence that successful treatment for glucose intolerance can reduce hepatocarcinogenesis. In an animal model, blood glucose control suppressed the incidence of squamous cell carcinoma in alloxan-induced diabetic rats through suppressing inflammation (35). However, the direct relationship between the treated DM and carcinogenesis still remains controversial. In our subjects, we did not have any detailed follow-up data and, therefore, it is difficult to evaluate whether the good control of blood glucose levels can reduce the occurrence of HCC.

In conclusion, this study revealed that DM pattern identified by the 75 g OGTT could be an independent risk factor of hepatocarcinogenesis in patients with HCV. These results indicate that an accurate evaluation of glucose intolerance would therefore be useful for estimating the risk of hepatocarcinogenesis in HCV patients. It is necessary to determine how the control of

hyperglycaemia reduces the potential of hepatocarcinogenesis and to determine how the incidence of HCC can be reduced in HCV patients with DM.

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Alimentary Pharmacology & Therapeutics

Clinical trial: extended treatment duration of peginterferon-alpha2b plus ribavirin for 72 and 96 weeks in hepatitis C genotype 1-infected late responders

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SUMMARY

Background
The benefits of prolonging peginterferon and ribavirin after 48 weeks of treatment to maximize sustained virological responses (SVR) in hepatitis C virus (HCV) genotype 1-infected patients remain to be understood.

Aim
To investigate whether extended treatment longer than 72 weeks may be superior to 72-week treatment.

Methods
A total of 120 treatment-naïve or retreated patients with HCV genotype 1 were treated with peginterferon-alpha-2b (1.5 μg/kg/week) plus weight-based ribavirin. We had 34 late responders, in whom HCV RNA first became undetectable at week 12–48, and randomized them into three groups receiving standard-dose peginterferon-alpha-2b plus low-dose ribavirin (200 mg/day) for extended 24 weeks (group A), receiving low-dose peginterferon-alpha-2b (0.75 μg/kg/week) plus low-dose ribavirin for extended 48 weeks (group B) or no extended treatment (group C), and evaluated the outcome according to their virological response.

Results
Multivariate analysis showed that the treatment for 96 weeks was identified as a significant, independent factor associated with SVR in HCV genotype 1-infected late responders in comparison with group A [odds ratio (OR), 10.002; $P = 0.080$] and group C (OR, 17.748; $P = 0.025$).

Conclusion
Extending the treatment duration from 48 weeks to 96 weeks improves SVR rates in genotype 1-infected patients with late virological response to peginterferon-alpha-2b and ribavirin.

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