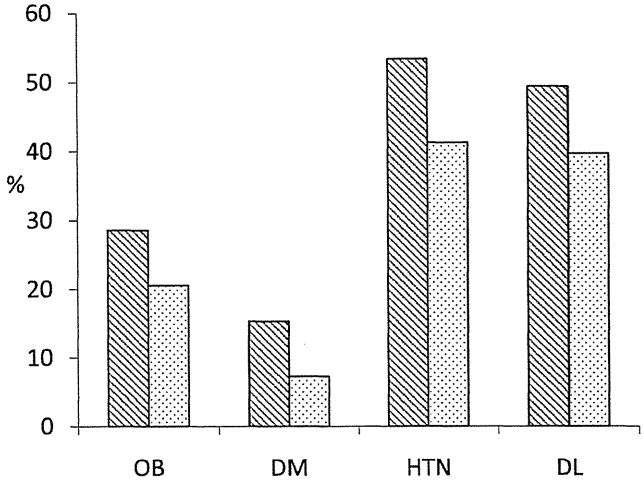

Figure 6 The complication rate of lifestyle-related diseases in Japan in 2007 to 2008. OB, obesity; DM, diabetes mellitus; HTN, hypertension; DL, dyslipidemia. ▨, Male; ▩, Female.

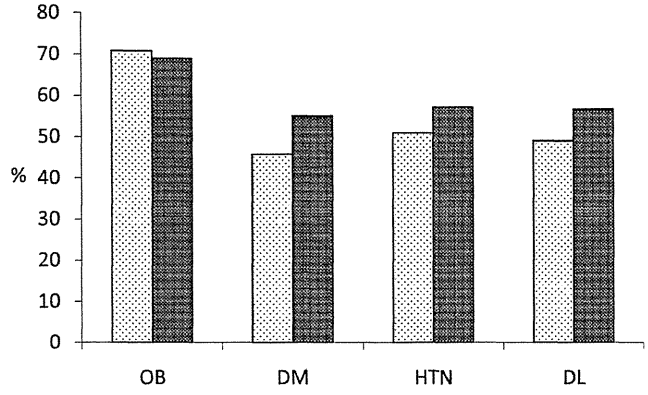

Figure 7 The complication rates of life-style related diseases among 283 NAFLD patients subjected to liver biopsy at Saiseikai Suita Hosptital from 2008 to 2010. SS, simple steatosis; NASH, nonalcoholic steatohepatitis; OB, obesity; DM, diabetes mellitus; HTN, hypertension; DL, dyslipidemia. ▨, SS n=96; ▩, NASH n=187.

women with NAFLD. In women, caution is required when the abdominal circumference is used instead of the visceral fat area.

Epidemiologically, it is clear that the risk of cardiovascular diseases is increased markedly in people with multiple risk factors for life-style related diseases. In addition, Hamaguchi *et al.* showed that NAFLD patients were at high risk of cardiovascular diseases and NAFLD, but not metabolic syndrome (MS), and showed a statistically significant correlation with cardiovascular disease in a multivariate model (OR = 4.12; 95% CI, 1.58–10.75).[10] Having multiple life-style related diseases is considered to be a risk factor for developing NASH.

The incidence of complications of life-style related diseases among 283 biopsy-proven NAFLD patients in Saiseikai Suita Hospital from April 2007 to March 2010 was high. However, no significant difference was seen in the incidence of individual factors between 187 NASH patients and 96 SS patients; obesity: NASH 69.0% *versus* SS 70.8%, diabetes: NASH 55.1% *versus* SS 45.8%, hypertension: NASH 57.2% *versus* SS 51.0%, and dyslipidemia: NASH 56.7% *versus* SS 49.0% (Fig. 7). Most NASH-cirrhotic patients have been reported to be obese with an average BMI of 27.6 ± 4.5 kg/m^2; the prevalence of the complications of diabetes and hypertension were 66.6% and 50.2%, respectively.[11]

Hamaguchi *et al.* also showed that the presence of metabolic syndrome was related to the new onset of NAFLD, with a 4-fold increase in men and an 11-fold increase in women compared to non-metabolic syndrome subjects.[5] Mitsumune *et al.* reported that obesity (6.3 fold), dyslipidemia (2.4 fold), hyperglycemia (1.8 fold), and hypertension (1.4 fold) all increased the odds of having NAFLD.

Obesity

The National Health and Nutrition Examination Survey[7] conducted in 2008 showed that the proportion of obese subjects with a BMI of 25 kg/m^2 or more was 28.6% of men and 20.6% of women. Classified by age, this category accounted for over 29% of men aged 30–69 years, whereas, for women, the obesity rate increased with age: 11.8% in their 30s, 18.0% in their 40s, 21.1% in their 50s, 24.4% in their 60s, and 26.8% in their 70s. The prevalence of fatty liver according to age shows the same tendency as obesity. Fatty liver was noted in only 2.7% of non-obese subjects with a BMI less than 23 kg/m^2 and was 10.5% in those with a BMI ≥ 23 but < 25 kg/m^2, 34.6% in those with a BMI ≥ 25 but < 30 kg/m^2, and 77.6% in highly obese subjects (BMI > 30 kg/m^2).[12] Kojima *et al.* carried out an analysis of 39 151 people who underwent a health check-up over a period of 12 years from 1989 to 2000. They reported that the grade of obesity correlated with the development of fatty liver. The prevalence was 12.8% in non-obese subjects (BMI < 25 kg/m^2), 51.4% in subjects with BMI ≥ 25 but < 30 kg/m^2, and 80.4% in highly obese subjects (BMI > 30 kg/m^2). In subjects with BMI values of 25 kg/m^2, the odds ratio for fatty liver was 6.3 compared with of non-obese subjects.[3]

Hamaguchi *et al.* reported that, in a group receiving a health check-up, 18% showed NAFLD at the time of the initial health check-up, and 10% (14% of men and 5% of women) developed NAFLD during the follow-up period of an average 414-days.[5] Patients with newly developed NAFLD showed weight gain of 1.7 ± 1.7 kg for men and 1.3 ± 1.4 kg for women. Logistic regression analysis showed that weight gain was an independent risk factor for the newly developed NAFLD, with an OR = 1.51 (95% CI, 1.40–1.63) for men and OR = 1.62 (95% CI, 1.39–1.89) for women.

Diabetes/impaired glucose tolerance

In the National Health and Nutrition Examination Survey conducted in Japan in 2007, 8 900 000 people were strongly suspected of diabetes (HbA1c ≥ 6.1%, or currently under treatment); the number of people with an undeniable possibility of diabetes (HbA1c ≥ 5.6% but < 6.1%) was 13 200 000, in total, the number of people possibly with diabetes was 22 100 000, which was 1.6-fold higher than 10 years earlier.[13] Kojima *et al.* reported that the prevalence of fatty liver was 18.6% in subjects with normal glucose metabolism (FBS < 110 mg/dL), 43.7% in borderline subjects (FBS ≥ 110 but < 126 mg/dL), and 53.3% in diabetic

patients (FBS ≥ 126 mg/dL). FBS ≥ 110 mg/dL was an independent risk factor for fatty liver (OR = 3.1).[3] Likewise, Jimba *et al.* reported that the overall prevalence of NAFLD was 29% among 1950 Japanese people receiving a health check-up; the prevalence was 27% in the normal glucose metabolism group (FBG < 6.1 mmol/L) and rose to 43% for the borderline type (FPG ≥ 6.1 but < 7.0 mmol/L) and 62% for the diabetic type (FBG ≥ 7.0 mmol/L or a medical history of diabetes). In addition, the incidence of complications with abnormal glucose metabolism (borderline type and diabetic type) was 19.1% in NAFLD patients, which was higher than the 5.6% of patients without NAFLD ($P < 0.001$).[14]

Miyaaki *et al.* examined the relationship between the stage of hepatic fibrosis and the prevalence of diabetes in Japanese patients. In the mild fibrosis group, 42% were complicated with diabetes, whereas in the severe fibrosis (bridging fibrosis or cirrhosis) group, the prevalence was as high as 71% ($P = 0.020$). Diabetes might be a factor responsible for the development of hepatic fibrosis in NAFLD.[15] Shiga *et al.* performed a 75-g oral glucose tolerance test on the participants of a health check-up. They found that blood glucose levels at one and two hours after glucose load showed a closer relationship with NAFLD than the fasting blood glucose level. Therefore, they stated the importance of the evaluation of impaired glucose tolerance (IGT) in detecting NAFLD.[16]

Hypertension

According to the criteria of the Japanese Society of Hypertension, systolic blood pressure under 130 mm Hg/diastolic blood pressure under 85 mm Hg is normal, pressure higher than 140/90 mm Hg is diagnosed as hypertension, and pressure 130–139/85–89 mm Hg is high-normal blood pressure.

In the National Health and Nutrition Examination Survey conducted in 2007, the prevalence of subjects with hypertension (including 24.0% currently under treatment) was 46.2%, the prevalence of high-normal blood pressure was 13.8%, and the normal pressure group was 40.0%.

Hypertension is frequently seen in NASH/NAFLD patients, but there are no reports describing the prevalence of NAFLD among hypertensive patients in Japan. Reports from overseas stated that systolic and diastolic blood pressures were correlated with liver fat content, and that the risk of developing NAFLD was 2-fold higher in patients with systolic hypertension, and 1.7-fold higher in patients with diastolic hypertension.[17,18] In addition, Donati *et al.* reported that even in non-obese, non-diabetic high blood pressure patients, the prevalence of fatty liver was three times higher than in healthy individuals. Further such patients showed high levels of HOMA-IR, indicating insulin resistance.[19] The pathogenesis of hypertension is influenced by various factors, such as salt intake, and also is associated with insulin resistance. It is important to know that even non-obese high blood pressure patients with no other lifestyle-related diseases are likely to develop NAFLD if they have insulin resistance. In Japan, large-scale studies on hypertension and NAFLD are currently underway, including among subjects with chronic kidney disease (CKD).

Recently, we reported the prevalence of CKD in 174 NAFLD patients. The prevalence of CKD was significantly higher in NASH patients (19 of 92; 21%) than SS patients (5 of 82; 6%), and associated with a higher body mass index and the presence of hypertension.[20]

Dyslipidemia

Dyslipidemia is a generic term describing a clinical condition in which the levels of cholesterol esters or triglycerides increase in the blood: high levels of triglycerides (150 mg/dL or higher) and LDL cholesterol (140 mg/dL or higher), with decreased levels of HDL cholesterol (less than 40 mg/dL) are each risk factors for other diseases. In the National Health and Nutrition Examination Survey conducted in 2007, the percentage of subjects suspected of dyslipidemia (including 9.7% currently under treatment) was 44.1%, and that of normal subjects was 55.9%.

Dyslipidemia in NAFLD often involves hypertriglyceridemia and decreased blood levels of HDL cholesterol. This is due to the insufficient effects of lipoprotein lipase (LPL), which leads to a decreased metabolism of triglyceride-rich lipoproteins into HDL cholesterol. In addition, there is also an increased synthesis of very-low density lipoprotein (VLDL).

Pediatric NASH/NAFLD

The incidence of pediatric NAFLD in Japan is increasing in proportion to the increase in the prevalence of childhood obesity. In a previous study conducted on children aged 6–15 years, Tominaga *et al.* reported that the prevalence of NAFLD was 3.4% in children aged 6–10 years and 5.2% in those aged 11–15 years.[21] In addition, the prevalence of NAFLD in children who met the diagnostic criteria for pediatric metabolic syndrome was 40.0% in those diagnosed with pre-metabolic syndrome, and 76.8% in those who fulfilled the criteria for metabolic syndrome. Tsuruta *et al.* also reported that, in a similar study conducted in 2007 on 288 junior high school students (13–15 years old), 5.9% were obese, the prevalence of NAFLD was 4.5%, and obesity and ALT levels of 30 IU/L or higher were independent risk factors for NAFLD in children.[22] In addition, the prevalence of complications with obesity (degree of obesity ((body weight—standard body weight)/standard body weight × 100) ≥ 20%) showed a higher frequency in NAFLD patients (58.3%) than those without NAFLD (5.7%, $P < 0.001$).

Pathophysiology and genetic background

Racial differences might affect the onset and pathophysiology of NAFLD. Weston *et al.* reported that the prevalence of obesity, dyslipidemia, and diabetes in NAFLD was similar among racial and ethnic groups, except that body mass index was lower in Asians compared to Whites, Hispanics, and African Americans ($P < 0.001$). Compared with the base population, Hispanics with NAFLD were overrepresented and Whites were underrepresented.[23] In addition, Mohanty *et al.* reported that African Americans showed a lower degree of steatosis than Whites. In contrast, it has been considered that Asians showed higher grades of ballooning and Hispanics showed higher grades of Mallory-Denk bodies, than Whites and other ethnicities combined.[24] These findings indicate the importance of racial differences for the development and progression of NAFLD.

There are many reports concerning the genetic predisposition to the development of NASH and NAFLD, and most of them refer to functional genetic polymorphisms. Tumor necrosis factor-alpha (TNF-α) is known to be produced by adipocytes in visceral fat and Kupffer cells in the liver. It inhibits insulin receptor substrate-1 (IRS-1) of target cells, and insulin receptor kinase in skeletal muscles and adipocytes, thereby cause or exacerbating insulin resistance. Increased blood levels of TNF-α have been reported in NAFLD and NASH patients whose BMI and insulin resistance were matched, thereby suggesting a relationship between increased levels of TNF-α and the development of NAFLD or the progression of NASH.[25] It has been reported in Japanese subjects that functional genetic polymorphisms of TNF-α are present at positions T-1031C and C-856A in the promoter region, and these were more frequent in patients with NASH, potentially mediating progression of the disease.[26]

Adiponectin has an insulin sensitivity effect by opposing fatty acid accumulation which causes insulin-resistance, an anti-atheriosclerotic effect, and an anti-inflammatory effect. Therefore, hypoadiponectinemia associated with obesity has been considered to play a crucial role in the development of metabolic syndromes. In addition, the serum adiponectin level has been shown to be lower in NASH patients than in healthy groups and simple fatty liver groups.[27] The presence of functional polymorphisms G45T and G276T is the adiponectin gene have been reported to be associated with diabetes.[28,29] Regarding Japanese subjects with NASH, it has been reported that the G/G homo-allele at the 45th base of the exon of adiponectin was more frequent in NASH with advanced fibrosis than that in mild fibrosis, and that insulin resistance was distinctly more prominent.[30] Yoneda *et al.* reported that genetic variations in angiotensin II type1 receptor (ATGR1) may influence the risk of NAFLD and liver fibrosis in NAFLD.[31]

Functional polymorphisms in the β3-adrenergic receptor gene, microsomal triglyceride transfer protein (MTP), phosphatidylethanolamine *N*-methyltransferase (PEMT), interleukin-1β (IL-1β), and manganese superoxide dismutase (MnSOD) have also been reported in Japan.[32–34] MTP and PEMT are important factors for the metabolism in triglyceride.

In addition, sex hormones are involved in gender differences in the incidence of NAFLD, and in postmenopausal women the decreased level of estrogen results in the accumulation of visceral fat and insulin resistance.[35] This may explain why postmenopausal women appear to be at a higher risk for the development of NAFLD.

Pathological diagnosis

NAFLD can be diagnosed in patients from whom hepatitis virus infection, alcoholic liver disease and autoimmune hepatitis have been excluded when over 5% of hepatocytes contain fatty droplets.

NAFLD encompasses a histological spectrum ranging from simple steatosis (SS) to NASH, the latter showing hepatocyte degeneration (ballooning hepatocyte), necrosis, inflammation and fibrosis.[36]

Recently, Matteoni *et al.* categorized NAFLD into four types; type 1 (simple fatty liver), type 2 (steatohepatitis), type 3 (steatonecrosis) and type 4 (steatonecrosis + Mallory-Denk body (MDB) or fibrosis). They proposed that types 1 and 2 should be categorized as SS, and types 3 and 4 as NASH, according to the prognosis based on their follow-up study.[37] Actually we sometimes encounter difficulty in the differential diagnosis between type 2 and type 3 NAFLD, and between type 3 and type 4 NAFLD. This is because the criteria of ballooning hepatocytes and presence of pericentral and pericelluar fibrosis are unclear when these morphological changes are very mild.

In 2005, Kleiner *et al.* proposed a new scoring system, the so-called NAFLD activity score (NAS), according to the extent of the three features: steatosis, hepatocellular ballooning and lobular inflammation. By the NAS, NASH is defined as having a score of five or more.[38] This score is based on disease activity and the evaluation of fibrosis is excluded; this might be not suitable for the diagnosis of advanced staged NASH. Brunt and others proposed a grading and staging system according to the grade of inflammation and fibrosis,[39] and this method is widely accepted in Japan.

Ten to 30% of NASH cases have the potential to develop to cirrhosis within 10 years. However, much attention should be paid to so-called "burn-out NASH", in which fatty droplets have disappeared during the progression of hepatic fibrosis, resulting in difficulty making a precise diagnosis of NASH. In such a case, we must make an effort to collect the detailed background and previous patient history. This difficulty could lead to an underestimation of the prevalence of NASH-cirrhosis the Mallory-Denk bodies (MDB) are one of the morphological hallmarks for the diagnosis of type 4 NAFLD: they are an abnormal flocculent producter in degenerated hepatocytes and are comprised of intermediate filaments (IF).[40] We consider that the frequency of MDB in Japanese NASH is lower than in western countries.

Biochemical markers: non-invasive score assessment

Liver histology is the gold standard for the diagnosis of NASH; however, it is invasive and there is a risk of sampling errors in some cases. It has been anticipated that it should be possible to use serum biochemical markers to diagnose NASH, and various parameters reflecting oxidative stress, insulin resistance, inflammation, apoptosis, and fibrosis have been proposed to discriminate between SS and NASH. A NASH test that allows prediction on the basis of 13 parameters has been reported in Europe but, in recent years, Gholam *et al.* designed a more convenient differential formula based on only two criteria: the AST level and the presence or absence of diabetes mellitus (DM).[41] Campos *et al.* proposed a clinical scoring system for NASH[42] in which the scored criteria consist of hypertension (HTN), type 2 DM, AST, ALT, sleep apnea syndrome, and race (exception for blacks). However, these reports are from Europe and the USA. Recently, it was reported that the serum level of soluble fraction in cytokeratin 18 (soluble CK-18) was able to discriminate between SS and NASH,[43] and this has been adopted for our Japanese patients (unpublished data).

We reported previously the importance of serum ferritin and thioredoxin levels, reflecting status of oxidative stress, in the differential diagnosis between SS and NASH.[44,45] Recently, Sumida *et al.* proposed the NAFIC (NASH, Ferritin, Insulin, Collagen) scoring using Japanese patients. This comprises three measurements: serum ferritin, insulin, and type-4 collagen 7s.[46] To determine the utility of this score, we conducted a validation study in collaboration with ten centers all over Japan (Japan Study Group of NAFLD; JSG-NAFLD).[46]

Various indicators have been proposed for the evaluation of the degree of fibrosis in NASH. From a study based on the analysis of 50 NASH patients including nine with cirrhosis, Fujii *et al.* reported that the cirrhosis determinant score (CDS) and the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) model were valuable for the differentiation of cirrhosis induced by NASH and HCV infection.[47] A French group proposed the BAAT score[48] and Fibrotest,[49] which assign one point to each of the following items: BMI, ALT, age, and triglycerides. Angulo *et al.* proposed the NAFLD fibrosis score which can be calculated from parameters such as age, platelet count, albumin, AST/ALT ratio, fasting hyperglycemia/DM, and BMI.[50] The NAFLD fibrosis score is simple and has advantages. However, the major problem is that liver biopsy cannot be avoided in around 25% cases, which are classified as intermediate because of scores halfway between the high cut-off level and the low cut-off level. Harrison *et al.* proposed the simple and easy BARD score based on BMI $\geq$ 28 kg/m^2, AST/ALT ratio, and DM; and reported that the odds ratio increased 17-fold for cases with scores of two points or higher, associated with F3 or higher stages of fibrosis.[51] However, Fujii *et al.* suggested that the BARD score was not useful in Japanese patients because the average BMI is significantly lower than western people.[52] The N (Nippon) score[15] is very simple; it can be calculated on the basis of only gender, age, and the presence or absence of type 2 DM and HTN, and has been evaluated by a multicenter study in Japan.[16] Recently we showed that senescence marker protein 30 (SMP-30), which has an antiapoptotic activity and an effect on Ca^{++} efflux, was significantly decreased in NASH compared to SS. Thus, SMP-30 is a useful marker for the differential diagnosis between SS and NASH. However, at present we cannot detect it in serum.[53]

Prognosis

It has been reported that cardiovascular-related death and liver-related death are significantly higher in NAFLD patients than with the general population.[54] A cohort study conducted in 2006, reported a development of cancers among 97 771 individuals in the general Japanese population; 6.7% of men and 3.1% of women had DM, in diabetes patients, the hazard ratio of developing liver cancer was 2.24 (95% CI, 1.64–3.04) in men, and 1.94 (95% CI, 1.00–3.73) in women during an average follow-up period of 10.7-years.[55]

In a comparative study between HCV and NASH cirrhosis matched by gender and age, obesity, diabetes, and dyslipidemia were significantly more frequent in NASH cirrhosis. The 5-year cancer rate was 11.3% in NASH cirrhosis and 30.5% in HCV cirrhosis.[55] The leading cause of death in these two types of cirrhosis was HCC, 47% in NASH and 68% in HCV, and the second cause was hepatic failure, 32% in NASH and 25% in HCV.[56,57] The annual incidence of HCC in Japan is 2.2% in NASH cirrhosis and 6.1% in HCV cirrhosis. Meanwhile, Ascha *et al.* reported that the annual incidence of HCC was 2.6% in patients with NASH cirrhosis, compared to 4.0% in HCV cirrhosis in the USA.[58]

Treatment

Diet and exercise therapy

Weight loss achieved by diet and exercise is the most important aspect of treatment in obese patients with NAFLD, including NASH. In those treated weight, blood biochemical data such as ALT, albumin, cholinesterase, total cholesterol and fasting blood glucose values, and steatosis decreased significantly after significant weight loss.[59] The recommended daily energy intake is 25–35 kcal/kg, daily protein intake is 1.0–1.5 g/kg and fat should be less than 20% of total calories.

Antihyperlipidemic drugs

Saibara *et al.* showed that bezafibrate for tamoxifen-induced NASH resulted in biochemical and histological improvement.[60] Dohmen *et al.* reported that administration of fenofibrate for fatty liver complicated with dyslipidemia improved dyslipidemia and led to a decrease in the levels of ALP, whereas the levels of ALT showed no significant change.[61] Hyogo *et al.* reported that atorvastatin led to an improvement in liver function, fibrosis marker, adipocytokine, and improvement of fatty liver and hepatic inflammation.[62] Nozaki *et al.* reported the utility of ezetimibe and acarbose in mouse models of NAFLD.[63] Recently, we also demonstrated the histological improvement using 96 weeks of ezetimibe monotherapy in the 45 biopsy-proven NAFLD patients.[64]

Angiotensin II type-1 receptor blockers (ARB)

There also have been a number of reports from Japan regarding the utility of angiotensin II type-1 blockers (ARB) in NASH. This application is derived from basic studies which showed the inhibitory effect of ARB on the progression of fibrosis via inhibition of the activation of hepatic stellate cells.[65–67]

Antidiabetic drugs

Morita *et al.* demonstrated the effect of nateglinide on glucose metabolism, liver function, and liver histology in NASH patients with type 2 diabetes.[68] The effects of metformin and thiazolidine derivatives such as pioglitazone and rosiglitazone on NASH were reported in Japan, however, the numbers were small and the trials were uncontrolled.

Other drugs

There is the possibility that combination therapies using pantethine and probucol,[69] colestimide[70] and α-tocopherol[71] are useful for NASH; however, the subjects were in small numbers and there was no histological analysis after treatment. Recently, Sanyal *et al.* reported that administration of vitamin E for 96 weeks administration for non-DM NASH patients significantly improved liver histology compared to placebo, this result being more promising than pioglitazone administration.[72] Phlebotomy might be effective in NASH with excessive iron deposition in the liver.[73]

Group survey in NASH in Japan

As mentioned above, the Japan NASH Study Group founded in April 2008 (the representative: Takeshi Okanoue, Table 1), has started the following research projects: (i) nationwide study of 5000 cases of diabetes mellitus; (ii) SNP study of 1000 cases of SS and NASH; (iii) long-term follow-up study of 1000 cases of SS

Table 1 The members of Japan NASH study group

The representative of this study group:
Takeshi Okanoue MD, PhD., Hepatology Unit, Saiseikai Suita Hospital
The members of this study group:
Yutaka Kohgo M.D, PhD., Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College,
Sumio Kawata M.D, PhD., Department of Gastroenterology, Yamagata University Faculty of Medicine,
Kazuhiko Koike, M.D., PhD, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo,
Kohjiro Ueki, M.D., PhD., Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo,
Shigeki Arii, M.D., PhD., Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University,
Etsuko Hashimoto, M.D., PhD., Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University,
Sumio Watanabe, M.D., PhD., Department of Gastroenterology, Juntendo University School of Medicine,
Toshinari Takamura, M.D., PhD., Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science
Kohichiroh Yasui, M.D., PhD., Department of Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine,
Fumihiko Matsuda, PhD, Center for Genomic Medicine, Kyoto University Graduate School of Medicine,
Tetsuo Takehara, M.D., PhD., Gastroenterology and Hepatology, Osaka University Graduate School of Medicine,
Toshiji Saibara, M.D., PhD., Department of Gastroenterology and Hepatology, Kochi Medical School,
Hirofumi Uto, M.D., PhD., Department of Digestive and Life-Style Related Disease, Kagoshima University Graduate School of Medical and Dental Sciences

and NASH; (iv) collection of 100 cases of NASH-HCC; (v) biochemical markers of differential diagnosis between SS and NASH; and (vi) therapeutic guidelines based on the individual pathophysiology. Projects i, ii, iii, and iv are going well and we are expecting to present these results, including SNPs, in the near future.

Conclusion

Recently, much attention has been paid to NAFLD in Japan because the number of NAFLD patients has been increasing, while non-B, non-C HCC also is increasing gradually. We suspect that NASH might be responsible for this increase in HCC in Japan; however, the precise cause of the increased non B, non C HCC has not yet been established. In this review, we have described the epidemiology and the present status of clinical and basic aspects of NASH/NAFLD in Japan.

Acknowledgments

This study was funded by the grant from by the Ministry of Labor and Welfare Japan. The authors thank all members of the Japan NASH Study Group.

References

1 Hotta N, Nakamura J, Iwamoto Y *et al.* Causes of death in Japanese diabetics based on the results of a survey of 18 385 diabetics during 1991–2000. Report of Committee on Cause of Death in Diabetes Mellitus.*J. Jpn. Diabetes Soc.* 2007; **50**: 47–61.
2 Dock JSoN. The report of 2008 National Summary of Ningen Dock,The 50th Scientific Meetings of Japan Society of Ningen Dock (in Japanese). *Official J. Jpn. Soc. Ningen Dock.* 2009; **24**: 322.
3 Kojima S, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol.* 2003; **38**: 954–61.
4 Tanaka H, Saibara T. Genetic background of NASH and environmental factor. *KAN·TAN·SUI (in Japanese).* 2006; **53**: 301–8.
5 Hamaguchi M, Kojima T, Takeda N *et al.* The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann. Intern. Med.* 2005; **143**: 722–8.
6 Syndrome TECoCfM. The definition and criteria of metabolic syndrome. (in Japanese). *J. Jpn. Soc. Intern. Med.* 2005; **94**: 794–809.
7 Welfare MoHLa. *National Health and Nutrition Examination Survey in Japan* (in Japanese). 2008.
8 Ishibashi E, Eguchi Y, Eguchi T *et al.* Waist circumference correlates with hepatic fat accumulation in male Japanese patients with non-alcoholic fatty liver disease, but not in females. *J. Gastroenterol. Hepatol.* 2008; **23**: 908–13.
9 Sakurai M, Takamura T, Miura K, Kaneko S, Nakagawa H. Middle-aged Japanese women are resistant to obesity-related metabolic abnormalities. *Metabolism* 2009; **58**: 456–9.
10 Hamaguchi M, Kojima T, Takeda N *et al.* Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J. Gastroenterol.* 2007; **13**: 1579–84.
11 Michitaka K, Nishiguchi S, Aoyagi Y, Hiasa Y, Tokumoto Y, Onji M. Etiology of liver cirrhosis in Japan: a nationwide survey. *J. Gastroenterol.* 2010; **45**: 86–94.
12 Hepatology TJSo. *The Medical Guide of NASH·NAFLD* (in Japanese). 2010.
13 Welfare MoHLa. *National Health and Nutrition Examination Survey in Japan* (in Japanese). 2007.
14 Jimba S, Nakagami T, Takahashi M *et al.* Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. *Diabet. Med.* 2005; **22**: 1141–5.
15 Miyaaki H, Ichikawa T, Nakao K *et al.* Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. *Liver Int.* 2008; **28**: 519–24.
16 Shiga T, Moriyoshi Y, Nagahara H, Shiratori K. Nonalcoholic fatty liver is a risk factor for postprandial hyperglycemia, but not for impaired fasting glucose. *J. Gastroenterol.* 2009; **44**: 757–64.
17 Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44–52.
18 Fallo F, Dalla Pozza A, Sonino N *et al.* Nonalcoholic fatty liver disease, adiponectin and insulin resistance in dipper and nondipper essential hypertensive patients. *J. Hypertens.* 2008; **26**: 2191–7.
19 Donati G, Stagni B, Piscaglia F *et al.* Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance. *Gut* 2004; **53**: 1020–3.
20 Yasui K, Sumida Y, Mori Y *et al.* Nonalcoholic steatohepatitis and increased risk of chronic kidney disease. *Metabolism* 2010; DOI: 10.1016/j.metabol.2010.07.022.
21 Tominaga K, Fujimoto E, Suzuki K, Hayashi M, Ichikawa M, Inaba Y. Prevalence of non-alcoholic fatty liver disease in children and

relationship to metabolic syndrome, insulin resistance, and waist circumference. *Environ. Health Prev. Med.* 2009; **14**: 142–9.
22 Tsuruta G, Tanaka N, Hongo M *et al.* Nonalcoholic fatty liver disease in Japanese junior high school students: its prevalence and relationship to lifestyle habits. *J. Gastroenterol.* 2010; **45**: 666–72.
23 Weston SR, Leyden W, Murphy R *et al.* Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; **41**: 372–9.
24 Mohanty SR, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J. Hepatol.* 2009; **50**: 797–804.
25 Jarrar MH, Baranova A, Collantes R *et al.* Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* 2008; **27**: 412–21.
26 Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J. Hepatol.* 2007; **46**: 1104–10.
27 Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46–54.
28 Takahashi M, Arita Y, Yamagata K *et al.* Genomic structure and mutations in adipose-specific gene, adiponectin. *Int. J. Obes. Relat. Metab. Disord.* 2000; **24**: 861–8.
29 Stumvoll M, Tschritter O, Fritsche A *et al.* Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 2002; **51**: 37–41.
30 Tokushige K, Hashimoto E, Noto H *et al.* Influence of adiponectin gene polymorphisms in Japanese patients with non-alcoholic fatty liver disease. *J. Gastroenterol.* 2009; **44**: 976–82.
31 Yoneda M, Hotta K, Nozaki Y *et al.* Association between angiotensin II type 1 receptor polymorphisms and the occurrence of nonalcoholic fatty liver disease. *Liver Int.* 2009; **29**: 1078–85.
32 Nozaki Y, Saibara T, Nemoto Y *et al.* Polymorphisms of interleukin-1 beta and beta 3-adrenergic receptor in Japanese patients with nonalcoholic steatohepatitis. *Alcohol. Clin. Exp. Res.* 2004; **28**: S106–10.
33 Namikawa C, Shu-Ping Z, Vyselaar JR *et al.* Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J. Hepatol.* 2004; **40**: 781–6.
34 Dong H, Wang J, Li C *et al.* The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. *J. Hepatol.* 2007; **46**: 915–20.
35 Suzuki A, Abdelmalek MF. Nonalcoholic fatty liver disease in women. *Womens Health (Lond Engl)* 2009; **5**: 191–203.
36 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842–5.
37 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413–9.
38 Kleiner DE, Brunt EM, Van Natta M *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313–21.
39 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.* 1999; **94**: 2467–74.
40 Okanoue T, Ohta M, Ou O *et al.* Relationship of Mallory bodies to intermediate filaments in hepatocytes. A scanning electron microscopy study. *Lab. Invest.* 1985; **53**: 534–40.
41 Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am. J. Gastroenterol.* 2007; **102**: 399–408.
42 Campos GM, Bambha K, Vittinghoff E *et al.* A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. *Hepatology* 2008; **47**: 1916–23.
43 Yilmaz Y, Dolar E, Ulukaya E *et al.* Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. *World J. Gastroenterol.* 2007; **13**: 837–44.
44 Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K. Correlation of hepatic steatosis with body mass index, serum ferritin level and hepatic fibrosis in Japanese patients with chronic hepatitis C. *Hepatol. Res.* 2007; **37**: 263–9.
45 Sumida Y, Nakashima T, Yoh T *et al.* Serum thioredoxin elucidates the significance of serum ferritin as a marker of oxidative stress in chronic liver diseases. *Liver* 2001; **21**: 295–9.
46 Sumida Y, Kanemasa K, Itoh Y, Yoshikawa T. A proposal of simple scoring system to differentiate nonalcoholic steatohepatitis from nonalcoholic fatty liver disease: we originally named NAFIC score composed of serum ferritin, fasting insulin, type? collagen 7S. *Kanzo* 2008; **49**: 279–81.
47 Fujii H, Enomoto M, Fukushima W *et al.* Noninvasive laboratory tests proposed for predicting cirrhosis in patients with chronic hepatitis C are also useful in patients with non-alcoholic steatohepatitis. *J. Gastroenterol.* 2009; **44**: 608–14.
48 de Andrade AR, Cotrim HP, Alves E *et al.* Nonalcoholic fatty liver disease in severely obese individuals: the influence of bariatric surgery. *Ann. Hepatol.* 2008; **7**: 364–8.
49 Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of liver fibrosis. *Gastroenterol. Clin. Biol.* 2008; **32**: 22–39.
50 Angulo P, Hui JM, Marchesini G *et al.* The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846–54.
51 Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441–7.
52 Fujii H, Enomoto M, Fukushima W, Tamori A, Sakaguchi H, Kawada N. Applicability of BARD score to Japanese patients with NAFLD. *Gut* 2009; **58**: 1566–7. author reply 7.
53 Park H, Ishigami A, Shima T *et al.* Hepatic senescence marker protein-30 is involved in the progression of nonalcoholic fatty liver disease. *J. Gastroenterol.* 2010; **45**: 426–34.
54 Adams LA, Lymp JF, St Sauver J *et al.* The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113–21.
55 Inoue M, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch. Intern. Med.* 2006; **166**: 1871–7.
56 Yatsuji S, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2009; **24**: 248–54.
57 Hashimoto E, Yatsuji S, Tobari M *et al.* Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *J. Gastroenterol.* 2009; **44** (Suppl. 19): 89–95.
58 Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in

patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972–8.
59 Ueno T, Sugawara H, Sujaku K *et al.* Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J. Hepatol.* 1997; **27**: 103–7.
60 Saibara T, Onishi S, Ogawa Y, Yoshida S, Enzan H. Bezafibrate for tamoxifen-induced non-alcoholic steatohepatitis. *Lancet* 1999; **353**: 1802.
61 Dohmen K, Wen CY, Nagaoka S *et al.* Fenofibrate-induced liver injury. *World J. Gastroenterol.* 2005; **11**: 7702–3.
62 Hyogo H, Tazuma S, Arihiro K *et al.* Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism* 2008; **57**: 1711–8.
63 Nozaki Y, Fujita K, Yoneda M *et al.* Long-term combination therapy of ezetimibe and acarbose for non-alcoholic fatty liver disease. *J. Hepatol.* 2009; **51**: 548–56.
64 Park H, Shima T, Okanoue T. Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease. *J. Gastroenterol.* 2010; DOI: 10.1007/s00535-010-0291-8.
65 Yoshiji H, Noguchi R, Ikenaka Y *et al.* Losartan, an angiotensin-II type 1 receptor blocker, attenuates the liver fibrosis development of non-alcoholic steatohepatitis in the rat. *BMC Res. Notes* 2009; **2**: 70.
66 Fujita K, Yoneda M, Wada K *et al.* Telmisartan, an angiotensin II type 1 receptor blocker, controls progress of nonalcoholic steatohepatitis in rats. *Dig. Dis. Sci.* 2007; **52**: 3455–64.
67 Yokohama S, Tokusashi Y, Nakamura K *et al.* Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. *World J. Gastroenterol.* 2006; **12**: 322–6.
68 Morita Y, Ueno T, Sasaki N *et al.* Nateglinide is useful for nonalcoholic steatohepatitis (NASH) patients with type 2 diabetes. *Hepatogastroenterology* 2005; **52**: 1338–43.
69 Tokushige K, Hashimoto E, Yatsuji S, Taniai M, Shiratori K. Combined pantethine and probucol therapy for Japanese patients with non-alcoholic steatohepatitis. *Hepatol. Res.* 2007; **37**: 872–7.
70 Taniai M, Hashimoto E, Tobari M *et al.* Treatment of nonalcoholic steatohepatitis with colestimide. *Hepatol. Res.* 2009; **39**: 685–93.
71 Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. *Aliment. Pharmacol. Ther.* 2001; **15**: 1667–72.
72 Sanyal AJ, Chalasani N, Kowdley KV *et al.* Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* 2010; **362**: 1675–85.
73 Sumida Y, Kanemasa K, Fukumoto K *et al.* Effect of iron reduction by phlebotomy in Japanese patients with nonalcoholic steatohepatitis: a pilot study. *Hepatol. Res.* 2006; **36**: 315–21.

ORIGINAL ARTICLES—LIVER, PANCREAS, AND BILIARY TRACT

Characteristics of Patients With Nonalcoholic Steatohepatitis Who Develop Hepatocellular Carcinoma

KOHICHIROH YASUI,* ETSUKO HASHIMOTO,‡ YASUJI KOMORIZONO,§ KAZUHIKO KOIKE,‖ SHIGEKI ARII,¶ YASUHARU IMAI,# TOSHIHIDE SHIMA,** YOSHIHIRO KANBARA,** TOSHIJI SAIBARA,‡‡ TAKAHIRO MORI,§§ SUMIO KAWATA,‖‖ HIROFUMI UTO,¶¶ SHIRO TAKAMI,## YOSHIO SUMIDA,*** TOSHINARI TAKAMURA,‡‡‡ MIWA KAWANAKA,§§§ TAKESHI OKANOUE*,** and the Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan

*Department of Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto; ‡Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo; §Department of Hepatology, Nanpuh Hospital, Kagoshima; ‖Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, Tokyo; ¶Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Tokyo; #Department of Internal Medicine, Ikeda Municipal Hospital, Ikeda; **Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita; ‡‡Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi; §§Department of Gastroenterology, Osaka Railway Hospital, Osaka; ‖‖Department of Gastroenterology, Yamagata University School of Medicine, Yamagata; ¶¶Digestive Disease and Life-style Related Disease Health Research, Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima; ##Department of Gastroenterology, Otsu Municipal Hospital, Otsu; ***Center for Digestive and Liver Diseases, Nara City Hospital, Nara; ‡‡‡Department of Disease Control and Homeostasis, Kanazawa University, Graduate School of Medical Science, Kanazawa; and §§§Center of Liver Diseases, Kawasaki Hospital, Kawasaki Medical School, Okayama, Japan

This article has an accompanying continuing medical education activity on page e50. Learning Objectives—At the end of this activity, the learner should identify the clinical features of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma and the role of hepatic fibrosis in the development of hepatocellular carcinoma.

See related article, Villanueva A et al, on page 1501 in *Gastroenterology*.

BACKGROUND & AIMS: Nonalcoholic steatohepatitis (NASH) can progress to hepatocellular carcinoma (HCC). We aimed to characterize the clinical features of NASH patients with HCC. **METHODS:** In a cross-sectional multicenter study in Japan, we examined 87 patients (median age, 72 years; 62% male) with histologically proven NASH who developed HCC. The clinical data were collected at the time HCC was diagnosed. **RESULTS:** Obesity (body mass index ≥25 kg/m^2), diabetes, dyslipidemia, and hypertension were present in 54 (62%), 51 (59%), 24 (28%), and 47 (55%) patients, respectively. In nontumor liver tissues, the degree of fibrosis was stage 1 in 10 patients (11%), stage 2 in 15 (17%), stage 3 in 18 (21%), and stage 4 (ie, liver cirrhosis) in 44 (51%). The prevalence of cirrhosis was significantly lower among male patients (21 of 54, 39%) compared with female patients (23 of 33, 70%) (P = .008). **CONCLUSIONS: Most patients with NASH who develop HCC are men; the patients have high rates of obesity, diabetes, and hypertension. Male patients appear to develop HCC at a less advanced stage of liver fibrosis than female patients.**

Keywords: Liver Cancer; Incidence; Sex; Retrospective Study.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer mortality.[1] HCC mostly occurs within an established background of chronic liver disease and cirrhosis. Although the risk factors for HCC, including infection with hepatitis B and C viruses as well as alcohol consumption, are well-defined, 5%–30% of patients with HCC lack a readily identifiable risk factor for their cancer. It has been suggested that a more severe form of nonalcoholic fatty liver disease (NAFLD), namely nonalcoholic steatohepatitis (NASH), might account for a substantial portion of cryptogenic cirrhosis and HCC cases.[2]

NAFLD is one of the most common causes of chronic liver disease in the world.[3,4] NAFLD is associated with obesity, diabetes, dyslipidemia, and insulin resistance and is recognized as a hepatic manifestation of metabolic syndrome. The spectrum of NAFLD ranges from a relatively benign accumulation of lipid (simple steatosis) to progressive NASH associated with fibrosis, necrosis, and inflammation. Despite its common occurrence and potentially serious nature, relatively little is known about the natural history or prognostic significance of NAFLD. Although prospective studies on the natural history of NAFLD and NASH with a larger cohort are awaited, these

Abbreviations used in this paper: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CT, computed tomography; DCP, des-γ-carboxy prothrombin; γ-GTP, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

1542-3565/$36.00
doi:10.1016/j.cgh.2011.01.023

studies might be limited by the long and asymptomatic clinical course of these diseases, by their high prevalence in the general population, and by the lack of serologic markers for NASH. The evidence suggesting that NASH can progress to HCC comes from (1) case reports and case series,[5-8] (2) retrospective studies,[9-12] and (3) prospective studies.[13-17] These studies generally examined limited numbers of cases and follow-ups; therefore, the incidence of HCC and risk factors for HCC in NASH patients remain unclear.

The Japan NASH Study Group (representative, Takeshi Okanoue)[18] was established in 2008 by the Ministry of Health, Labour and Welfare of Japan to address unmet research needs in the area of liver diseases. As a part of this mandate, the study group conducted a cross-sectional multicenter study to characterize the clinical features of histologically proven NASH patients who developed HCC.

Methods

Patients

We retrospectively identified and reviewed 87 Japanese patients with NASH, who developed HCC between 1993 and 2010, at 15 hepatology centers that belong to the Japan NASH Study Group[18] and their affiliated hospitals in Japan. The diagnosis of NASH was based on (1) the histologic features of steatohepatitis, (2) negligible alcohol consumption, and (3) exclusion of liver diseases of other etiology. To determine alcohol consumption as accurately as possible, we reviewed medical records in our institutions, and when patients had been transferred from other institutions, we also reviewed a summary of medical records from those institutions. According to the medical records, alcohol consumption was assessed on the basis of a detailed history that was obtained by physicians and by interviewing family members. Exclusion criteria included consumption of more than 20 g of alcohol per day, positivity for hepatitis B virus surface antigen, positivity for anti-hepatitis C virus antibody, the presence of other types of liver diseases (eg, primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, or hemochromatosis), previous treatment with drugs known to produce hepatic steatosis, and a history of gastrointestinal bypass surgery. The sections of nontumor liver tissues were reanalyzed by experienced hepatopathologists (T.O., E.H.) who were blinded to the laboratory parameters and clinical data. We excluded patients whose histologic diagnosis of NASH was not confirmed by central review and patients with insufficient or inconclusive information concerning alcohol consumption, body mass index (BMI), and laboratory data including fasting glucose and lipid.

Of the 87 patients, 14 patients had been previously diagnosed as NAFLD or NASH and had been followed at our institutions; 73 patients had been transferred from other institutions to our institutions for investigation and treatment of HCC. Most patients had been identified as having HCC during screening, which included ultrasound and/or computed tomography (CT) of the liver and alpha-fetoprotein (AFP) testing.

The diagnosis of HCC was based on liver histology and, in the absence of histology, on typical features of HCC as assessed by dynamic CT or magnetic resonance imaging (MRI) (ie, hypervascular with washout in the portal/venous phase).[19] Of the 87 patients, 49 patients were diagnosed as HCC after hepatic resection, 21 patients were diagnosed after ultrasound-guided tumor biopsy, and 17 patients were diagnosed by dynamic CT or MRI.

The Ethics Committees of each participating center approved this study. Informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

Clinical Assessment and Laboratory Tests

The clinical and laboratory data were collected at the time HCC was diagnosed. BMI was calculated by using the following formula: weight in kilograms/(height in meters)^2. Obesity was defined as BMI $\geq$25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity.[20] Diabetes was defined as fasting plasma glucose concentration of $\geq$126 mg/dL or 2-hour plasma glucose concentration of $\geq$200 mg/dL during an oral glucose (75 g) tolerance test or by the use of insulin or oral hypoglycemic agents to control blood glucose.[21] Hypertension was defined as systolic blood pressure $\geq$130 mm Hg or diastolic blood pressure $\geq$85 mm Hg or by the use of antihypertensive agents.[22] Dyslipidemia was defined as serum concentrations of triglycerides $\geq$150 mg/dL or high-density lipoprotein (HDL) cholesterol $<$40 mg/dL and $<$50 mg/dL for men and women, respectively, or by the use of specific medication.[22]

Venous blood samples were taken in the morning after 12-hour overnight fast. The laboratory evaluations included blood cell count and measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), fasting plasma glucose, HbA1c, total cholesterol, HDL cholesterol, triglyceride, ferritin, hyaluronic acid, AFP, and des-γ-carboxy prothrombin (DCP). These parameters were measured by using standard clinical chemistry techniques.

Histopathologic Examination

Nontumor liver tissues were obtained from all 87 patients to diagnose the background liver tissue at the time HCC was diagnosed. In 49 patients who underwent hepatic resection for HCC, we examined nontumor liver tissues that were surgically resected. In 21 patients who underwent ultrasound-guided tumor biopsy, nontumor liver tissues far from HCC tumors were biopsied separately. In 17 patients who were diagnosed as HCC by dynamic CT or MRI and did not undergo either hepatic resection or tumor biopsy, only nontumor liver tissues far from HCC tumors were obtained by ultrasound-guided biopsy.

The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, with Masson trichrome, and by silver impregnation. NASH was defined as steatosis with lobular inflammation, hepatocellular ballooning, and Mallory's hyaline (Mallory's body) or fibrosis.[23-25] The necroinflammatory grade and the degree of fibrosis were evaluated and scored according to the criteria proposed by Brunt et al.[26]

Statistical Analysis

Results are presented as numbers with percentages in parentheses for qualitative data or as the medians and ranges (25th-75th percentiles) for quantitative data. Comparisons were made by using a χ^2 test for qualitative factors or a Mann-

Table 1. Patient Characteristics

Characteristic	Total (n = 87)	Male (n = 54)	Female (n = 33)	*P* value[a]
Age (*y*)	72 (69–75)	72 (69–75)	72 (68–75)	.52
BMI (kg/m^2)	26.0 (23.8–28.3)	26.0 (23.8–28.8)	26.2 (23.9–27.7)	.54
Obesity	54 (62%)	35 (65%)	19 (58%)	.50
Diabetes	51 (59%)	31 (57%)	20 (61%)	.77
Dyslipidemia	24 (28%)	13 (24%)	11 (33%)	.35
Hypertension	47 (54%)	22 (41%)	25 (76%)	.001
Platelet count ($\times 10^4/\mu L$)	13.9 (10.1–18.0)	14.5 (11.7–18.0)	10.9 (7.8–18.0)	.05
AST (*IU/L*)	47 (30–59)	46 (27–60)	47 (35–58)	.45
ALT (*IU/L*)	36 (26–55)	43 (26–69)	34 (26–42)	.11
γ-GTP (*IU/L*)	75 (40–115)	68 (36–177)	75 (40–115)	.90
Fasting glucose (*mg/dL*)	114 (99–145)	112 (99–144)	120 (97–152)	.59
HbA1c (%)	6.1 (5.4–7.1)	5.9 (5.4–7.0)	6.3 (5.2–7.1)	.78
Total cholesterol (*mg/dL*)	169 (147–202)	169 (147–202)	169 (147–202)	.62
HDL cholesterol (*mg/dL*)	50 (41–60)	45 (41–58)	55 (50–73)	.03
Triglyceride (*mg/dL*)	100 (76–138)	118 (80–147)	96 (74–116)	.06
Ferritin (*ng/dL*)[b]	197 (74–401)	273 (154–703)	98 (23–172)	.005
Hyaluronic acid (*ng/mL*)[c]	166 (67–241)	151 (69–244)	174 (61–332)	.85
AFP (*ng/mL*)	7.1 (5.0–18.0)	6.0 (4.0–14.7)	10.8 (5.9–18.0)	.02
DCP (*mAU/mL*)	66 (22–298)	48 (22–243)	81 (21–942)	.42
HCC tumor size (*cm*)	3.0 (2.0–4.0)	3.1 (2.2–4.5)	2.6 (1.9–4.0)	.18
Number of HCC tumors				.78
1	65 (75%)	39 (72%)	26 (79%)	
2 or 3	16 (18%)	11 (20%)	5 (15%)	
≥4	6 (7%)	4 (8%)	2 (6%)	
Background liver tissue				
Steatosis grade				.64
0: <5%	1 (1%)	1 (2%)	0 (0%)	
1: 5%–33%	60 (69%)	36 (67%)	24 (73%)	
2: 34%–66%	19 (22%)	11 (20%)	8 (24%)	
3: >66%	7 (8%)	6 (11%)	1 (3%)	
Necroinflammatory grade[d]				.22
1: mild	31 (35%)	22 (41%)	9 (27%)	
2: moderate	45 (52%)	26 (48%)	19 (58%)	
3: severe	11 (13%)	6 (11%)	5 (15%)	
Fibrosis stage[d]				.003
1	10 (11%)	10 (18%)	0 (0%)	
2	15 (17%)	10 (18%)	5 (15%)	
3	18 (21%)	13 (25%)	5 (15%)	
4	44 (51%)	21 (39%)	23 (70%)	

NOTE. Values are medians (25th–75th percentiles) or numbers (%). Where no other unit is specified, values refer to number of patients.
[a]χ^2 test or Mann–Whitney *U* test.
[b]Missing data for 27 patients.
[c]Missing data for 29 patients.
[d]According to reference 26.

Whitney *U* test on ranks for quantitative factors with non-equal variance. *P* values less than .05 from two-sided tests were considered to be significant. All statistical analyses were performed by using SPSS 15.0 software (SPSS Inc, Chicago, IL).

Results

The characteristics of the 87 NASH patients who developed HCC are summarized in Table 1. The median age was 72 years (25th percentile, 69; 75th percentile, 75); the mean age (standard deviation) was 71.2 (6.7) years. There were 54 male patients (62%) and 33 female patients (38%); the male:female ratio was 1.6:1. The median BMI was 26.0 kg/m^2, and 54 patients (62%) were obese (BMI ≥25 kg/m^2). Diabetes, dyslipidemia, and hypertension were present in 51 (59%), 24 (28%), and 47 (55%) patients, respectively.

The diagnosis of NASH was proved by histologic examination of nontumor liver tissues at the time HCC was diagnosed. The degree of steatosis was grade 1 (5%–33%) in 60 patients (69%), grade 2 (34%–66%) in 19 (22%), and grade 3 (>66%) in 7 (8%). One patient who showed less than 5% steatosis was diagnosed as "burn-out" NASH, because a previous liver biopsy that was performed before development of HCC had demonstrated typical histologic features of NASH. The necroinflammatory grade was mild (grade 1) in 31 patients (35%), moderate (grade 2) in 45 (52%), and severe (grade 3) in 11 (13%). The degree of fibrosis was stage 1 in 10 patients (11%), stage 2 in 15 (17%), stage 3 in 18 (21%), and stage 4 (ie, liver cirrhosis) in 44 (51%).

The median diameter of HCC tumors was 3.0 cm (25th percentile, 2.0; 75th percentile, 4.0). A single HCC lesion was present in 65 of 87 patients (75%).

Data were stratified according to sex (Table 1). Compared with female patients, male patients had significantly less hypertension, lower HDL cholesterol and AFP, higher ferritin, and a less advanced stage of fibrosis. The prevalence of cirrhosis was significantly lower in male patients (21 of 54, 39%) than in female patients (23 of 33, 70%) ($P = .008$).

Discussion

In this cross-sectional multicenter study in Japan, we showed the clinical features of a relatively large number (n = 87) of NASH patients with HCC. The male:female ratio was 1.6:1. Men have higher HCC rates than women in almost all populations, with male:female ratios usually averaging between 2:1 and 4:1.[2] In the latest nationwide survey of HCC in Japan,[27] this ratio was 2.5:1. The reasons underlying higher rates of HCC in men might relate to sex-specific differences in exposure to risk factors. Men are more likely to be infected with hepatitis B and C viruses, consume alcohol, smoke cigarettes, and have increased iron stores.[2] Moreover, androgens are considered to influence the development of HCC. With regard to the male: female ratio of HCC associated with NASH, a male:female ratio of 1.3:1 was reported in a summary of 16 published cases of HCC associated with NASH.[28] Ratios of 2.8:1 and 0.67:1 were reported in 2 retrospective studies of HCC arising from cryptogenic cirrhosis in Italy (n = 44)[10] and the United States (n = 30),[9] respectively, and a ratio of 1.6:1 was reported for 36 cases of NASH-associated HCC from a single center in Japan.[15] Overall, NASH patients with HCC are more often men. However, these male:female ratios might be lower than the ratios for HCC of other etiologies, including viral hepatitis and alcohol consumption.

Although it is well-known that male gender is a risk factor for HCC in patients infected with hepatitis B and C viruses,[2] it remains unclear whether male gender is a factor associated with the development of HCC in NASH patients. It is now suspected that there is an even distribution of NASH among men and women.[29] In another study by our group,[30] the male:female ratio was 0.85:1 in 342 NASH patients without cirrhosis and HCC. The male:female ratio (1.6:1) of NASH patients with HCC in the present study is higher than this ratio. In agreement with our observations, a case-control study showed that the male: female ratio was 1.6:1 in 34 NASH patients with HCC, whereas the ratio was 0.69:1 in 348 NASH patients without HCC.[15] A recent prospective study indicated that older age and alcohol consumption were independent risk factors for the development of HCC in patients with NASH-cirrhosis and that male gender tended to be associated with the development of HCC, although this trend did not reach statistical significance.[17]

The median age of our patients was 72 years. There was no significant difference in age between men and women. Although the global age distribution of HCC varies by geographic region, sex, and etiology, in almost all areas the peak female age group in HCC patients is 5 years older than in male HCC patients.[2] In a nationwide survey of HCC in Japan,[27] the mean ages were 65.5 years for men and 69.4 years for women. The male patients in the present study are slightly older than the mean ages reported in these previous studies.

Consistent with the literature,[9-12] more than half of our patients displayed obesity, diabetes, and hypertension. Obesity constitutes a significant risk factor for cancer mortality in general and is an increasingly recognized risk factor for HCC in particular.[31,32] In the present study, body weight was measured at the time HCC was diagnosed. Because advanced HCC might cause weight loss, it is likely that our patients were obese before the development of HCC. Diabetes has also been proposed as a risk factor for HCC.[2] Thus, HCC shares 2 major risk factors, obesity and diabetes, with NASH.

Once cirrhosis and HCC are established, it is difficult to identify pathologic features of NASH. As NASH progresses to cirrhosis, steatosis tends to disappear, so-called burn-out NASH.[5] As expected, the grade of steatosis was mild in most of our cases. It was possible to diagnose 1 case without steatosis as burn-out NASH, because a previous liver biopsy specimen (liver biopsy was performed 25 years prior) was preserved and available. It is likely that many cases of NASH-associated HCC might have been missed because of loss of the telltale sign of steatosis.

Most HCC arises on a background of cirrhosis. It is less clear whether cirrhosis is a necessary predisposition for the development of HCC in patients with NASH. Case reports of HCC arising from NAFLD and NASH patients without fibrosis or cirrhosis have been accumulating.[33-36] Cirrhosis (fibrosis stage 4) was present in 51% of cases, and advanced stages of fibrosis (stage 3 or 4) were found in 72% of cases in the present study. Indeed, cirrhosis or advanced fibrosis appeared to be the predominant risk factors for HCC development. However, in the remaining 28% of cases, HCC developed in patients with less fibrosis (stage 1 or 2). Interestingly, male patients developed HCC at a less advanced stage of fibrosis than female patients, and the prevalence of cirrhosis was significantly lower in men (39%) than in women (70%). Although the reason for the sex differences is unclear, these findings indicate that screening for HCC is needed not only in NASH patients with advanced fibrosis but also in those with less fibrosis, particularly if they are men. Further studies are needed to confirm this potentially important observation. Paradis et al[37] reported that in patients whose only risk factors for chronic liver disease are features of metabolic syndrome, HCC usually occurs in the absence of significant liver fibrosis. In addition, they found that some of these HCCs developed on preexisting liver cell adenomas. However, no preexisting adenomas were observed in the present cases.

Compared with female patients, male patients had significantly higher serum ferritin value. The normal value for ferritin varies according to the age and gender of the individual. Adult men have serum ferritin values averaging approximately 100 ng/mL (range, 75–250), whereas adult women have levels averaging approximately 30 ng/mL (range, 20–75).[38] Thus, normal men have higher ferritin levels than women. Elevation of ferritin levels is associated with NASH.[39] Because we excluded patients with alcohol consumption as rigorously as possible, we believe that alcohol consumption did not contribute to the elevation of ferritin levels in our patients.

The median diameter of the HCCs in the present study was 3.0 cm, which is equal to or smaller than the size of previously reported HCCs.[9,10,12,28,37] This is probably because most of our patients had been identified as having HCC during screening. A single HCC lesion was present in 75% of patients. For early detection of NASH-associated HCC, vigilant screening is important,[9] and the development of serologic markers for NASH is necessary.

The mechanisms of carcinogenesis in NASH remain to be elucidated. Possible mechanisms include hyperinsulinemia

caused by insulin resistance in NASH, increased levels of insulin-like growth factor that promotes tumor growth, increased susceptibility of the steatotic liver to lipid peroxidation, production of reactive oxygen species and subsequent DNA mutations, disordered energy and hormonal regulation in obesity, and aberrations in regenerative processes occurring in cirrhosis.[25]

Certain limitations should be considered in the interpretation of our findings. First, the cross-sectional study design hinders the ability to draw inferences regarding the causality of NASH in HCC. Second, the study did not include a control group of HCC patients with other liver diseases. Third, there might be a bias in patient selection, because patients were retrospectively identified as having NASH-associated HCC. Finally, although our patients were negative for hepatitis B virus surface antigen, it is still possible that occult hepatitis B virus infection might be associated with the development of HCC in some of our cases.

In summary, we showed the clinical features of NASH patients with HCC. NASH patients with HCC were more often men and frequently displayed obesity, diabetes, and hypertension. Our results suggest that male patients might develop HCC at a less advanced stage of fibrosis than female patients. Further prospective studies with a longer follow-up time and larger cohorts are needed to determine the causal association of NASH with HCC and to identify risk factors for the development of HCC in NASH patients.

References

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–543.
2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–2576.
3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99–S112.
4. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–1231.
5. Powell EE, Cooksley WG, Hanson R, et al. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74–80.
6. Cotrim HP, Paraná R, Braga E, et al. Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 2000;95:3018–3019.
7. Zen Y, Katayanagi K, Tsuneyama K, et al. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. Pathol Int 2001;51:127–131.
8. Shimada M, Hashimoto E, Taniai M, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002;37:154–160.
9. Marrero JA, Fontana RJ, Su GL, et al. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002;36:1349–1354.
10. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123: 134–140.
11. Ratziu V, Bonyhay L, Di Martino V, et al. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 2002;35:1485–1493.
12. Regimbeau JM, Colombat M, Mognol P, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 2004;10:S69–S73.
13. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121.
14. Sanyal AJ, Banas C, Sargeant C, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. Hepatology 2006;43:682–689.
15. Hashimoto E, Yatsuji S, Tobari M, et al. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. J Gastroenterol 2009;44:89–95.
16. Yatsuji S, Hashimoto E, Tobari M, et al. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. J Gastroenterol Hepatol 2009;24:248–254.
17. Ascha MS, Hanouneh IA, Lopez R, et al. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology 2010;51:1972–1978.
18. Okanoue T, Umemura A, Yasui K, et al. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan. J Gastroenterol Hepatol 2011;26(Suppl 1):153–162.
19. Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.
20. Japan Society for the Study of Obesity. New criteria of obesity (in Japanese). J Jpn Soc Study Obes 2000;6:18–28.
21. Kuzuya T, Nakagawa S, Satoh J, et al. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002;55:65–85.
22. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–2497.
23. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419.
24. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
25. Brunt EM. Non-alcoholic fatty liver disease. In: Burt AD, Portmann BC, Ferrell LD, eds. MacSween's pathology of the liver. 5th ed. London: Churchill Livingstone, 2006:367–397.
26. Brunt EM, Janney CG, Di Bisceglie AM, et al. Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–2474.
27. Ikai I, Arii S, Okazaki M, et al. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. Hepatol Res 2007;37:676–691.
28. Bugianesi E. Non-alcoholic steatohepatitis and cancer. Clin Liver Dis 2007;11:191–207.
29. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology 2003;37:1202–1219.
30. Sumida Y, Yoneda M, Hyogo H, et al. A simple clinical scoring system using ferritin, fasting insulin and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. J Gastroenterol 2011;46:257–268.
31. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–1638.
32. Caldwell S, Park SH. The epidemiology of hepatocellular cancer: from the perspectives of public health problem to tumor biology. J Gastroenterol 2009;44:96–101.
33. Bullock RE, Zaitoun AM, Aithal GP, et al. Association of non-alcoholic steatohepatitis without significant fibrosis with hepatocellular carcinoma. J Hepatol 2004;41:685–686.
34. Ichikawa T, Yanagi K, Motoyoshi Y, et al. Two cases of non-alcoholic steatohepatitis with development of hepatocellular carcinoma without cirrhosis. J Gastroenterol Hepatol 2006;21:1865–1866.
35. Guzman G, Brunt EM, Petrovic LM, et al. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? Arch Pathol Lab Med 2008;132:1761–1766.

36. Kawada N, Imanaka K, Kawaguchi T, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. J Gastroenterol 2009;44:1190–1194.
37. Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009; 49:851–859.
38. Adamson JW. Hematopoietic disorders. In: Fauci AS, Braunwald E, Kasper DL, et al, eds. Harrison's principles of internal medicine. 17th ed. New York: McGraw-Hill Companies, 2008:628–634.
39. Bonkovsky HL, Jawaid Q, Tortorelli K, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol 1999;31:421–429.

Reprint requests

Address requests for reprints to: Takeshi Okanoue, MD, PhD, Director, Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, 1-2 Kawazono-cho, Suita 5640013, Japan. e-mail: okanoue@suita.saiseikai.or.jp; fax: +81-6-6382-1524.

Conflicts of interest

The authors disclose no conflicts.

Funding

This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H20-hepatitis-008 to Takeshi Okanoue).

Hepatology Research 2011; 41: 1145–1152 doi: 10.1111/j.1872-034X.2011.00886.x

Original Article

Hepatic steatosis in chronic hepatitis C patients infected with genotype 2 is associated with insulin resistance, hepatic fibrosis and affects cumulative positivity of serum hepatitis C virus RNA in peginterferon and ribavirin combination therapy

Yoshito Itoh,[1] Takeshi Nishimura,[1] Kanji Yamaguchi,[1] Chihiro Yokomizo,[1] Hideki Fujii,[1] Masahito Minami,[1] Yasuyuki Nagao,[2] Yoshio Sumida,[4] Hiroaki Hashimoto,[3] Atsushi Umemura,[3] Toshihide Shima,[3] Takeshi Okanoue[3] and Toshikazu Yoshikawa[1]

[1]*Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto,* [2]*Department of Gastroenterology, Matsushita Memorial Hospital,* [3]*Hepatology Center, Saiseikai Suita Hospital, Osaka, and* [4]*Department of Gastroenterology and Hepatology Nara City Hospital, Nara, Japan*

Aim: Hepatic steatosis is one of the factors limiting the virological response to interferon-based antiviral therapy for chronic hepatitis C (CH-C) patients infected with genotype 1, while contradictory results have been reported for genotype 2. We aimed to clarify the effect of hepatic steatosis on therapeutic outcome and cumulative positivity of serum HCV RNA in CH-C patients infected with genotype 2 treated by peginterferon (PEG-IFN)α2b and ribavirin (RBV) combination therapy.

Methods: A total of 74 treatment-naïve non-cirrhotic CH-C patients infected with genotype 2 who received PEG-IFNα2b and RBV according to the standard regimen were divided into hepatic steatosis 0–10% and >10% groups. The clinical backgrounds, sustained virological response (SVR) rates and cumulative positivity of serum HCV RNA were compared between the two groups.

Results: Among the 74 patients, 61 (82.4%) had hepatic steatosis 0–10% and 13 (17.6%) had hepatic steatosis >10%. Scores of homeostasis model assessment-insulin resistance and hepatic fibrosis were higher in patients with hepatic steatosis >10% than hepatic steatosis 0–10% ($P = 0.040$ and 0.042, respectively). Non-SVR was more frequent in patients with hepatic steatosis >10% than hepatic steatosis 0–10% ($P = 0.003$). Cumulative positivity of serum HCV RNA was significantly higher in patients with hepatic steatosis >10% than hepatic steatosis 0–10% ($P = 0.004$).

Conclusions: In CH-C patients infected with genotype 2 treated by PEG-IFNα2b and RBV combination therapy, hepatic steatosis >10% was associated with increased insulin resistance, advanced hepatic fibrosis and higher cumulative positivity of serum HCV RNA, which lead to a higher risk of non-SVR.

Key words: chronic hepatitis C, genotype 2, hepatic fibrosis, hepatic steatosis, homeostasis model assessment-insulin resistance

INTRODUCTION

WITH ADVANCES IN the practice of clinical hepatology, around 50% of chronic hepatitis C (CH-C) patients infected with genotype 1, and more than 80% of genotype 2, can be cured by peginterferon (PEG-IFN) and ribavirin (RBV) combination therapy.[1,2] However, although PEG-IFN and RBV combination therapy is a powerful tool for the treatment of CH-C patients infected with genotype 2 worldwide, a significant number remain viremic.

Because of the high rate of sustained virological response (SVR) to PEG-IFN and RBV combination therapy in CH-C patients infected with genotype 2, the clinical backgrounds associated with resistance to therapy have not been evaluated in detail. Previously, CH-C patients infected with genotype 2 who had

Correspondence: Dr Yoshito Itoh, Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kawaramachi-Hirokouji, Kamigyou-ku, Kyoto, 602-8566, Japan. Email: yitoh@koto.kpu-m.ac.jp
Received 4 March 2011; revision 19 July 2011; accepted 4 August 2011.

hepatic steatosis were reported to be resistant to interferon (IFN) monotherapy[3,4] and PEG-IFN and RBV combination therapy,[5] but several recent publications have contradicted these earlier reports.[6,7]

A recent trend in PEG-IFN and RBV combination therapy for CH-C is response-guided therapy. Several trials have evaluated short courses of treatment for CH-C patients infected with genotype 2 or genotype 3 with a rapid virological response (RVR) but controversial results also have been reported.[8–13] Recent papers on genotype 2/3 patients reported that low platelet counts and a body mass index (BMI) of 30 or higher are associated with relapse of RVR after a short regimen of 12 weeks,[14] and a body weight greater than 75 kg also affected the probability of relapse,[15] whereas the impact of hepatic steatosis on the anti-viral effect was not fully studied. In the present study, we investigated the effects of hepatic steatosis on clinical backgrounds of CH-C patients infected with genotype 2. Specifically, we investigated the impact of hepatic steatosis on cumulative positivity of serum HCV RNA during PEG-IFN and RBV combination therapy and therapeutic outcome.

METHODS

Patients

THIS STUDY WAS conducted at the university hospital of Kyoto Prefectural University of Medicine, Kyoto, Japan and related hospitals in Kinki Area (Kyoto, Osaka, Nara, Shiga Prefecture). The study protocol was approved by the ethical committee of each institution in 2005. Written informed consent was obtained from all patients before treatment. Enrollment of the patients began in January 2006 and ended in December 2008 and the follow up study was completed in August 2009. Patients with liver cirrhosis, co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease were excluded. Patients with uncontrollable hypertension or diabetes mellitus, BMI ≥30 kg/m^2, and those with a history of alcohol abuse also were excluded. The criteria for enrollment were platelet count (PLT) greater than 10×10^4/mm^3, neutrophil count greater than 1100/mm^3, and hemoglobin concentration greater than 11.0 g/dL.

Among the 136 patients enrolled, 10 dropped out because of severe adverse effects and eight were lost to follow up. Forty-four patients without liver biopsy also were excluded. Finally, 74 eligible and previously untreated Japanese CH-C patients infected with genotype 2, aged 27 to 73 years and who had high viral loads (≥100 KIU/mL by the Amplicor HCV RNA kit, version 2.0, Roche Diagnostics, Tokyo, Japan), were studied.

All 74 patients had provided a liver biopsy within a year prior to treatment. Liver biopsy was performed using a Sure-cut needle guided by ultrasound. Liver biopsy specimens were fixed in 10% formalin and stained with hematoxylin and eosin and Masson's trichrome. Histopathological diagnosis was based on the scoring of New Inuyama classification.[16] The fibrosis scores were F0: no fibrosis, F1: portal fibrous widening, F2: portal fibrous widening with bridging fibrosis, F3: bridging fibrosis plus lobular distortion. The inflammation scores were A0: none to minimal, A1: mild, A2: moderate, A3: severe. Evaluation of the percentage of hepatic steatosis was carried out by two expert hepatologists who were blinded to the treatment outcome of each patient and classified as ≥0% and ≤1%, >1% and ≤5%, >5% and ≤10%, >10% and ≤15%, >15% and ≤20%, >20% and ≤25%, >25% and ≤30%.

Study design

All patients received weekly injections of PEG-IFNα2b (PEG-INTRON; Shering-Plough, Kenilworth, NJ, USA) of 1.5 μg/kg·bw and oral administration of RBV (Rebetol; Shering-Plough) of 600 to 1000 mg/day according to the 24 week standard regimen. The amount of RBV was adjusted based on body weight (bw): 600 mg for <60 kg.bw, 800 mg for ≥60 kg.bw and <80 kg.bw, 1000 mg for ≥80 kg.bw. The dose of PEG-IFNα2b was decreased by 50% when the PLT count fell below 8×10^4/mm^3 or the neutrophil count fell below 750/mm^3. The dose of RBV was lowered by 200 mg/day when the hemoglobin concentration fell below 10 g/dL. The full dose was reinstated when the adverse events improved.

Hepatitis C virus RNA negativity at treatment week 4, based on a qualitative polymerase chain reaction (PCR) assay, was defined as RVR. HCV RNA negativity at 24 weeks after the cessation of combination therapy was defined as SVR. Those who failed to attain SVR were defined as non-SVR patients. All patients were examined serially (at 2, 4, 8, 12, 24 weeks) by qualitative HCV RNA assays and again 24 weeks after termination of the therapy.

Statistical analysis

All data analyses were carried out using the SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL, USA). Individual characteristics were compared between the groups using the Mann-Whitney *U*-test or Fisher's exact test. For some variables, receiver operating characteristic analysis was performed followed by proper cat-

egorization of the data. Cumulative positivity of serum HCV RNA was calculated by the Kaplan–Meier method and analyzed by the log-rank test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Evaluation of hepatic steatosis in chronic hepatitis C patients infected with genotype 2

RECEIVER OPERATING CHARACTERISTIC curve analysis was performed to examine the relationship between hepatic steatosis and SVR (Fig. 1). Because the sum of sensitivity and specificity was maximum when the hepatic steatosis was 11.50% (data not shown), we classified the patients into hepatic steatosis 0–10% and hepatic steatosis >10% groups. Based on these results, the degree of hepatic steatosis was classified into seven categories from ≥0% and ≤1% to >25% and ≤30% (Fig. 2) and presented separately for the SVR and non-SVR groups (Table 1).

Correlation between hepatic steatosis and clinical and other histological features

The baseline characteristics of the 74 patients (31 male and 43 female) with chronic hepatitis C infected with genotype 2 are shown in Table 2. There were no significant differences between the patients with hepatic steatosis 0–10% and hepatic steatosis >10% in clinical backgrounds such as gender, age, baseline HCV RNA load and other laboratory data, except that homeostasis model assessment-insulin resistance (HOMA-IR) was significantly ($P = 0.040$) higher in patients with hepatic steatosis >10%. BMI and γ-glutamyl transferase (γ-GTP) also tended to be higher in patients with hepatic steatosis >10%; however, statistical significances were not demonstrated ($P = 0.052$ and $P = 0.050$, respectively). The scores of hepatic fibrosis, but not of hepatic inflammation, were significantly ($P = 0.042$) higher in patients with hepatic steatosis >10% (Table 3). Although we investigated the factors associated with hepatic steatosis >10% by multivariate regression analysis, neither HOMA-IR nor hepatic fibrosis were independently associated with it.

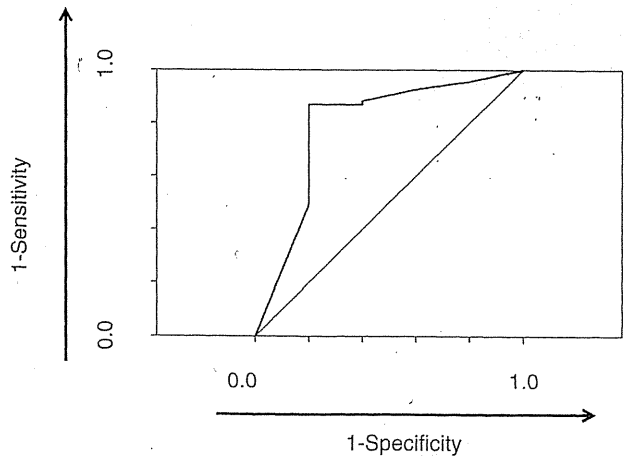

Figure 1 Receiver operating characteristic curve analysis of hepatic steatosis and sustained virological response (SVR). Receiver operating characteristic curve analysis was performed to examine the relationship between hepatic steatosis and SVR in all patients. Because the sum of sensitivity and specificity was maximum when the hepatic steatosis was 11.50% (data not shown), we classified the patients into hepatic steatosis 0–10% and hepatic steatosis >10% groups.

Table 1 Hepatic steatosis in chronic hepatitis C patients infected with genotype 2

Hepatic steatosis (%)	No. patients		
	SVR	non-SVR	Total
–1%	34	1	35
–5%	17	0	17
–10%	9	0	9
–15%	1	1	2
–20%	3	1	4
–25%	2	1	3
–30%	3	1	4
	69	5	74
IL28B major homo/ hetero (total)	10/0 (10)	3/2 (5)	

The degree of hepatic steatosis was classified as ≥0% and ≤1%, >1% and ≤5%, >5% and ≤10%, >10% and ≤15%, >15% and ≤20%, >20% and ≤25%, >25% and ≤30% and presented for the SVR and non-SVR groups.
SVR, sustained virological response.

Impact of hepatic steatosis on therapeutic response

The relationship between hepatic steatosis and SVR or RVR ratio is shown in Table 3. The SVR ratio of patients with hepatic steatosis 0–10% was significantly ($P = 0.003$) higher than that of patients with hepatic steatosis >10% and the RVR ratio of patients with hepatic steatosis 0–10% also tended to be higher; however, statistical significance was not demonstrated ($P = 0.055$). This result tempted us to investigate the impact of hepatic steatosis on the cumulative positivity of serum HCV RNA in the early phase of PEG-IFNα2b and RBV combination therapy.

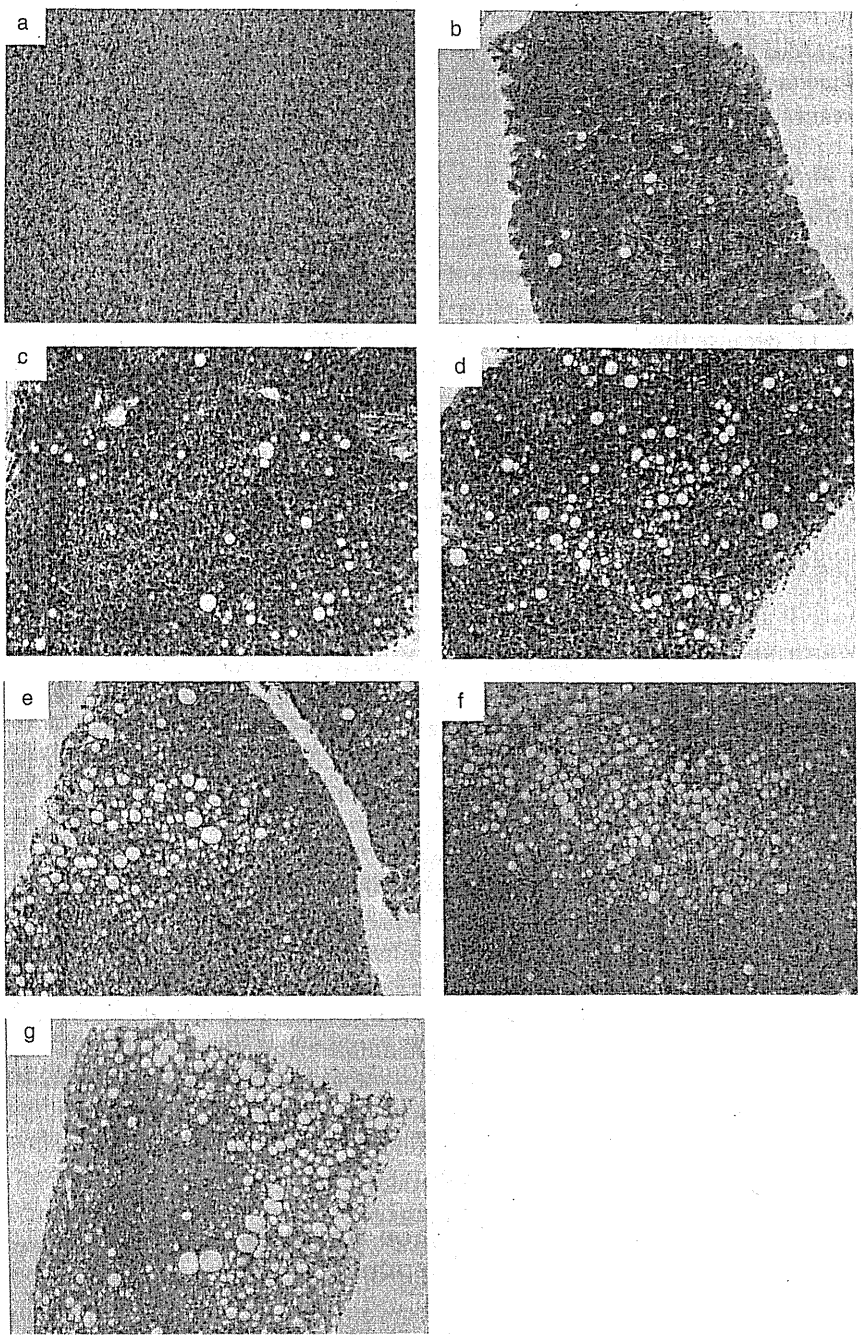

Figure 2 Histological classification of hepatic steatosis. Typical histological classification was presented. The degree of hepatic steatosis was classified as ≥0% and ≤1% (a), >1% and ≤5% (b), >5% and ≤10% (c), >10% and ≤15% (d), >15% and ≤20% (e), >20% and ≤25% (f), >25% and ≤30% (g) by two hepatologists who were blinded to the treatment outcome of each patient.

Impact of hepatic steatosis on cumulative positivity of serum HCV RNA

Here, we divided the patients based on receiver operating characteristic curve analysis and demonstrated that hepatic steatosis >10% was associated with resistance to PEG-IFNα2b and RBV combination therapy for CH-C patients infected with genotype 2. To support this finding, we compared the cumulative positivity of serum HCV RNA between the patients with hepatic steatosis 0–10% and those with hepatic steatosis >10% in the early phase of PEG-IFNα2b and RBV combination therapy using the Kaplan–Meier method.

The patients with hepatic steatosis >10% had a significantly ($P = 0.004$) higher cumulative positivity of HCV RNA than the patients with hepatic steatosis

Table 2 Relationship between hepatic steatosis (0–10% or >10%) and the clinical backgrounds of the patients

Hepatic steatosis	>10% (13) Median [range]	0–10% (61) Median [range]	*P*-value
Genotype 2a/2b/N.D.	5/6/2	28/13/10	0.148
Gender (M/F)	5/8	26/35	0.782
Age (years)	56 [33.0–70.0]	54 [27.0–73.0]	0.430
BMI (kg/m^2)	23.9 [19.9–28.8]	22.0 [17.5–29.6]*	0.052
HCVRNA (KIU/mL)	1850 [120–5000]†	1300 [130–5000]†	0.439
Hb (g/dL)	14.5 [11.8–15.1]	13.9 [11.3–17.4]	0.691
Plt (×10^4/mm^3)	19.2 [10.6–24.0]	18.9 [10.4–37.9]	0.551
WBC (×10^3/mm^3)	5.20 [3.33–7.40]	4.90 [2.30–9.40]	0.612
ALT (IU/L)	78 [16.0–248]	47 [15.0–377]	0.125
rGTP (IU/L)	56 [19–285]	23 [8–158]*	0.050
T-chol (mg/dL)	187 [130–250]	178 [108–274]	0.227
Feritin (ng/dL)	150 [22–1107]	103 [20–664]‡	0.330
HOMA-IR	4.6 [1.5–6.5]	2.0 [0.3–11.3]*	0.040
Creatinine (mg/dL)	0.6 [0.5–0.9]	0.7 [0.3–1.1]	0.172

The clinical backgrounds of the 74 patients were compared by Mann–Whitney *U*-test or Fisher's exact test. Continuous variables are presented as medians (ranges). Individual characteristics between the groups were evaluated using the Mann–Whitney *U*-test. All patients with >10% hepatic steatosis were evaluated for each parameter. In patients with 0–10% hepatic steatosis *60 patients were evaluated for body mass index (BMI), 56 patients for γ-glutamyl transferase (γ-GTP) and homeostasis model assessment-insulin resistance (HOMA-IR). †The upper limit of measurement is 5000 KIU/mL. ‡The lower limit of measurement is 20 ng/dL.
ALT, alanine aminotransferase; F, female; Hb, hemoglobin; HCV, hepatitis C virus; M, male; N.D., not determined; PLT platelet count; T-chol, total cholesterol; WBC, white blood cell count.

0–10% (Fig. 3). To exclude the possibility that adherence to PEG-IFNα2b or RBV influenced the changes in serum HCV RNA in this study, we examined the cumulative positivity of serum HCV RNA in patients who achieved ≥80% adherence (as a percentage of the expected total dose) to both PEG-IFNα2b and RBV. Patients with hepatic steatosis >10% also had significantly ($P = 0.045$) higher cumulative positivity of serum HCV RNA than those with hepatic steatosis 0–10% (data not shown). The SVR ratio of patients with ≥80% adherence to both PEG-IFNα2b and RBV did not differ significantly from those without (data not shown).

DISCUSSION

PREVIOUS PAPERS[3–5] REPORTED that hepatic steatosis affected the efficacy of IFN-based therapy for CH-C patients infected with genotype 2, whereas recent papers by Poustchi *et al.*[6] and Rodriguez-Torres *et al.*[7] denied it. Because steatosis was roughly graded (for example, less than 5%, 5–33%, 34–66%, and more than 67%) in these studies, we considered that tight grading of hepatic steatosis may lead to a different result. As we have expected, in the present study, tight grading of hepatic steatosis revealed a close relation between

Table 3 Relationship between hepatic steatosis (0–10% or >10%) and other histological features and therapeutic response

Hepatic steatosis	>10%	0–10%	*P*-value
Hepatic inflammation (0,1/2,3)	5/8	37/24	0.143
Hepatic fibrosis (0,1/2,3)	5/8	42/19	0.042
Hepatic iron deposit Yes/No/N.D.	3/5/5	16/39/6	0.455
Rapid virological response Yes/No	6/7	45/16	0.055
Sustained virological response Yes/No	9/4	60/1	0.003

The histological findings of the 74 patients were evaluated according to the scoring system of New Inuyama classification.[16] Histological features such as inflammation, fibrosis and iron deposition, were compared between the hepatic steatosis 0–10% and >10% groups by Fisher's exact test. The ratio of rapid virological response and sustained virological response were compared between the hepatic steatosis 0–10% and >10% groups by Fisher's exact test.
N.D., not determined.

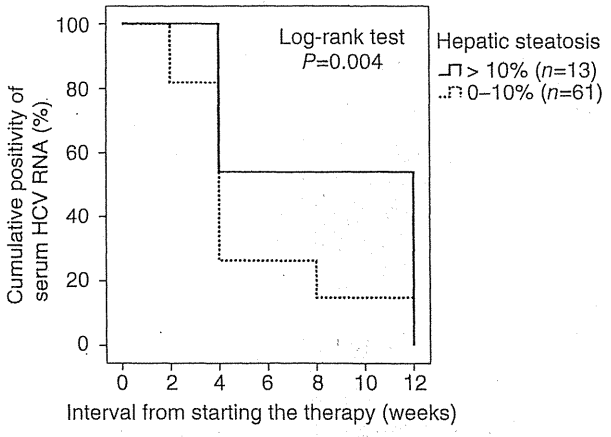

Figure 3 Cumulative positivity of serum hepatitis C virus (HCV) RNA during the early phase of pegylated interferon (PEG-IFN)α2b and ribavirin (RBV) combination therapy. Cumulative positivity of serum HCV RNA as determined by qualitative HCV RNA assay was compared between the hepatic steatosis >10% and hepatic steatosis 0–10% groups using the Kaplan–Meier method, and then analyzed by log-rank test. A *P*-value of <0.05 was considered to be statistically significant. (⌐¬): >10% ($n = 13$); (···): 0–10% ($n = 61$).

hepatic steatosis and HOMA-IR, hepatic fibrosis, and especially, cumulative positivity of serum HCV RNA during the PEG-IFN/RBV combination therapy, which was not studied as far as we investigated.

In response-guided therapy for hepatitis C, early disappearance of HCV RNA from the serum is the best predictor of SVR.[17] Therefore, our finding that >10% hepatic steatosis affected the cumulative positivity of serum HCV RNA during the PEG-IFN/RBV combination therapy for genotype 2 infected patients is useful to establish more accurate prediction of therapeutic outcome. So, we think that evaluation of hepatic steatosis before PEG-IFN/RBV combination therapy is very important.

The percentage of patients with hepatic steatosis >10% varies according to the papers; however, our result that patients with hepatic steatosis >10% was 17.6% (13/74) do not seem to be extraordinary considering a report by Rodriguez-Torres *et al.*[7] or a recent paper by Kurosaki *et al.*[18] Our results showing that hepatic steatosis >10% was associated with higher HOMA-IR and advanced hepatic fibrosis (Tables 2,3) are reasonable, judging from previous reports.[19,20] While the number of non-SVR patients was small, hepatic steatosis >10% was associated with resistance to PEG-IFN and RBV combination therapy (Table 3). To support this finding, we compared the cumulative positivity of serum HCV RNA between the patients with hepatic steatosis >10% and ≤10% and found that it was significantly ($P = 0.004$) higher in patients with hepatic steatosis >10%, compared to those without.

Patton *et al.*[20] demonstrated first that hepatic steatosis reduced the likelihood of achieving SVR in CH-C patients infected with genotype 1. This finding was confirmed by Lok *et al.*[21] in non-diabetic patients. Our result that hepatic steatosis >10% was associated with resistance to combination therapy in CH-C patients infected with genotype 2 is supported by the report by Poynard *et al.*[5] showing that absence of hepatic steatosis was associated with a higher SVR ratio to PEG-IFN and RBV combination therapy, except for genotype 3. In our study, in addition, increased cumulative positivity of HCV RNA was demonstrated in patients with hepatic steatosis >10% (Fig. 3). We speculate that there is a close relationship between hepatic steatosis >10% and impaired IFN-mediated anti-HCV activity in CH-C patients infected with genotype 2.

It is well known that metabolic diseases such as diabetes mellitus can trigger hepatic steatosis and HCV infection also causes hepatic steatosis by way of increased expression of genes including sterol regulatory element-binding protein 1c.[22] Recently, Vanni *et al.*[23] demonstrated that HCV infected patients with more hepatic steatosis revealed higher intra-hepatic lipid oxidation, which in turn stimulated gluconeogenesis and induced higher suppressor of cytokine signaling-3 (SOCS-3) expression resulting in increased insulin resistance. Because IFN unresponsiveness is, in part, linked to upregulated hepatic expression of SOCS-3 and insulin resistance as shown by increased HOMA-IR,[24] we hypothesize that enhanced hepatic staetosis in CH-C patients infected with genotype 2 may be associated with increased insulin resistance and increased hepatic expression of SOCS-3, which might have interfered with IFN signal transduction pathway.[25] It should be clarified whether or not hepatic steatosis in CH-C patients infected with genotype 2 really links to IFN unresponsiveness through this molecular pathway in the next study.

To exclude the possibility that adherence to PEG-IFNα2b or RBV may have influenced the response to the therapy, we compared the cumulative positivity of serum HCV RNA among the patients with ≥80% adherence to both drugs and demonstrated that patients with hepatic steatosis >10% showed a higher cumulative positive serum HCV RNA (data not shown).

Recently, an interleukin 28B (IL28B) gene polymorphism has been reported to be strongly associated with

the response to PEG-IFN and RBV combination therapy for CH-C.[26–28] In Japanese patients, in particular, a single nucleotide polymorphism (SNP rs8099917) near the IL28B gene on chromosome 19 was shown to be strongly associated with non-virological response in genotypes 1.[27] However, in genotype 2, this association was weak especially in genotype 2a.[29,30] A recent paper from Japan reported that CH-C patients infected with genotype 1b who showed hepatic steatosis >10% were likely to harbor IL28B minor allele (TG or GG genotype).[18] In our preliminary retrospective data, two out of five (40%) non-SVR patients had the TG genotype and ten out of ten (100%) of SVR patients had the TT genotype (Table 1). The relationship between hepatic steatosis and IL28B polymorphism may be an interesting subject for further investigation.

In conclusion, CH-C patients infected with genotype 2 with hepatic steatosis >10% showed resistance to PEG-IFNα2b and RBV combination therapy. Considering cost minimization, a variable-duration regimen is recommended.[31] We advocate that CH-C patients infected with genotype 2 should be evaluated for hepatic steatosis by biopsied liver specimens before starting PEG-IFN and RBV combination therapy. In combination with other factors such as mutations in HCV and IL28B gene polymorphism,[30] evaluation of hepatic steatosis will enable us to more accurately predict the outcome of CH-C patients infected with genotype 2 in PEG-IFN and RBV combination therapy.

ACKNOWLEDGMENTS

WE ARE GRATEFUL to the members of the Hepatology Research Unit in Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan, and related hospitals for their contribution to this work.

REFERENCES

1 Manns MP, McHutchinson JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–65.
2 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–82.
3 Akuta N, Suzuki F, Tsubota A *et al.* Efficacy of interferon monotherapy to 394 consecutive naïve cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartile interaction of viral, host and interferon treatment-related factors. *J Hepatol* 2002; 37: 831–6.
4 Akuta N, Suzuki F, Suzuki Y *et al.* Hepatocyte steatosis is an important predictor of response to interferon (IFN) monotherapy in Japanese patients infected with HCV genotype 2a: virological features of IFN-resistant cases with hepatocyte steatosis. *J Med Virol* 2005; 75: 550–8.
5 Poynard T, Ratziu V, McHutchison J *et al.* Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003; 38: 75–85.
6 Poustchi H, Negro F, Hui J *et al.* Insulin resistance and response to therapy in patients with chronic hepatitis C virus genotype 2 and 3. *J Hepatol* 2008; 48: 28–34.
7 Rodriguez-Torres M, Govindarajan S, Diago M *et al.* Hepatic steatosis in patients with chronic hepatitis C virus genotype 2 or 3 does not affect viral response in patients treated with peginterferon alpha-2a (40KD) (PEGASYS) plus ribavirin (COPEGUS) for 16 0r 24 weeks. *Liver Int* 2009; 29: 237–41.
8 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; 352: 2609–17.
9 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; 129: 522–7.
10 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; 357: 124–34.
11 Dalgard O, Bjøro K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; 47: 35–42.
12 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; 47: 1837–45.
13 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; 56: 553–9.
14 Mangia A, Minerva N, Bacca D *et al.* Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. *Hepatology* 2009; 49: 358–63.
15 Rodriguez Torres M, Rios Bedoya CF, Ortiz-Lasanta G, Purcell-Arevalo D, Marxuach-Cuetara A, Jimenez-Rivera J. Weight affect relapse rates in latinos with genotype 2/3 chronic hepatitis C (CHC) treated with peg IFN alfa-2a (Pegasys) 180mcg/week and 800 mg daily of ribavirin for 24 weeks. *J Med Virol* 2008; 80: 1576–80.