In this study, HCV-KT9 showed no virus production ability *in vitro*. Recently, Kato *et al.* (2007) reported that the genotype 1b HCV clone CG1b replicated in Huh7.5.1 cells and produced infectious HCV. It will be of interest to create chimeric viruses of HCV-KT9 and HCV-CG1b, and to determine the mutations that are important for virus production *in vitro*.

In summary, we established an infection model of a genotype 1b HCV clone using human hepatocyte chimeric mice. This model will be useful for studies of HCV replication, particularly the mechanism underlying the variable resistance of HCV genotypes to IFN therapy.

ACKNOWLEDGEMENTS

The authors thank Rie Akiyama and Kana Kunihiro for their technical help and Dr Francis V. Chisari for providing the Huh7.5.1 cells. This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Education, Sports, Culture and Technology and the Ministry of Health, Labor and Welfare, Japan.

REFERENCES

Beard, M. R., Abell, G., Honda, M., Carroll, A., Gartland, M., Clarke, B., Suzuki, K., Lanford, R., Sangar, D. V. & Lemon, S. M. (1999). An infectious molecular clone of a Japanese genotype 1b hepatitis C virus. *Hepatology* **30**, 316–324.

Bukh, J., Pietschmann, T., Lohmann, V., Krieger, N., Faulk, K., Engle, R. E., Govindarajan, S., Shapiro, M., St Claire, M. & other authors (2002). Mutations that permit efficient replication of hepatitis C virus RNA in Huh-7 cells prevent productive replication in chimpanzees. *Proc Natl Acad Sci U S A* **99**, 14416–14421.

Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Ogura, Y., Izumi, N., Marumo, F. & other authors (1996). Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* **334**, 77–81.

Friebe, P. & Bartenschlager, R. (2002). Genetic analysis of sequences in the 3′ nontranslated region of hepatitis C virus that are important for RNA replication. *J Virol* **76**, 5326–5338.

Fried, M. W., Shiffman, M. L., Reddy, K. R., Smith, C., Marinos, G., Goncales, F. L., Jr, Haussinger, D., Diago, M., Carosi, G. & other authors (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* **347**, 975–982.

Gale, M. J., Jr, Korth, M. J., Tang, N. M., Tan, S. L., Hopkins, D. A., Dever, T. E., Polyak, S. J., Gretch, D. R. & Katze, M. G. (1997). Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. *Virology* **230**, 217–227.

Gojobori, T., Ishii, K. & Nei, M. (1982). Estimation of average number of nucleotide substitutions when the rate of substitution varies with nucleotide. *J Mol Evol* **18**, 414–423.

Heller, T., Saito, S., Auerbach, J., Williams, T., Moreen, T. R., Jazwinski, A., Cruz, B., Jeurkar, N., Sapp, R. & other authors (2005). An in vitro model of hepatitis C virion production. *Proc Natl Acad Sci U S A* **102**, 2579–2583.

Hiraga, N., Imamura, M., Tsuge, M., Noguchi, C., Takahashi, S., Iwao, E., Fujimoto, Y., Abe, H., Maekawa, T. & other authors (2007). Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon. *FEBS Lett* **581**, 1983–1987.

Kato, T., Matsumura, T., Heller, T., Saito, S., Sapp, R. K., Murthy, K., Wakita, T. & Liang, T. J. (2007). Production of infectious hepatitis C virus of various genotypes in cell cultures. *J Virol* **81**, 4405–4411.

Kneteman, N. M., Weiner, A. J., O'Connell, J., Collett, M., Gao, T., Aukerman, L., Kovelsky, R., Ni, Z. J., Zhu, Q. & other authors (2006). Anti-HCV therapies in chimeric scid-Alb/uPA mice parallel outcomes in human clinical application. *Hepatology* **43**, 1346–1353.

Lindenbach, B. D., Meuleman, P., Ploss, A., Vanwolleghem, T., Syder, A. J., McKeating, J. A., Lanford, R. E., Feinstone, S. M., Major, M. E. & other authors (2006). Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro. *Proc Natl Acad Sci U S A* **103**, 3805–3809.

Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L. & Bartenschlager, R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* **285**, 110–113.

Mercer, D. F., Schiller, D. E., Elliott, J. F., Douglas, D. N., Hao, C., Rinfret, A., Addison, W. R., Fischer, K. P., Churchill, T. A. & other authors (2001). Hepatitis C virus replication in mice with chimeric human livers. *Nat Med* **7**, 927–933.

Okamoto, H., Kojima, M., Okada, S., Yoshizawa, H., Iizuka, H., Tanaka, T., Muchmore, E. E., Peterson, D. A., Ito, Y. & other authors (1992). Genetic drift of hepatitis C virus during an 8.2-year infection in a chimpanzee: variability and stability. *Virology* **190**, 894–899.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Simmonds, P., Holmes, E. C., Cha, T. A., Chan, S. W., McOmish, F., Irvine, B., Beall, E., Yap, P. L., Kolberg, J. & other authors (1993). Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* **74**, 2391–2399.

Takamizawa, A., Mori, C., Fuke, I., Manabe, S., Murakami, S., Fujita, J., Onishi, E., Andoh, T., Yoshida, I. & other authors (1991). Structure and organization of the hepatitis C virus genome isolated from human carriers. *J Virol* **65**, 1105–1113.

Tateno, C., Yoshizane, Y., Saito, N., Kataoka, M., Utoh, R., Yamasaki, C., Tachibana, A., Soeno, Y., Asahina, K. & other authors (2004). Near completely humanized liver in mice shows human-type metabolic responses to drugs. *Am J Pathol* **165**, 901–912.

Thomson, M., Nascimbeni, M., Gonzales, S., Murthy, K. K., Rehermann, B. & Liang, T. J. (2001). Emergence of a distinct pattern of viral mutations in chimpanzees infected with a homogeneous inoculum of hepatitis C virus. *Gastroenterology* **121**, 1226–1233.

Tsuge, M., Hiraga, N., Takaishi, H., Noguchi, C., Oga, H., Imamura, M., Takahashi, S., Iwao, E., Fujimoto, Y. & other authors (2005). Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus. *Hepatology* **42**, 1046–1054.

Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Krausslich, H. G. & other authors (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* **11**, 791–796.

Yi, M. & Lemon, S. M. (2003). 3′ Nontranslated RNA signals required for replication of hepatitis C virus RNA. *J Virol* **77**, 3557–3568.

You, S. & Rice, C. M. (2008). 3′ RNA elements in hepatitis C virus replication: kissing partners and long poly(U). *J Virol* **82**, 184–195.

Zhong, J., Gastaminza, P., Cheng, G., Kapadia, S., Kato, T., Burton, D. R., Wieland, S. F., Uprichard, S. L., Wakita, T. & other authors (2005). Robust hepatitis C virus infection *in vitro*. *Proc Natl Acad Sci U S A* **102**, 9294–9299.

Risk Factors for Hepatocellular Carcinoma in a Japanese Population: A Nested Case-Control Study

Waka Ohishi,[1] Saeko Fujiwara,[1] John B. Cologne,[2] Gen Suzuki,[1,5] Masazumi Akahoshi,[1] Nobuo Nishi,[3] Ikuno Takahashi,[1] and Kazuaki Chayama[4]

Departments of [1]Clinical Studies, [2]Statistics, and [3]Epidemiology, Radiation Effects Research Foundation; [4]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; and [5]Department of Environmental Health, National Institute of Public Health, Wako, Japan

Abstract

Background: **Epidemiologic studies have shown effects of lifestyle-related factors on risk for hepatocellular carcinoma. However, few cohort studies have incorporated, in a strict and in-depth manner, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections or investigated synergism between such factors.**

Methods: **We conducted a nested case-control study using sera stored before hepatocellular carcinoma diagnosis in the longitudinal cohort of atomic bomb survivors. The study included 224 hepatocellular carcinoma cases and 644 controls that were matched to the cases on gender, age, city, time of serum storage, and method of serum storage, and countermatched on radiation dose.**

Results: **Univariate analysis showed that HBV and HCV infections, alcohol consumption, smoking habit, body mass index (BMI), and diabetes mellitus were associated with increased hepatocellular carcinoma risk, whereas coffee drinking was associated with decreased hepatocellular carcinoma risk. Multivariate relative risks of hepatocellular carcinoma (95% confidence interval) were 45.8 (15.2-138), 101 (38.7-263), 70.7 (8.3-601), 4.36 (1.48-13.0), and 4.57 (1.85-11.3), for HBV infection alone, HCV infection alone, both HBV and HCV infections, alcohol consumption of ≥40 g of ethanol per day, and BMI of >25.0 kg/m^2 10 years before diagnosis, respectively. HBV and HCV infection and BMI of >25.0 kg/m^2 remained independent risk factors even after adjusting for severity of liver fibrosis. Among HCV-infected individuals, the relative risk of hepatocellular carcinoma for a 1 kg/m^2 increase in BMI was 1.39 (P = 0.003).**

Conclusions: **To limit the risk for hepatocellular carcinoma, control of excess weight may be crucial for individuals with chronic liver disease, especially those with chronic hepatitis C.** (Cancer Epidemiol Biomarkers Prev 2008;17(4):846–54)

Introduction

Hepatocellular carcinoma is one of the most common cancers worldwide. Chronic infections with hepatitis B virus (HBV) or with hepatitis C virus (HCV) are recognized as critically important risk factors for hepatocellular carcinoma. In addition, a large number of epidemiologic studies have shown that environmental factors such as dietary aflatoxin, smoking, alcohol consumption, and oral contraceptive intake are associated with increased risk for hepatocellular carcinoma (1, 2). It is generally considered that effects of these environmental factors are modified by gender, age, and race of patients (2-4).

Obesity and diabetes mellitus have recently received increased attention as risk factors for hepatocellular carcinoma (5-9). A large number of epidemiologic studies have shown that obesity and diabetes mellitus increase risks of a variety of cancers, including colon, renal, prostate, postmenopausal breast, and ovarian, in Asian and Western countries (7, 10, 11). Several recent epidemiologic studies indicated that obesity might be associated with an increased risk for hepatocellular carcinoma, but few cohort studies have incorporated HBV and HCV infection status in a strict and in-depth manner. A recent study of liver cirrhosis showed that, although obesity [body mass index (BMI), >30 kg/m^2] is an independent risk factor for hepatocellular carcinoma among patients with alcoholic cirrhosis or cryptogenic cirrhosis, it is not a significant risk factor for hepatocellular carcinoma in patients with chronic HBV and/or HCV infections (12).

Compared with viral etiologic factors, alcohol consumption, smoking, obesity, and diabetes mellitus may have less effect on hepatocellular carcinoma occurrence (13, 14); however, most epidemiologic studies have indicated that such factors promote development from chronic hepatitis to hepatocellular carcinoma (6, 8). Alcohol consumption, obesity, and diabetes mellitus have been shown to be involved in the progression of liver fibrosis; it is possible that liver fibrosis results from advanced oxidative stress due to hepatic steatosis and iron overload (15-17). Liver cirrhosis characterized by severe liver fibrosis may underlie the occurrence of hepatocellular carcinoma, specifically in the presence of chronic hepatitis C, nonalcoholic steatohepatitis, and

Received 11/16/07; revised 1/15/08; accepted 2/1/08.

Grant support: Japanese Ministry of Health, Labor and Welfare grant H16-Cancer Prevention-012 and Japanese Ministry of Education, Culture, Sports, Science and Technology grant 18590626.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Waka Ohishi, Department of Clinical Studies, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima 732-0815, Japan. Phone: 81-82-261-3131; Fax: 81-82-261-3259. E-mail: nwaka@rerf.or.jp

doi:10.1158/1055-9965.EPI-07-2806

alcoholic liver diseases (3, 8). On the other hand, several recent large-scale studies have indicated that coffee drinking suppressed the progression of liver fibrosis and inhibited the development of hepatocellular carcinoma (18, 19).

The fact that liver cirrhosis is not a necessary condition for hepatocellular carcinoma occurrence was already known, not only from clinical findings but also from genetic findings. Among hepatocellular carcinoma cases with HBV, a part of the HBV genome has been shown to be integrated into the host's intracellular DNA, thereby causing hepatocellular carcinoma (20). Among hepatocellular carcinoma cases with HCV, the HCV core protein seems to directly contribute to the mechanism of carcinogenesis by elevating oxidative stress (21). In light of the aforementioned findings, for the purpose of determining independent risk factors for hepatocellular carcinoma, careful analyses are needed controlling for severity of liver fibrosis, as well as for viral etiologic factors.

With the aim of determining whether HBV or HCV infections, alcohol consumption, smoking, coffee drinking, BMI, and diabetes mellitus are independent risk factors for hepatocellular carcinoma, and how the effects of these factors might change after adjusting for severity of liver fibrosis, we conducted a nested case-control study among the Adult Health Study longitudinal cohort using stored sera. We also evaluated whether viral etiology and increase of BMI exert synergistic effects on the risk for hepatocellular carcinoma.

Materials and Methods

Cohorts. The Atomic Bomb Casualty Commission and its successor, the Radiation Effects Research Foundation, established the Adult Health Study longitudinal cohort in 1958, in which 20,000 age-, gender-, and city-matched proximal and distal atomic bomb survivors and persons not present in the cities at the time of bombings have been examined biennially in outpatient clinics in Hiroshima and Nagasaki.

Study Population. Serum samples obtained from the study participants on each occasion of visiting outpatient clinics have been collected and stored systematically since 1969 (22). Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, supplemented by additional cases detected via pathologic review of related diseases (23). There were 359 primary hepatocellular carcinoma cases among Adult Health Study participants diagnosed between 1970 and 2002, who visited our outpatient clinics before their diagnosis. Of these, 130 cases were excluded because of nonavailability of stored serum or having only one stored sample. The other 229 cases had serum samples obtained within 6 years before hepatocellular carcinoma diagnosis. After excluding five cases with inadequate stored serum, 224 cases remained for our study. For each case, three controls were selected from the cohort in nested case-control fashion. Nested control selection was random among those who matched the case on gender, age (±2 years), city, time of serum storage (±2 years), and method of serum storage, and countermatched on radiation exposure (24). Although the total number of potential matched control serum samples is 672, because of occasional lack of subjects with stored sera who met the matching and countermatching criteria, the total number of control serum samples actually used was 644.

Laboratory Tests. HBV surface antigen and antibody to hepatitis B core antigen were measured by enzyme immunoassay, and anti-HCV antibody was measured by second-generation enzyme immunoassay as previously described (22, 25). Qualitative detection of HCV RNA among anti-HCV–positive samples was done using a thermocycler (Whatman Biometra) with two sets of PCR primers corresponding to the 5′-untranslated region, as previously described (25). Qualitative detection of HCV RNA was conducted at least twice. HBV infection (HBV+) status was defined as positive for HBV surface antigen or having a high titer of the antibody to hepatitis B core antigen. HCV infection (HCV+) status was defined as positive for HCV RNA (25). Hyaluronic acid and type IV collagen as liver fibrosis markers were measured using an autoanalyzer (Hitachi 7180, Hitachi, Ltd.) and latex agglutination–turbidimetric immunoassay (Fujirebio, Inc., Daiichi Pure Chemicals Co. Ltd.). Ferritin was measured using an autoanalyzer (Hitachi 7180, Hitachi) and colloidal gold immunoassay (Alfresa Pharma Corporation). Platelet count was measured using an automatic blood cell counter at the time of serum storage.

Information on Covariates. Self-administered questionnaires on various lifestyle factors were given to participants in 1965 during attendance at the Adult Health Study examination and in 1978 by mail survey. Information from the 1978 survey was obtained before hepatocellular carcinoma diagnosis for all but 19 (15%) of the cases. Information on alcohol consumption was obtained from the 1965 questionnaire when available, with missing data complemented using the 1978 survey. Alcohol consumption per volume of each type of alcoholic beverage was quantified as previously described (26), and mean ethanol amounts were calculated as grams per day. Information on smoking habits was obtained from the 1965 questionnaire; subjects were divided into the following categories: never, prior, and current smoker. Information on coffee drinking was obtained from the 1978 survey; subjects were divided into the following categories of frequency of coffee consumption: never, 1 day per week, 2 to 4 days per week, and almost daily. Disease diagnoses were based on the International Classification of Diseases (ICD) codes: diabetes mellitus was defined by ICD-7 code 260, ICD-8 code 250, ICD-9 code 250, and ICD-10 codes E10 through E14. BMI (kg/m^2) was calculated from height and weight measured at the Adult Health Study examination.

Subjects were classified based on BMI quintiles with cut points of 19.5, 21.2, 22.9, and 25.0. The number of hepatocellular carcinoma cases with BMI of >30.0 kg/m^2 was too small to be analyzed in detail. Following the recommendations for Asian people by the WHO, the International Association for the Study of Obesity, and the International Obesity Task Force (27), 21.3 to 22.9 kg/m^2 was considered as normal, 23 to 25 kg/m^2 as overweight, and >25.0 kg/m^2 as obese in the present study. We used information on diabetes mellitus and BMI obtained 10 years before the time of hepatocellular

Table 1. Characteristics of hepatocellular carcinoma cases and controls

Study variables	Hepatocellular carcinoma cases (n = 224)			Controls (n = 644)		
	Complete data (%)	n (%)	Mean (SD)	Complete data (%)	n (%)	Mean (SD)
Matched variables						
Gender	100			100		
Male		136 (60.7)			387 (60.1)	
Female		88 (39.3)			257 (39.9)	
Age at hepatocellular carcinoma diagnosis (y)	100		67.6 (10.1)	—		—
City	100			100		
Hiroshima		155 (69.2)			444 (68.9)	
Nagasaki		69 (30.8)			200 (31.1)	
Age at serum storage (y)	100		66.4 (10.2)	100		63.7 (9.8)
Unmatched variables						
Etiology (HBV/HCV status)	94.2			99.4		
HBV−/HCV−		45 (21.3)			579 (90.5)	
HBV+/HCV−		29 (13.7)			18 (2.8)	
HBV−/HCV+		132 (62.6)			41 (6.4)	
HBV+/HCV+		5 (2.4)			2 (0.3)	
Fibrosis markers	94.2			99.4		
Hyaluronic acid (ng/mL)			288.6 (284.6)			69.1 (108.3)
Type IV collagen (ng/mL)			245.2 (136.9)			148.8 (122.1)
Platelet count ($\times 10^4/\mu L$)	67.4		13.0 (6.0)	70.0		22.4 (6.2)
Ferritin (ng/mL)	92.0		250.5 (278.6)	98.6		136.7 (151.0)
Alcohol consumption (g of ethanol per day)	88.8			89.6		
>0 and <20		37 (18.6)			130 (22.5)	
≥20 and <40		20 (10.1)			64 (11.1)	
≥40		45 (22.6)			68 (11.8)	
Current smoking		107 (53.8)			262 (45.3)	
Prior smoking	88.8	12 (6.0)		89.8	33 (5.7)	
Daily coffee drinking	62.1	38 (27.3)		73.3	175 (37.1)	
BMI (kg/m^2) 10 y before diagnosis	93.8			98.3		
≤19.5		38 (18.1)			122 (19.3)	
19.6-21.2		33 (15.7)			136 (21.5)	
21.3-22.9		36 (17.2)			142 (22.4)	
23-25		49 (23.3)			124 (19.6)	
>25		54 (25.7)			109 (17.2)	
Diabetes 10 y before diagnosis	100	18 (8.0)		100	33 (5.1)	
Radiation dose to the liver (Gy)	91.1		0.46 (0.69)	94.1		0.34 (0.56)

carcinoma diagnosis or control matching because these conditions are subject to change because of disease progression in the later stages before diagnosis of hepatocellular carcinoma. Atomic bomb radiation dose was estimated for each subject according to the Dosimetry System DS02 (28).

Ethical Consideration. This nested case-control study was based on RERF Research Protocol 1-04 and approved by the Human Investigation Committee of Radiation Effects Research Foundation.

Statistical Analyses. The nested case-control design is analyzed using a partial likelihood method analogous to that used for cohort follow-up studies (29), which is, in practice, the same as the conditional binary data likelihood for matched case-control studies (30) except that the subjects (cases and controls) in the study are not completely independent because of the possibility of repeated selection. All factors other than radiation were analyzed using relative risks estimated by a log-linear model. The population attributable fraction was estimated for individual factors that increased the risk for hepatocellular carcinoma in the present study. Population attributable fraction was calculated as $pd \times [(mRR - 1) / mRR]$, where mRR is the multivariate adjusted relative risk for the covariates and pd is the proportion of cases exposed to the risk factor. Statistical interaction between viral infection and BMI was tested by adding the product of the two factors to the log-linear model, which tests departure from a multiplicative relationship. Reported P values and confidence limits are based on Wald statistics. Although radiation exposure could have been adjusted by matching on radiation dose as an additional matching factor in the control selection (31), in addition to assessing effects of lifestyle factors and viral hepatitis, another purpose of the present study was to examine effects of radiation exposure after adjustment for possible confounding and interaction by these factors, so matching on radiation, which prevents analysis of radiation risk, was not desirable; rather, we countermatched on radiation (29, 32). Radiation risk was analyzed by using an excess relative risk model as has been done previously (33).

Results

Characteristics of Study Population. Characteristics of the 224 hepatocellular carcinoma cases and 644 comparison subjects are shown in Table 1. The mean age of the cases was 67.6 years, and 61% were men. Cases and controls were comparable with respect to gender, age, city, time of serum storage, and method of serum storage by design. Virological and biochemical assays were done on 211 case and 640 control sera because 13 case samples and 4 control samples had insufficient stored sera for these assays. Hepatocellular carcinoma

case sera evidenced a higher prevalence of HBV or HCV infection status, higher values of fibrosis markers and ferritin, and lower platelet counts compared with control sera. Greater proportions of hepatocellular carcinoma cases had a history of alcohol consumption of ≥40 g of ethanol per day, were current smokers, were obese, had diabetes mellitus, and received high radiation doses compared with the controls. In addition, hepatocellular carcinoma cases were less likely than controls to be daily coffee drinkers. There were no important differences in characteristics such as gender, age at hepatocellular carcinoma diagnosis, city, or BMI between hepatocellular carcinoma cases excluded because of nonavailability of stored serum and those included in this study.

Risk Factors for Hepatocellular Carcinoma Development. Table 2 shows the results of univariate and multivariate analyses using HBV and HCV infection status, alcohol consumption, smoking habit, coffee drinking, BMI, diabetes mellitus, and radiation dose. Strong association was found between hepatocellular carcinoma and hepatitis virus infection, resulting in unadjusted relative risks of 33.7 [95% confidence interval (95% CI), 12.7-89.6] for HBV+/HCV− status and 64.5 (95% CI, 29.1-143) for HBV−/HCV+ status. As expected, the risk for hepatocellular carcinoma for alcohol consumption was significant, with an unadjusted relative risk of 1.34 (95% CI, 1.12-1.60) per 20 g of ethanol per day using continuous alcohol consumption and 2.66 (95% CI, 1.55-4.55) at ≥40 g of ethanol per day using grouped alcohol consumption. Although the grouped results suggest that a simple log-linear model in continuous alcohol consumption may not be adequate, a quadratic term did not significantly improve the model (data not shown). Current smoking was significantly associated with hepatocellular carcinoma risk, with an unadjusted relative risk of 1.87 (95% CI, 1.14-3.07). Daily coffee drinking was associated with decreased risk for hepatocellular carcinoma, with an unadjusted relative risk of 0.51 (95% CI, 0.29-0.90). The presence of obesity and diabetes mellitus 10 years before diagnosis were statistically associated with increased risk for hepatocellular carcinoma, resulting in unadjusted relative risks of 1.88 (95% CI, 1.13-3.13) and 1.88 (95% CI, 1.01-3.50), respectively. The relative risk for a 1-unit difference in BMI was 1.04 (95% CI, 0.99-1.09). Radiation exposure was marginally significantly associated with increased risk for hepatocellular carcinoma ($P = 0.055$).

The risks for viral infection in multivariate analysis did not meaningfully differ from those obtained in the univariate analysis. Alcohol consumption of ≥40 g of ethanol per day and obesity remained significant risk factors for hepatocellular carcinoma even after adjusting for viral infection status and the other factors, whereas the effects of current smoking and diabetes mellitus became nonsignificant after adjustment. Daily coffee drinking was marginally significantly associated with decreased risk for hepatocellular carcinoma after adjustment for viral infection and the other factors. The adjusted relative risk for a one unit difference in BMI, 1.12 (95% CI, 1.03-1.22), was statistically significant, but a quadratic term was not significant.

Table 3 shows the estimated population attributable fraction based on the multivariate adjusted relative risks in the present study. The proportion of hepatocellular

Table 2. Relative risks of hepatocellular carcinoma for individual factors

Variables	Unadjusted		Multivariate adjusted	
	RR (95% CI)	*P*	RR (95% CI)*	*P*
Etiology (HBV/HCV status)				
HBV−/HCV−	1	—	1	—
HBV+/HCV−	33.7 (12.7-89.6)	<0.001	45.8 (15.2-138)	<0.001
HBV−/HCV+	64.5 (29.1-143)	<0.001	101 (38.7-263)	<0.001
HBV+/HCV+	42.4 (6.2-291)	<0.001	70.7 (8.3-601)	<0.001
Alcohol consumption (g of ethanol per day)				
Never	1	—	1	—
>0 and <20	1.11 (0.69-1.78)	>0.5	1.27 (0.56-2.87)	>0.5
≥20 and <40	1.07 (0.57-1.99)	>0.5	1.02 (0.34-3.05)	>0.5
≥40	2.66 (1.55-4.55)	<0.001	4.36 (1.48-13.0)	0.008
Continuous (per 20-g ethanol per day)	1.34 (1.12-1.60)	<0.001	1.73 (1.19-2.52)	0.004
Smoking habit				
Never	1	—	1	—
Current smoking	1.87 (1.14-3.07)	0.014	2.03 (0.82-4.98)	0.13
Prior smoking	1.80 (0.81-3.99)	0.15	1.12 (0.25-5.07)	>0.5
Coffee drinking				
Never	1	—	—	—
Daily	0.51 (0.29-0.90)	0.016	0.40 (0.16-1.02)	0.055
BMI (kg/m^2) 10 y before diagnosis				
≤19.5	1.24 (0.73-2.11)	0.43	1.31 (0.51-3.34)	>0.5
19.6-21.2	0.97 (0.55-1.70)	>0.5	1.24 (0.43-3.54)	>0.5
21.3-22.9	1	—	1	—
23-25	1.61 (0.96-2.70)	0.074	2.51 (0.99-6.37)	0.053
>25	1.88 (1.13-3.13)	0.016	4.57 (1.85-11.3)	<0.001
Continuous (+1 kg/m^2 difference)	1.04 (0.99-1.09)	0.087	1.12 (1.03-1.22)	0.010
Diabetes 10 y before diagnosis	1.88 (1.01-3.50)	0.047	1.98 (0.63-6.27)	0.24

Abbreviation: RR, relative risk.
*Adjusted for hepatitis virus infection, continuous alcohol consumption, smoking habit, coffee drinking, BMI, diabetes mellitus, and radiation dose to the liver.

Table 3. Estimated population attributable fraction of hepatocellular carcinoma for risk factors in this study population

Variables*	Proportion of cases exposed (%)	Multivariate-adjusted RR	Population attributable fraction (%)
Etiology (HBV/HCV status)			
HBV+/HCV−	13.7	45.8	13.4
HBV−/HCV+	62.6	101	62.0
HBV+/HCV+	2.4	70.7	2.4
Alcohol consumption			
≥40-g ethanol per day	22.6	4.36	17.4
BMI 10 y before diagnosis			
>25 kg/m^2	25.7	4.57	20.1

*Population attributable fraction was estimated only for the significant hepatocellular carcinoma risk factors.

carcinoma cases that is attributable to HBV+/HCV−, HBV−/HCV+, HBV+/HCV+, alcohol consumption of ≥40 g of ethanol per day, and obesity were 13.4%, 62.0%, 2.4%, 17.4%, and 20.1%, respectively. These values are not mutually exclusive because some cases were exposed to more than one risk factor.

Analyses with Adjustment for Variables Associated with Severity of Liver Fibrosis. Table 4 shows results for univariate analyses incorporating biomarkers associated with progression of liver fibrosis, such as hyaluronic acid and type IV collagen of fibrosis markers, platelet count, and ferritin. Large statistically significant differences in the mean values of these variables were observed between hepatocellular carcinoma cases and controls. Figure 1 shows a comparison of multivariate analysis results with or without adjustment for ln(type IV collagen) and platelet count using HBV and HCV infection status, alcohol consumption, smoking habit, coffee drinking, BMI, diabetes mellitus, and radiation dose as adjustment variables. We evaluated type IV collagen and platelet count as surrogate markers associated with severity of liver fibrosis. Hepatocellular carcinoma risk for hepatitis virus infection status after adjusting for liver fibrosis meaningfully decreased compared with the results indicated in the previous multivariate analysis, with relative risks of 20.8 (95% CI, 4.8-90.3) and 37.8 (95% CI, 12.4-115) for HBV+/HCV− status and HBV−/HCV+ status, respectively (Fig. 1A). Effects of ≥40 g of ethanol per day and daily coffee drinking decreased and disappeared, respectively, so that adjustment for liver fibrosis decreased the effect of these factors on risk for hepatocellular carcinoma. Current smoking became marginally significantly associated with increased risk for hepatocellular carcinoma after adjusting for liver fibrosis. Obesity remained a significant risk factor independent of adjustment for severity of liver fibrosis, and the relative risk for diabetes mellitus did not meaningfully differ from that without such adjustment (Fig. 1B).

Interaction between Hepatitis Virus Infection Status and Increase of BMI. Table 5 shows the joint effects of hepatitis virus infection status and BMI, with adjustment for alcohol consumption, smoking habit, coffee drinking, diabetes mellitus, and radiation dose. Although being obese was clearly a risk factor for hepatocellular carcinoma subjects with adjustment for viral factors, it was not a significant risk factor in those with HBV−/HCV− status. However, despite the appearance of a trend with BMI, only 15 hepatocellular carcinoma cases were identified among HBV−/HCV− individuals with obesity. Among hepatocellular carcinoma subjects with HBV−/HCV+ status, the relative risk increased dramatically with increasing BMI. Linear (P = 0.003) and quadratic (P = 0.013) terms in continuous BMI were

Table 4. Relative risks of hepatocellular carcinoma for variables associated with severity of liver fibrosis: unadjusted relative risk and 95% CI

Variables	Hepatocellular carcinoma cases/controls	Unadjusted	
		RR (95% CI)	P
Liver fibrosis markers	211/640		
Hyaluronic acid (+per 10 ng/mL)		1.10 (1.08-1.12)	<0.001
ln(hyaluronic acid) (+per 1 unit)		5.43 (4.04-7.30)	<0.001
Type IV collagen (+per 10 ng/mL)		1.14 (1.10-1.17)	<0.001
ln(type IV collagen) (+per 1 unit)		80.9 (35.8-183)	<0.001
Platelet count	151/448		
+Per 10^4/μL		0.75 (0.71-0.80)	<0.001
≥25.0 (×10^4/μL)	4/133	1	
20.0-24.9 (×10^4/μL)	19/163	4.5 (1.3-1.6)	0.02
15.0-19.9 (×10^4/μL)	26/105	11.8 (3.2-43)	<0.001
10.0-14.9 (×10^4/μL)	52/42	61 (16-232)	<0.001
<10.0 (×10^4/μL)	50/5	822 (125-5400)	<0.001
Ferritin	206/635		
+ Per 10 ng/mL		1.03 (1.02-1.04)	<0.001
ln(ferritin) (+per 1 unit)		1.51 (1.25-1.82)	<0.001

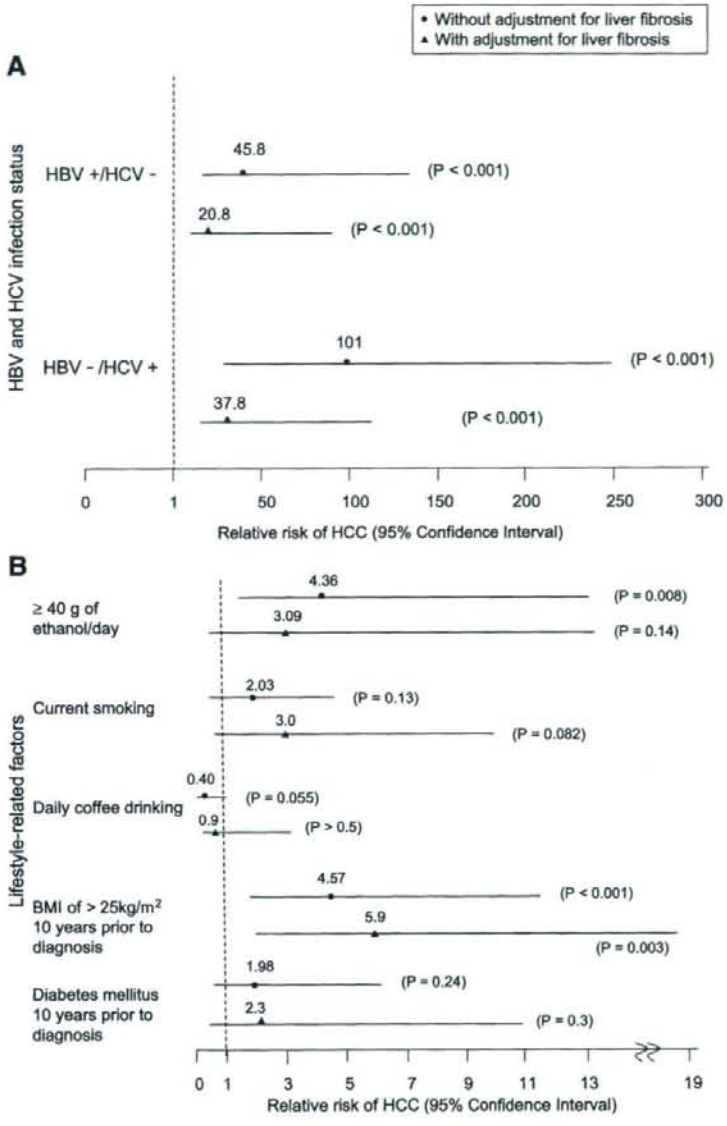

Figure 1. Multivariate relative risk for hepatocellular carcinoma for individual risk factors, with and without adjustment for variables associated with severity of liver fibrosis. Each relative risk was analyzed with and without adjustment for ln(type IV collagen) and platelet count, using HBV and HCV infection status, continuous alcohol consumption, smoking habit, coffee drinking, BMI, diabetes mellitus, and radiation dose as adjustment variables. **A.** HBV and HCV infection status. **B.** Lifestyle-related factors. *HCC*, hepatocellular carcinoma.

significant among HBV−/HCV+ individuals. Among hepatocellular carcinoma subjects with HBV+/HCV− status, the relative risk for hepatocellular carcinoma did not show evidence of an increase with increased BMI, although the examination of a joint effect of HBV infection and BMI was based on only one hepatocellular carcinoma case out of three subjects who were HBV+/HCV− and obese. The reason for the relatively small unadjusted relative risk for obesity (Table 2) might have been due to the small number of cases and controls with HBV+/HCV− status, which apparently offset the increase observed in HBV−/HCV+ status individuals.

Discussion

This nested case-control study indicated that HBV and HCV infection, alcohol consumption of ≥40 g of ethanol per day, and obesity 10 years before hepatocellular carcinoma diagnosis were independent risk factors for hepatocellular carcinoma, and that obesity as well as hepatitis virus infection remained independent risk factors for hepatocellular carcinoma after taking into account the severity of liver fibrosis. Furthermore, significant multiplicative interaction in hepatocellular carcinoma risk between viral etiology and increased BMI was observed in HCV-infected individuals. The

population attributable fraction of 62.0% for hepatocellular carcinoma cases with HCV infection was highest, and hepatocellular carcinoma cases with HBV infection, alcohol consumption of ≥40 g of ethanol per day, or obesity had population attributable fractions in the range of 13.4% to 20.1%. These are only approximate estimates of the potential for reducing hepatocellular carcinoma occurrence, as we do not know what effect removal of one risk factor would have on the distribution of the other risk factors.

Multivariate analysis after adjusting for severity of liver fibrosis indicated that hepatocellular carcinoma risk for HBV and HCV infections significantly decreased, which is consistent with the existing notion that hepatocellular carcinoma risk increases with progression from chronic hepatitis B and C to liver cirrhosis. A large-scale meta-analysis (34) and a case-control study (35) showed a combined effect of HBV and HCV infections on hepatocellular carcinoma risk, whereas our study did not detect similar effects among those with HBV+/HCV+ status. This difference may be partly attributable to the extremely limited number of coinfected subjects with HBV and HCV among our study population. It may be also partly because most past epidemiologic studies have defined chronic HCV infection by either anti-HCV antibody positivity or by HCV RNA positivity in serum (34, 35).

Several epidemiologic studies and clinical trials revealed an association between obesity and hepatocellular carcinoma risk (9-12), but few population-based cohort studies have been conducted with precise adjustment for HBV and HCV infection status, the major risk factors for hepatocellular carcinoma. Obesity was recently found to be one of the etiologic factors for nonalcoholic steatohepatitis, which is considered a non-B, non-C liver disease, and it has been shown to be a risk factor for hepatocellular carcinoma (12, 16). Although many clinical studies showed that, among chronic hepatitis C patients, obesity was associated with progression of inflammation, insulin resistance, hepatic steatosis, and liver fibrosis (17, 36), a study by Nair et al. (12) reported that obesity was not an independent risk factor for hepatocellular carcinoma among liver cirrhosis patients with HBV and HCV. On the other hand, a recent Western cohort study showed that being overweight (BMI, 25 to <30 kg/m^2) or obese (BMI, ≥30 kg/m^2) was an independent risk factor for hepatocellular carcinoma (37).

In the present study, we adjusted for potentially confounding factors including hepatitis virus infection and also found that being obese 10 years before hepatocellular carcinoma diagnosis was associated with a 4.57-fold increase in hepatocellular carcinoma risk. Furthermore, we observed a statistically significant, positive, multiplicative interaction between HCV infection and increased BMI on the risk for hepatocellular carcinoma, which indicates decisively that the joint effect of the two factors is greater than additive.

Obesity contributes to a high rate of visceral fat storage, accelerating production of tumor necrosis factor-α, interleukin 6, resistin, and leptin, and decreasing production of adiponectin (16). These cytokines presumably foster insulin resistance (16), cause hepatic steatosis and oxidative stress, and eventually promote hepatocellular carcinoma occurrence. A large number of studies pointed out association between progression of liver fibrosis and insulin resistance or hepatic steatosis (15-17), but authenticity of any connection is now being questioned (8, 36). Interestingly, in this study, obesity remained an independent risk factor for hepatocellular carcinoma even after adjusting for all confounding factors including severity of liver fibrosis. The following are the possible reasons why obesity increases hepatocellular carcinoma risk irrespective of severity of liver fibrosis: Several animal experiments showed that liver tumors were not always accompanied by advanced fibrosis among a variety of genetically engineered mouse models with steatohepatitis (38), and some reports indicated several nonalcoholic steatohepatitis–derived human cancer cases without significant liver fibrosis (39). The findings suggest that significant liver fibrosis is not essential for the carcinogenic process, but that steatohepatitis itself is a state conferring a risk for high carcinogenicity. With regard to the proven relationship between obesity and such malignant tumors as colon, breast, and ovarian cancers (10), the cell proliferation activity of insulin due to hyperinsulinemia is believed to play a role in a common carcinogenic mechanism (5).

It is well documented that obesity induces insulin resistance, with a tendency to cause diabetes mellitus. In the case of hepatic cirrhosis accompanied by highly advanced liver fibrosis, glucose intolerance tends to lead to diabetes mellitus. A recent animal experiment showed that HCV contributed to progression of insulin resistance, resulting in diabetes mellitus (40). The present study failed to show that diabetes mellitus 10 years before hepatocellular carcinoma diagnosis was an independent risk factor for hepatocellular carcinoma, but an adjustment for all factors, except alcohol consumption and BMI, brought about a 30% increase in the effect of diabetes on hepatocellular carcinoma risk (data not shown). Such findings suggest a relationship between diabetes mellitus and alcohol consumption, as well as BMI. Therefore, by taking into account the proven association between alcohol consumption, obesity, and increased risk for hepatocellular carcinoma, our results will not likely refute an association between diabetes mellitus and hepatocellular carcinoma risk.

A large number of epidemiologic studies showed that heavy alcohol consumption was an independent risk factor for hepatocellular carcinoma and that there was

Table 5. Interaction between hepatitis virus infection status and increase of BMI on hepatocellular carcinoma risk (joint hepatitis virus/BMI)

Viral etiology	BMI (kg/m^2)	RR* (95% CI)†	Likelihood ratio P †
HBV−/HCV−	+1	1.05 (0.95-1.17)	0.33
HBV+/HCV−	+1	0.89 (0.64-1.23)	0.50
HBV−/HCV+	+1	1.39 (1.11-1.83)	0.003‡
HBV+/HCV+	+1	—§	—§

*Adjusted for continuous alcohol consumption, smoking habit, coffee drinking, diabetes mellitus, and radiation dose to the liver.
†Likelihood bounds and P values for relative risks estimated separately within each BMI/hepatitis virus category.
‡A quadratic term was also significant for HBV−/HCV+ individuals (P = 0.013). However, only the relative risk for the linear model in continuous BMI is shown because it is not possible to express the risk as a single value with a two-parameter linear-quadratic model.
§Neither could the joint effect of obesity and simultaneous HBV+/HCV+ status be estimated because of small numbers of jointly affected cases and controls.

correlation between increased risk for hepatocellular carcinoma and amount of alcohol consumed (3, 9, 13, 14). In addition, in some case-control studies of hepatocellular carcinoma risk, synergistic interactions between alcohol consumption and hepatitis virus infection, or between obesity and diabetes mellitus, have been observed (9, 13, 14). In the present study, after adjusting for other factors such as hepatitis virus infection and BMI, alcohol consumption of ≥40 g of ethanol produced a 4.36-fold increase in hepatocellular carcinoma risk. A few recent case-control studies suggested that ethanol consumption of <50 to 60 g/d (41, 42) or alcohol exposure <1,500 gram-years (9) had protective effects on the progression of liver fibrosis and risk for developing hepatocellular carcinoma. Reasons for such discrepancy between our result and former reports are unclear, but factors such as gender, age, race (43), hereditary predisposition, and etiology of liver disease presumably affect the severity of alcohol-related liver diseases. Our study also showed that effects of alcohol consumption of ≥40 g of ethanol per day on hepatocellular carcinoma risk were reduced after adjusting for all confounding factors including severity of liver fibrosis. The finding suggests that alcohol consumption may contribute to hepatic carcinogenesis by enhancing oxidative stress and aggravating liver fibrosis.

As a result of recent assessments by the IARC, hepatocellular carcinoma has been positioned as a smoking-related malignant disease (44). However, it has yet to be determined whether smoking itself has direct hepatic carcinogenic effects or whether smoking contributes to hepatic carcinogenesis by way of progression of liver fibrosis. A case-control study showed that 4-aminobiphenyl DNA adducts contained in tobacco smoke are a liver carcinogen (45). In the present study, we adjusted for potential confounding factors including hepatitis virus infection and failed to detect significant smoking effects on hepatocellular carcinoma risk; however, a multivariate analysis that excluded hepatitis virus infection showed significant effects of smoking (data not shown). With adjustment for all factors including severity of liver fibrosis, effects of smoking on hepatocellular carcinoma risk were found to be marginally significant. These findings suggest the possibility that smoking, in conjunction with hepatitis virus infection, further enhances the risk for hepatocellular carcinoma and might directly contribute to the mechanism of liver carcinogenesis.

Several epidemiologic studies indicated the involvement of coffee in decreased alanine aminotransferase activity and γ-glutamyltransferase level, suppression of progression to liver cirrhosis, and inhibited development of hepatocellular carcinoma (18, 19). Such oxidation inhibitors as caffeine, coffee diterpenes, and chlorogenic acid are among candidate substances in coffee that potentially reduce the risk for hepatocellular carcinoma, and several animal experiments have shown that such substances have direct inhibitory effects on hepatic carcinogenesis (46). Adjusting for all potential confounding factors including hepatitis virus infection rendered the effects of coffee drinking on hepatocellular carcinoma risk marginally significant, whereas adjusting for all factors, except hepatitis virus infection, revealed significant effects of coffee drinking (data not shown). Furthermore, adjusting for all factors including severity of liver fibrosis erased the effects of coffee drinking on hepatocellular carcinoma risk. These findings suggest that coffee drinking may somehow suppress liver fibrosis and thereby indirectly reduce hepatocellular carcinoma risk.

The main strengths of our study are its prospective cohort-based, nested case-control design, which minimized selection bias and provided for the use of stored sera and a wealth of epidemiologic information obtained before hepatocellular carcinoma diagnosis. Indeed, the distributions of HBV and HCV infection status among hepatocellular carcinoma cases and controls and mean age at diagnosis among hepatocellular carcinoma cases were similar to those in previous reports on Japanese populations (2, 4). Another major strength of our study is that it incorporated, in a strict and in-depth manner, HBV and HCV infection status and showed the interrelationship between these and numerous other epidemiologic factors. It is difficult and expensive to perform full cohort serum analyses, whereas the nested case-control design used here can provide substantial reductions in cost and effort with little loss of statistical efficiency.

The main limitation of our study is that the severity of liver fibrosis could not be classified into fibrosis stage of F0 to F4 based on liver specimens. We used platelet counts and type IV collagen concentrations as surrogate, but independent, markers of liver fibrosis. Previous reports showed a strong correlation between platelet count and fibrosis stage in the presence of chronic hepatitis C (47) and a close association between levels of type IV collagen, a basic component of the hepatic basal membrane, and severity of liver fibrosis. Another limitation of our study is the usage of sera that had been stored for long periods of time. Proteins and HCV RNA trend to degrade during prolonged storage of either frozen or freeze-dried sera. However, we minimized this degradative effect by the selection of matched controls relative to time and method of serum storage. Furthermore, we have previously shown that the freeze-dried sera are interchangeable with frozen sera in serologic and molecular biological detection of HBV and HCV (22, 25). Finally, some hepatocellular carcinoma cases had to be excluded because of nonavailability of stored sera. We did not detect any differences between included and excluded cases in terms of demographic variables or BMI.

In conclusion, HBV and HCV infection and obesity were independent risk factors for hepatocellular carcinoma, even after taking into account the severity of liver fibrosis. Moreover, the combination of HCV infection and increased BMI exerted a synergistic effect on the risk for hepatocellular carcinoma. Alcohol consumption of ≥40 g of ethanol per day was also an independent risk factor for hepatocellular carcinoma, likely contributing to the development of hepatocellular carcinoma through liver fibrosis. The radiation effect on hepatocellular carcinoma risk was shown to be marginally significant in univariate analysis; whether the radiation effect is confounded with other factors will be closely examined in a separate report. A precise understanding of the mechanism by which obesity contributes to development of hepatocellular carcinoma should lead to better therapeutic strategies, public health policies, and cost-effectiveness.

Acknowledgments

We thank Naomi Masunari and Sachiko Teranishi for the collection and processing of the data; all members of division of clinical laboratories for their excellent assistance in experiments; and Michiko Yamada, Yoshimi Tatsukawa, and Kyoji Furukawa for helpful discussion. The Radiation Effects Research Foundation, Hiroshima and Nagasaki, Japan, is a private, nonprofit foundation funded by the Japanese Ministry of Health, Labour and Welfare and the U.S. Department of Energy, the latter through the National Academy of Sciences.

References

1. Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127:S72–8.
2. Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127:S17–26.
3. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. Cancer 2000;89:53–9.
4. Ohishi W, Kitamoto M, Aikata H, et al. Impact of aging on the development of hepatocellular carcinoma in patients with hepatitis C virus infection in Japan. Scand J Gastroenterol 2003;38:894–900.
5. Gupta K, Krishnaswamy G, Karnad A, Peiris AN. Insulin: a novel factor in carcinogenesis. Am J Med Sci 2002;323:140–5.
6. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–8.
7. 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.
8. Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. Gastroenterology 2004;127:S97–103.
9. Marrero JA, Fontana RJ, Fu S, Conjeevaram HS, Su GL, Lok AS. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. J Hepatol 2005;42:218–24.
10. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.
11. Oh SW, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. J Clin Oncol 2005;23:4742–54.
12. Nair S, Mason A, Eason J, Loss G, Perrillo RP. Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? Hepatology 2002;36:150–5.
13. Hassan MM, Hwang LY, Hatten CJ, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206–13.
14. Yuan JM, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer 2004;101:1009–17.
15. Ferrannini E. Insulin resistance, iron, and the liver. Lancet 2000;355:2181–2.
16. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 2005;42:987–1000.
17. Hu KQ, Kyulo NL, Esrailian E, et al. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. J Hepatol 2004;40:147–54.
18. Inoue M, Yoshimi I, Sobue T, Tsugane S; JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. J Natl Cancer Inst 2005;97:293–300.
19. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2005;128:24–32.
20. Yaginuma K, Kobayashi H, Kobayashi M, Morishima T, Matsuyama K, Koike K. Multiple integration site of hepatitis B virus DNA in hepatocellular carcinoma and chronic active hepatitis tissues from children. J Virol 1987;61:1808–13.
21. Moriya K, Nakagawa K, Santa T, et al. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001;61:4365–70.
22. Ohishi W, Fujiwara S, Suzuki G, et al. Feasibility of freeze-dried sera for serological and molecular biological detection of hepatitis B and C viruses. J Clin Microbiol 2006;44:4593–5.
23. Fukuhara T, Sharp GB, Mizuno T, et al. Liver cancer in atomic-bomb survivors: histological characteristics and relationships to radiation and hepatitis B and C viruses. J Radiat Res 2001;42:117–30.
24. Cologne JB, Sharp GB, Neriishi K, Verkasalo PK, Land CE, Nakachi K. Improving the efficiency of nested case-control studies of interaction by selecting controls using counter matching on exposure. Int J Epidemiol 2004;33:485–92.
25. Ohishi W, Fujiwara S, Suzuki G, Chayama K. Validation of the use of freeze-dried sera for the diagnosis of hepatitis B and C virus infections in a longitudinal study cohort. In: Mohan RM, editor. Research Advances in Microbiology 7. Kerala, India: Global Research Network; 2007. p. 1–9.
26. Sharp GB, Lagarde F, Mizuno T, et al. Relationship of hepatocellular carcinoma to soya food consumption: a cohort-based, case-control study in Japan. Int J Cancer 2005;115:290–5.
27. The World Health Organization Western Pacific Region; The International Association for the Study; The International Obesity Task Force. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney, Australia: Health Communications Australia Pty Limited; 2000.
28. Young RW, Kerr GD, editors. Reassessment of the atomic bomb radiation dosimetry for Hiroshima and Nagasaki, Dosimetry System 2002, Report of the Joint US-Japan Working Group. Hiroshima, Japan: Radiation Effects Research Foundation; 2005.
29. Langholz B, Borgan Ø. Counter-matching: a stratified nested case-control sampling method. Biometrika 1985;82:69–79.
30. Breslow NE, Day NE. Statistical methods in cancer research: volume 1—the analysis of case-control studies. Lyon: IARC; 1980.
31. Cologne JB, Shibata Y. Optimal case-control matching in practice. Epidemiology 1995;6:271–5.
32. Cologne J, Langholz B. Selecting controls for assessing interaction in nested case-control studies. J Epidemiol 2003;13:193–202.
33. Cologne JB, Tokuoka S, Beebe GW, Fukuhara T, Mabuchi K. Effects of radiation on incidence of primary liver cancer among atomic bomb survivors. Radiat Res 1999;152:364–73.
34. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75:347–54.
35. Kirk GD, Lesi OA, Mendy M, et al. The Gambia Liver Cancer Study: Infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. Hepatology 2004;39:211–9.
36. Perumalswami P, Kleiner DE, Lutchman G, et al. Steatosis and progression of fibrosis in untreated patients with chronic hepatitis C infection. Hepatology 2006;43:780–7.
37. Ioannou GN, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol 2007;5:938–45.
38. Fan CY, Pan J, Usuda N, Yeldandi AV, Rao MS, Reddy JK. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor α natural ligand metabolism. J Biol Chem 1998;273:15639–45.
39. Bullock RE, Zaitoun AM, Aithal GP, Ryder SD, Beckingham IJ, Lobo DN. Association of non-alcoholic steatohepatitis without significant fibrosis with hepatocellular carcinoma. J Hepatol 2004;41:685–6.
40. Shintani Y, Fujie H, Miyoshi H, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004;126:840–8.
41. Donato F, Tagger A, Gelatti U, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002;155:323–31.
42. Monto A, Patel K, Bostrom A, et al. Risks of a range of alcohol intake on hepatitis C-related fibrosis. Hepatology 2004;39:826–34.
43. Iwahashi K, Matsuo Y, Suwaki H, Nakamura K, Ichikawa Y. CYP2E1 and ALDH2 genotypes and alcohol dependence in Japanese. Alcohol Clin Exp Res 1995;19:564–6.
44. IARC. IARC Monographs on the evaluation of the carcinogenic risks to humans. Volume 83: tobacco smoke and involuntary smoking. Lyon: IARC; 2004.
45. Wang LY, Chen CJ, Zhang YJ, et al. 4-Aminobiphenyl DNA damage in liver tissue of hepatocellular carcinoma patients and controls. Am J Epidemiol 1998;147:315–23.
46. Tanaka T, Nishikawa A, Shima H, et al. Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. Basic Life Sci 1990;52:429–40.
47. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001;96:3142–6.

doi:10.1111/j.1440-1746.2008.05356.x

HEPATOLOGY

Prospective study of short-term peginterferon-α-2a monotherapy in patients who had a virological response at 2 weeks after initiation of interferon therapy

Soocheol Jeong,* Yoshiiku Kawakami,* Mikiya Kitamoto,† Hiroto Ishihara,‡ Keiji Tsuji,§ Shiomi Aimitsu,¶ Hiroiku Kawakami,** Kiminori Uka,* Shintaro Takaki,* Hideaki Kodama,* Koji Waki,* Michio Imamura,* Hiroshi Aikata,* Shoichi Takahashi* and Kazuaki Chayama*

*Department of Medicine and Molecular Science, Hiroshima University, †Department of Internal Medicine, Prefectural Hiroshima Hospital, ‡Department of Internal Medicine, Chuden Hospital, §Department of Internal Medicine, Hiroshima City Asa Hospital, ¶Second Department of Internal Medicine, Hiroshima Red Cross and Atomic Bomb Survivors' Hospital, **Kawakami Clinic, Hiroshima, Japan

Key words
adverse effects, clinical trial, efficacy, hepatitis C virus, peginterferon-α-2a.

Accepted for publication 16 December 2007.

Correspondence
Dr Yoshiiku Kawakami, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: kamy4419@hiroshima-u.ac.jp

Abstract

Background and Aims: Long-term interferon (IFN) therapy is effective in eliminating hepatitis C virus (HCV). However, it carries the risk of adverse effects and reduced quality of life. To assess whether short-term IFN therapy effectively eliminates HCV, we performed a prospective pilot study of pegylated (peg)IFN-α-2a therapy for 8 or 24 weeks.

Methods: After excluding patients with high titers of genotype-1, 55 HCV patients received pegIFN-α-2a. Patients who became negative for HCV-RNA at week 2 were allocated to either an 8-week ($n = 19$) or 24-week ($n = 15$) course of IFN. We evaluated the efficacy of and tolerance to IFN therapy.

Results: The sustained virological response rate was excellent in the two groups (8 weeks, 89.5% [17/19]; 24 weeks, 100% [15/15], respectively,). IFN dose reduction was required in one patient of the 8-week group, but in six patients of the 24-week group ($P = 0.028$). Treatment was completed by all patients of the 8-week group, but discontinued in five patients of the 24-week group ($P = 0.011$).

Conclusions: The 8-week IFN therapy is more tolerable than the 24-week therapy and had similar outcomes. Excluding the patients with high titers of genotype-1, we recommend switching to an 8-week course of pegIFN-α monotherapy once patients show an ultra rapid virological response at week 2 from the start of IFN therapy.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease with an estimated 170 million chronic carriers worldwide.[1] Chronic HCV infection is usually associated with liver cirrhosis (LC) and hepatocellular carcinoma (HCC).[2–6] In Japan, 60–70% of patients with HCC or LC are HCV carriers.[7] Antiviral therapy of interferon (IFN) is widely used for the treatment of chronic HCV infection and is assumed to prevent progression to LC and HCC, especially in patients who show a sustained virological response (SVR).

The reported total HCV-RNA elimination rate is approximately 30–40% in patients treated with conventional IFN monotherapy.[8–10] However, better results have been reported when pegylated (peg)IFN-α is used in both naive patients and in those who fail to respond to or relapse after conventional IFN-α monotherapy. In Japan, two kinds of pegIFN are available: pegIFN-α-2a and pegIFN-α-2b. PegIFN-α-2b can be used with ribavirin, a purine nucleoside analog, in naive patients with genotypes 1 and 2 with a high viral load (>100 KIU/mL of HCV-RNA) or patients with any viral load in whom previous IFN treatment did not eliminate HCV-RNA. PegIFN-α-2a has been used in Japan without ribavirin only since December 2003 because of health insurance restrictions. However, ribavirin combination therapy has been covered by public health insurance since March 2007 in Japan. The HCV elimination rate with pegIFN-α-2b plus ribavirin combination therapy is up to 54% in patients with genotype 1.[11] Several investigators have reported that pegIFN and ribavirin combination therapy for a period of 24 or 48 weeks ensures a viral clearance in most patients with HCV genotypes 2 or 3 infection.[12,13] However, ribavirin combination therapy frequently causes anemia and should be carefully used in the elderly, anemic, or pregnant young patients, and in those who require long-term treatment.[14] Apart from patients with a high viral load of genotype 1, IFN monotherapy is also effective in HCV elimination even when used without ribavirin. Previous studies suggest that the SVR achieved with pegIFN-α-2a is similar to that observed with pegIFN-α-2a combined with ribavirin in patients with hepatitis C.[15,16]

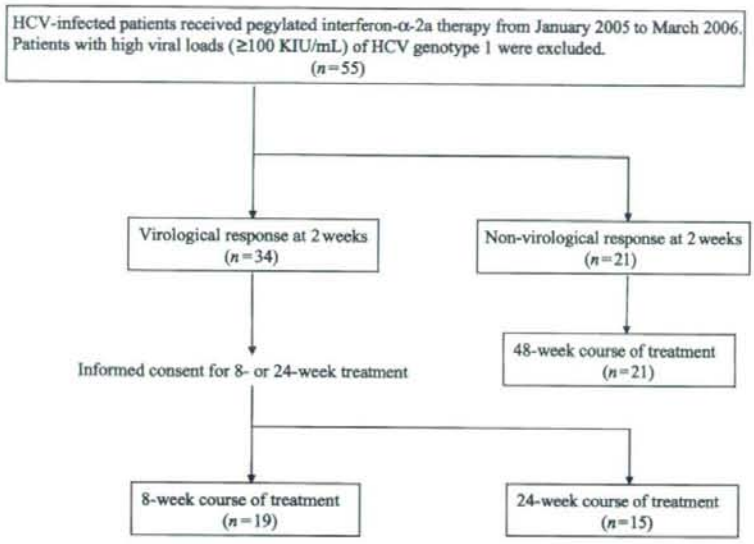

Figure 1 Flow diagram of the clinical trial. HCV, hepatitis C virus.

Although the tolerability of pegIFN is similar to that of the conventional IFN,[15] the 180 μg dose of pegIFN-α-2a therapy for 48 weeks is sometimes not tolerated by some patients. With the exception of those with high viral loads of genotype 1, the above regimen is expected to produce a high viral clearance rate, especially in patients with an early virological response. Several studies report the effectiveness of short-course IFN therapy (<24 weeks) for patients with an early virological response.[17–20] Therefore, a treatment duration of 48 weeks may be too long or more than sufficient for some patients, especially when one considers the undesirable adverse effects or the cost of treatment.

In the present study, we conducted a prospective controlled trial to compare the efficacy of an 8-week versus a 24-week course of pegIFN-α-2a (180 μg/time/week) for patients negative for HCV-RNA at 2 weeks after the initiation of therapy.

Methods

Patients

Between January 2005 and March 2006, a total of 55 HCV-infected patients received pegIFN-α-2a therapy at Hiroshima University Hospital (Hiroshima, Japan) and its associated hospitals in Japan. Patients with high viral loads (≥100 KIU/mL) of HCV genotype 1 were excluded from this study because of their low SVR rate. Among the 55 patients, 34 consecutive patients who showed a rapid virological response at 2 weeks were enrolled in this study (Fig. 1). Eligible patients had antibodies to HCV, were positive for HCV-RNA at study entry, and had not received previous IFN therapy. They included 21 men and 13 women, with a mean age of 53 years (range, 21–71 years). Their HCV genotypes were 1b, 2a, and 2b with variant HCV-RNA (5.1–400 KIU/mL by a reverse transcriptase-polymerase chain reaction [RT-PCR]). All patients underwent liver biopsies within 12 weeks before the start of IFN therapy and were confirmed to have chronic hepatitis by histopathological examination. Patients with any other cause of liver disease including coinfection with hepatitis-B virus or HIV, alcoholic hepatitis, fatty liver, autoimmune hepatitis, or previous organ transplantation were excluded from this study.

Study design

This multicenter prospective controlled study compared the efficacy and safety of 8 weeks versus 24 weeks of pegIFN-α-2a monotherapy in previously untreated patients with chronic hepatitis C who had a virological response at 2 weeks after the start of IFN. Patients with a virological response at 2 weeks were invited to sign a consent form accepting treatment with IFN for 8 weeks only. Those patients who refused consent received a 24-week course of treatment. The primary measure of efficacy was SVR, which was defined as undetectable HCV-RNA in the serum at 24 weeks after the cessation of treatment. All patients agreed to participate in the research protocol, which was approved by the hospital research ethics board, and gave written informed consent. The eligible patients received pegIFN-α-2a (Pegasys, F. Hoffmann-LaRoche, Basel, Switzerland) at 180 μg once per week subcutaneously, either for 8 weeks or 24 weeks, without ribavirin. Other patients who showed no rapid virological response at 2 weeks after the start of pegIFN-α-2a were treated for 24–48 weeks.

All patients were evaluated in an outpatient setting for safety, tolerance, and efficacy every week during the IFN treatment. Blood count was checked just before the IFN injection every week. The qualitative detection of HCV-RNA was performed by a standardized qualitative RT–PCR assay (Amplicor HCV monitor v2.0; Roche diagnostics Co., Tokyo, Japan) at the first 2 weeks and every 4 weeks during and after IFN treatment. The primary efficacy end point for this study was defined as a disappearance of detectable serum HCV-RNA at week 24 after the completion of the IFN treatment.

Journal of Gastroenterology and Hepatology **23** (2008) 541–545

Table 1 Patients' characteristics

	8-week group ($n = 19$)	24-week group ($n = 15$)
Age (years)	51† (21–71)	47† (25–58)
Sex (male/female)	14/5	7/8
Height (cm)	169† (147–178)	161† (139–178)
Weight (kg)	64.6† (40.6–85)	59† (47–92.4)
Body mass index (kg/m²)	23.4† (18.0–27.8)	21.5† (18.6–30.5)
Platelet count (×10⁴/μL)	19.5† (9.6–30.7)	18.1† (8.9–31.7)
Alanine aminotransferase (IU/L)	55† (22–152)	60† (21–184)
γ-Glutamyl transpeptidase (IU/L)	25† (9–155)	47† (14–137)
Creatinine (mg/dL)	0.76† (0.6–0.9)	0.68† (0.38–0.85)
Total cholesterol (mg/dL)	160† (116–219)	154† (125–201)
Fasting blood glucose (mg/dL)	90† (72–104)	96† (84–115)
Diabetes mellitus	0	1
Hyaluronic acid (ng/mL)	24† (13–72)	78† (16–191)
HCV genotype (1b/2a/2b)	2/15/2	2/10/3
HCV-RNA (KIU/mL)	45† (5.1–370)	43† (5.3–400)
Fibrosis (F1/F2/F3/F4)	6/8/5/0	5/6/4/0

†Median. HCV, hepatitis C virus.

Statistical analysis

We compared the response to an 8-week course of pegIFN-α-2a with that to a 24-week course of pegIFN-α-2a. The χ^2-test and Fisher's exact test were used for comparisons of categorical variables between groups, while Student's *t*-test and the Wilcoxon test were used for continuous and ordinal variables as appropriate. *P*-values less than 0.05 were considered to indicate statistical significance. The JMP version 5.1 statistical software package (SAS Institute, Cary, NC, USA) was used for the statistical analysis of data.

Results

Baseline characteristics

Thirty-four patients who became HCV-RNA-negative at week 2 subsequently received either an 8-week course ($n = 19$) or 24-week ($n = 15$) course of 180 μg pegIFN-α-2a. The baseline characteristics of the two groups at the start of the IFN therapy are summarized in Table 1. None of the patients had LC, based on clinical, laboratory, and histopathological findings. Table 2 also shows the data of 21 patients with a non-rapid virological response at 2 weeks after the start of pegIFN-α-2a. The pretreatment viral loads of non-rapid virological responders were significantly higher than those of rapid virological responders ($P < 0.0001$).

Tolerance of IFN therapy and adverse events

Among the 19 patients of the 8-week group, the dose was reduced by 50% (to 90 μg of pegIFN-α-2a) in one patient with SVR at 3 weeks due to a fall in platelet count. However, all other patients were able to complete the full 8-week course without discontinuation. In 15 patients of the 24-week course, the dose was reduced to half (90 μg of pegIFN-α-2a) in six patients due to neutropenia ($n = 2$; one patient at 8 weeks and one patient at 10 weeks), thrombocytopenia ($n = 3$; two patients at 9 weeks and one patient at 10 weeks) and epigastralgia ($n = 1$; at 14 weeks). Furthermore, IFN therapy was withdrawn in another five patients, including two patients at 8 weeks due to thrombocytopenia, two patients at 12 weeks due to generalized fatigue, and one patient at 18 weeks due to various neurological symptoms, such as hand numbness. Thus the proportion of patients who required a dose reduction was lower in the 8-week group than in the 24-week group ($P = 0.028$). Furthermore, the proportion of patients who completed the treatment was significantly higher in the 8-week group than the 24-week group ($P = 0.011$). We concluded that our patients with HCV could tolerate 8 weeks of IFN therapy better than 24 weeks.

Table 2 Characteristics of 21 patients who did not show a rapid virological response

Age (years)	51† (22–76)
Sex (male/female)	11/8
Height (cm)	164.5† (148–175.5)
Weight (kg)	58.5† (42.5–75)
Body mass index (kg/m2)	22.5† (16.9–27.3)
Platelet count (×104/_L)	20.5† (12–28.6)
Alanine aminotransferase (IU/L)	93† (17–157)
γ-Glutamyl transpeptidase (IU/L)	39† (10–145)
Creatinine (mg/dL)	0.58† (0.5–0.96)
Total cholesterol (mg/dL)	158† (111–214)
Fasting blood glucose (mg/dL)	87† (68–119)
Diabetes mellitus	0
Hyaluronic acid (ng/mL)	45.6† (10–100)
HCV genotype (1b/2a/2b)	0/15/6
HCV-RNA (KIU/mL)	660† (40–830)
Fibrosis (F1/F2/F3/F4)	12/6/3/0

†Median. HCV, hepatitis C virus.

Biochemical and virological responses to therapy

With regard to the alanine aminotransferase (ALT) response to IFN therapy, all patients of both groups showed biochemical normalization at the end of treatment and at 6 months after the end of treatment. There was no difference in the sustained ALT response between the 8-week group and 24-week group. With regard to the virological response to IFN therapy, all patients of both groups exhibited a rapid decrease in HCV-RNA, reaching undetectable levels (HCV-RNA ≤ 100 copies/mL) by week 2. All patients had negative HCV-RNA levels at the end of treatment and none showed a null response. There was no significant difference in the rate of fall of the virological load between patients who had a sustained response and those who had a relapse, as discussed later. The proportions of patients who showed a SVR in the 8-week group and 24-week group were not significantly different (89.5% [17/19] and 100% [15/15], respectively [$P = 0.195$]). Two patients of the 8-week group had viral relapse after the end of treatment; one who had HCV genotype 2a with 50 KIU/mL pretreatment viral load relapsed at 12 weeks after the end of the treatment while the other had genotype 2b with 230 KIU/mL pretreatment viral load and relapsed at 8 weeks after the end of the treatment. The non-

rapid virological responders had a lower SVR rate. Eight (38%) patients showed SVR, 11 (52%) patients developed relapse after discontinuation of IFN, and two (10%) patients had no virological response.

Discussion

In Japan, pegIFN-α-2a monotherapy has been covered by public health insurance since December 2003. The standard duration of treatment with pegIFN-α-2a is 24 weeks for patients with low viral loads of genotype HCV-1 and any viral loads of genotype HCV-2 infection. Recent studies have reported that a treatment duration of more than 24 weeks in such cases does not increase the SVR rate.[11,13,21,22] Moreover, patients with early virological response seem to have a high rate of SVR.[23–25] In those patients, to reduce unnecessary exposure to treatment and its potential side-effects and to reduce costs, short-term IFN therapy has been used by several groups.[17–21] However, details of the IFN regimen differ from those of others and there are no studies that use short-term of pegIFN-α-2a treatment. We therefore conducted a prospective pilot study on the efficacies of an 8-week and 24-week pegIFN-α-2a regimen for patients with low viral titers of genotype HCV-1 and any viral titers of genotype HCV-2 who exhibited a virological response at 2 weeks after the initiation of IFN. In our study, patients with a relatively low viral load before the start of the IFN therapy tended to have a very early virological response.

Our results demonstrated that the virological response to the 8-week treatment (89.5% [17/19]) was excellent and was similar to the 24-week course (100% [15/15]). This high SVR rate of 8-week pegIFN-α-2a monotherapy seems as high as that reported in another short course study of 14-week pegIFN plus ribavirin combination treatment for patients with HCV genotype HCV-2 or HCV-3.[26] This high SVR rate of the 8-week course of pegIFN-α-2a may be associated with a rapid viral disappearance. Several studies have indicated that negative HCV-RNA at week 2 after the commencement of IFN is a predictor of SVR.[19,20,27,28] Therefore, for patients with a low HCV-1 viral load or those with HCV-2 infection with any viral load, we recommend switching to an 8-week course of pegIFN-α-2a monotherapy once they show an ultra rapid virological response, that is, negative HCV-RNA at week 2 from the start of IFN therapy. Furthermore, a longer course of IFN therapy with or without ribavirin can be prescribed when HCV-RNA becomes positive after discontinuation of the 8-week course of IFN therapy.

Although in our study all of the patients in the 24-week course showed SVR, it seems that 24 weeks is a long treatment period for those patients who become negative for HCV-RNA by week 2 of treatment to ascertain SVR. Our results showed that all patients in the 8-week course completed the course to the end of treatment. However, 33% of the patients of the 24-week course did not continue their treatment to the end of the course. Because patients tend to adhere to shorter regimens, which are also better tolerated than longer treatment regimens, a shorter exposure will probably translate into a better benefit–risk ratio in patients with early virological response.

Our study identified two relapsers among patients of the 8-week course after discontinuation of pegIFN-α-2a therapy. These two patients had a negative history of exposure to new HCV infection. One patient who had genotype HCV-2b and a high pretreatment viral load (HCV-RNA: 230KIU/mL) relapsed at 8 weeks after the discontinuation of IFN therapy, while the other who had genotype HCV-2a and a low pretreatment viral load (HCV-RNA: 50KIU/mL) relapsed at 12 weeks after the discontinuation of IFN therapy. These two patients could have SVR after additional IFN therapy for 24 weeks (one patient; pegIFN-α-2a monotherapy, one patient; pegIFN-α-2b and ribavirin combination therapy). We could not identify a definite factor associated with SVR or relapse. Although pretreatment factors, like genotype, viral load, and grade of fibrosis can be used to predict the mean treatment outcome for study cohorts, they are often of limited value in individual patients.[29,30]

As mentioned earlier, a short course of pegIFN-α-2a therapy for 8 weeks could be recommended in those patients who show an ultra rapid virological response at week 2 after the initiation of IFN therapy. Although the study by Shiffman *et al.*[31] demonstrates the inferiority of a shorter regimen in a large-scale, randomized, controlled study, the characteristics of their patients were largely different from those of our study, including racial difference (mostly Caucasian patients versus Japanese, a heavier body weight for the Caucasians versus Japanese patients) and differences in pretreatment viral load (variable and higher HCV-RNA level versus relatively low viral load in our patients). Patients' selection was also different between the two studies; our study was carried out only in cases negative for HCV-RNA at 2 weeks after the start of IFN compared to their randomized study, irrespective of a rapid virological response.

In conclusion, patients chronically infected with low titers of HCV-1 and those with HCV-2, regardless of their viral loads, who achieve an ultra rapid virological response, that is, HCV-RNA negativity at week 2, can receive only 8 weeks of pegIFN-α-2a monotherapy without compromising the chance of SVR. The results of our prospective study are encouraging, although the study population was small and was based on non-randomized methodology. The data of the present study are not conclusive for patients with very high pretreatment viremia who might achieve a rapid virological response or for those patients who do not achieve a rapid virological response. Further clinical trials are required to optimize the treatment duration in these patients.

References

1 Alter MJ. Epidemiology of hepatitis C in the West. *Semin. Liver Dis.* 1995; **15**: 5–14.
2 Poynard T, Bedossa P, Opolon P, The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC Groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; **349**: 825–32.
3 Kiyosawa K, Sodeyama T, Tanaka E et el. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671–5.
4 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N. Engl. J. Med.* 1993; **28**: 1797–801.
5 Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C. A prospective study of 251 patients. *Hepatology* 1995; **21**: 650–5.
6 Shiratori Y, Shiina S, Imamura M *et al.* Characteristic difference of hepatocellular carcinoma between hepatitis-B and C-viral infection in Japan. *Hepatology* 1995; **22**: 1027–33.

7 Tomimatsu M, Ishiguro N, Taniai M *et al.* Hepatitis C virus antibody in patients with primary liver cancer (hepatocellular carcinoma, cholangiocarcinoma, and combined hepatocellular-cholangiocarcinoma) in Japan. *Cancer* 1993; **72**: 683–8.
8 Poynard T, Leroy V, Cohard M *et al.* Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. *Hepatology* 1996; **24**: 778–89.
9 Carithers RL Jr, Emerson SS. Therapy of hepatitis C: meta-analysis of interferon alfa-2b trials. *Hepatology* 1997; **26**: S83–8.
10 Orito E, Mizokami M, Nakano T *et al.* Serum hepatitis C virus RNA level as a predictor of subsequent response to interferon-alpha therapy in Japanese patients with chronic hepatitis C. *J. Med. Virol.* 1994; **44**: 410–14.
11 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; **358**: 958–65.
12 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* 2002; **347**: 975–82.
13 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med.* 2004; **140**: 346–55.
14 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061–9.
15 Zeuzem S, Feinman SV, Rasenack J *et al.* Peginterferon alfa-2a in patients with chronic hepatitis C. *N. Engl. J. Med.* 2000; **343**: 1666–72.
16 Lai MY, Kao JH, Yang PM *et al.* Long-term efficacy of ribavirin plus interferon alfa in the treatment of chronic hepatitis C. *Gastroenterology* 1996; **111**: 1307–12.
17 Wagner MV, Huber M, Berg T *et al.* Peginterferon-α-2a (40KD) and Ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; **129**: 522–7.
18 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.
19 Nomura H, Tsuchiya Y, Maruyama T, Miki K, Yokota T, Okubo H. Interferon therapy and hepatitis C virus: the effects of a high dose, short course of interferon on hepatitis C. *J. Gastroenterol. Hepatol.* 1999; **14**: 85–9.
20 Sato Y, Tokuue H, Kawamura N *et al.* Short-term interferon therapy for chronic hepatitis C patients with low viral load. *Hepatogastoenterology* 2004; **51**: 968–72.
21 Tabaru A, Narita R, Hiura M, Abe S, Otsuki M. Efficacy of short-term interferon therapy for patients infected with hepatitis C virus genotype 2a. *Am. J. Gastroenterol.* 2005; **100**: 862–7.
22 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients with HCV genotype 2 or 3. *J. Hepatol.* 2004; **40**: 993–9.
23 Bjøro K, Bell H, Hellum KB *et al.* Effect of combined interferon-alpha induction therapy and ribavirin on chronic hepatitis C virus infection: a randomized multicentre study. *Scand. J. Gastroenterol.* 2002; **37**: 226–32.
24 Bekkering FC, Stalgis C, McHutchison JG, Brouwer JT, Perelson AS. Estimation of early hepatitis C viral clearance in patients receiving daily interferon and ribavirin therapy using a mathematical model. *Hepatology* 2001; **33**: 419–23.
25 Zeuzem S. Clinical implications of hepatitis C viral kinetics. *J. Hepatol.* 1999; **31** (Suppl. 1): 61–4.
26 Dalgard O, Bjøro K, Hellum KB *et al.* Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* 2004; **40**: 1260–5.
27 Hino K, Okuda M, Konishi T, Ishiko H, Okita K. Serial assay of hepatitis C virus RNA in serum for predicting response to interferon-alpha therapy. *Dig. Dis. Sci.* 1995; **40**: 14–20.
28 Kakumu S, Aiyama T, Okumura A, Iwata K, Ishikawa T, Yoshioka K. Earlier loss of hepatitis C virus RNA in interferon therapy can predict a long-term response in chronic hepatitis C. *J. Gastroenterol. Hepatol.* 1997; **12**: 468–72.
29 Davis GL, Lau JY. Factors predictive of a beneficial response to therapy of hepatitis C. *Hepatology* 1997; **26**: 122S–127S.
30 Tong MJ, Blatt LM, McHutchison JG, Co RL, Conrad A. Prediction of response during interferon alfa 2b therapy in chronic hepatitis C patients using viral and biochemical characteristics: a comparison. *Hepatology* 1997; **26**: 1640–5.
31 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.

J Gastroenterol 2008; 43:550–557
DOI 10.1007/s00535-008-2194-5

Journal of Gastroenterology

Clinicopathological features of elderly patients with hepatitis C virus-related hepatocellular carcinoma

Daiki Miki[1], Hiroshi Aikata[1], Kiminori Uka[1], Hiromi Saneto[1], Tomokazu Kawaoka[1], Takahiro Azakami[1], Shintaro Takaki[1], Soo Cheol Jeong[1], Michio Imamura[1], Yoshiiku Kawakami[1], Shoichi Takahashi[1], Toshiyuki Itamoto[2], Toshimasa Asahara[2], Koji Arihiro[3], and Kazuaki Chayama[1]

Departments of [1]Medicine and Molecular Science and [2]Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
[3]Department of Anatomical Pathology, Hiroshima University Hospital, Hiroshima, Japan

Background. It is well known that the incidence of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV) correlates with progression of liver fibrosis. However, there is little information on the impact of aging on hepatocarcinogenesis. The aim of this study was to elucidate the clinicopathological features of elderly patients with HCV-related HCC. ***Methods.*** The study subjects were 693 consecutive patients newly diagnosed with HCC with anti-HCV. First, we divided them into a younger group (<70 years) and an elderly group (≥70 years) and compared clinicopathological features between the two groups. Next, we selected pure HCV-related HCC patients by excluding the patients with other probable factors for hepatocarcinogenesis (anti-HBc, interferon therapy, and alcohol) and compared the two groups again. ***Results.*** Higher platelet count, lower male/female ratio, lower rate of habitual alcohol consumption, and better Child-Pugh class were recognized in the elderly group thant the younger group, statistically. In 133 cases of hepatic resection, fibrosis stage was lower in the elderly than the younger group. After selection of pure HCV-related HCC patients, in a stepwise multivariate analysis, male sex and platelet count $<10 \times 10^4/mm^3$ were significant variables associated with age <70. Regarding the latency period to HCC development, the patients who received a blood transfusion at an older age developed HCC sooner despite their lower grade of fibrosis. ***Conclusions.*** The elderly patients developed HCC more often, despite their lower grade of fibrosis, compared with the younger patients. In addition to fibrosis, aging could be a factor affecting HCV-related hepatocarcinogenesis.

Key words: hepatocellular carcinoma, hepatitis C virus, elderly patients, aging, fibrosis

Received: September 23, 2007 / Accepted: March 20, 2008
Reprint requests to: H. Aikata

Introduction

Since the discovery of the hepatitis C virus (HCV) RNA in 1989,[1,2] there has been no doubt that HCV is associated with hepatocarcinogenesis based on the high prevalence of HCV infection among hepatocellular carcinoma (HCC) patients who are otherwise negative for hepatitis B virus (HBV) markers.[3–14] In Japan, HCV is considered one of the main causes of HCC.[15,16] Several studies have examined patients with HCV and identified age, the extent of liver fibrosis, male sex, alcohol consumption, positivity for hepatitis B surface antigen (HBsAg), and high alpha-fetoprotein (AFP) level as risk factors for hepatocarcinogenesis.[15–20] The annual incidence of HCC correlates with the severity of liver fibrosis, from 0.5% among patients with stage F0 or F1 fibrosis to 7.9% among patients with stage F4 fibrosis.[21] In patients with chronic hepatitis C, age at infection, alcohol consumption, and male sex are independent factors associated with increased rate of progression of liver fibrosis.[22] Progression of fibrosis is dependent mainly on age and duration of infection. Moreover, the highest rate of progression of liver fibrosis occurs in patients aged ≥50 years.[23] In Japan, we and other groups have reported a recent increase in the number of elderly HCC patients with HCV,[24–26] but the impact of aging on the incidence of HCC has not been investigated carefully.

Thus, we started analyzing HCV-related HCC patients who were positive for anti-HCV but negative for HBsAg to determine the correlation between aging and liver fibrosis in relation to the incidence of HCC with HCV. To date, several factors for affecting hepatocarcinogenesis have been reported, such as antibody to hepatitis B core antigen (anti-HBc), previous interferon therapy, and alcohol consumption. It was reported that previous HBV infection increased the risk for development of HCC in HCV-related liver cirrhosis.[3,27] Also, alcohol consumption also considered a sig-

nificant risk factor for development of HCC with HCV.[15,16,18] Conversely, interferon therapy reduces the risk for development of HCC.[19,21] Therefore, we also analyzed patients without these factors to study them as the nearest condition to the natural history of HCV-related HCC patients and considered them as pure HCV-related HCC patients. In this study, we elucidated the clinicopathological features of elderly patients with HCV-related HCC and examined the impact of aging on hepatocarcinogenesis.

Methods

Patients

A total of 1038 consecutive patients newly diagnosed with HCC were consulted or admitted to Hiroshima University Hospital from January 1988 to December 2005. Of these patients, 693 patients (474 men, 41–92 years of age; 219 women, 41–88 years of age), positive for anti-HCV but negative for HBsAg, were enrolled in the present study. The 693 patients were divided into two groups according to age at initial diagnosis: 440 patients (325 men, 115 women) were <70 years old (younger group), and 253 patients (149 men, 104 women) were ≥70 years old (elderly group). We recorded epidemiological data (age, sex, history of blood transfusion, history of habitual alcohol consumption) and chemical data (serum bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, and prothrombin time) for each patient. A detailed epidemiological questionnaire was administered during face-to-face interviews. Then, we compared the clinical and pathological features of these groups. All patients submitted informed consent for collection and subsequent use of data for research purposes, and the study was carried out in accordance with the Helsinki Declaration.

Selection of pure HCV-related HCC

As already mentioned, we first studied anti-HCV-positive and HBsAg-negative patients with HCC. However, this study included HBsAg-negative and anti-HBc-positive patients. It is recognized that previous HBV infection may be a cofactor for the development of HCC in HCV-related HCC patients.[27] Therefore, we excluded 167 patients (116 males, 51 females) who were positive for anti-HBc, the remainder being 526 patients (358 males, 168 females). Next, we excluded 57 patients (40 males, 17 females) who were negative for HCV-RNA. These patients probably became virus negative as a result of interferon therapy or spontaneously. The remainder included 469 patients (318 males, 151 females). There are some data that interferon reduces HCC development even in patients who do not achieve sustained eradication of HCV[19,21,28]; therefore, we also excluded 84 patients (67 males, 17 females) with history of interferon therapy. The remainder comprised 385 patients (251 males, 134 females). Habitual alcohol consumption is recognized as a risk factor of HCC development,[15,16,18] and in our study the proportion of patients with habitual alcohol consumption was greater in the younger group than in the elderly group. Thus, we excluded 48 patients (46 males, 2 females) with excessive alcohol consumption; the remainder included 337 patients (205 males, 132 females). We decided that 337 patients were appropriate to be the next subjects of our study as pure HCV-related HCC patients. We wanted to elucidate the relationship between the impact of aging and HCV-induced fibrosis for HCC development as the nearest condition to the natural history, so we removed those patients with other probable factors affecting hepatocarcinogenesis.

HCC diagnosis

The diagnosis of HCC was based on hypervascularity, confirmed by dynamic computed tomography (CT), magnetic resonance imaging, angiography, or CT angiography, when the serum levels of HCC-related tumor markers, such as alpha-fetoprotein or protein induced in the absence of vitamin K or antagonist II (PIVKA-II), were increased or a mass lesion was detected by ultrasonography. When a nodule was not proven to be hypervascular, percutaneous biopsy under ultrasonography was performed for confirmation of the diagnosis of HCC. Staging adopted in this study was the revised version of the Liver Cancer Study Group of Japan in 2000.[29]

Blood biochemical tests and viral markers

Routine serum biochemical tests were carried out with automated techniques. Anti-HCV in sera was assayed using the Anti-HCV-EIA Cobas Core Test (Hoffmann La Roche, Basel, Switzerland). All serum samples collected were analyzed with second- or third generation anti-HCV tests. If the index was ≥1.0, anti-HCV was considered positive. HBsAg and anti-HBc in sera were assayed using the HBsAg-EIA Cobas Core Test (Hoffmann La Roche) and the Anti-HBc-EIA Cobas Core Test (Hoffmann La Roche), respectively. HCV-RNA in sera was detected by qualitative reverse transcription-polymerase chain reaction (RT-PCR) (Hoffmann La Roche). For cases before March 1990, serum samples frozen at –20°C were retrieved from storage and tested for anti-HCV using the aforementioned method.

Alcohol consumption

The average quantity of alcohol consumed per day was evaluated regardless of type of alcohol beverage. Habitual alcohol consumption was defined as regular consumption of at least one alcohol drink per week over 10 years. In particular, excessive habitual drinkers were defined as persons consuming more than 86 g ethanol per day.

Platelet count

We evaluated the platelet count, which is known to correlate significantly with the stage of liver fibrosis in patients with chronic hepatitis C; also, platelet count $<10 \times 10^4/mm^3$ has been used as a marker for liver cirrhosis.[30-35]

Stage of liver fibrosis and activity grade of chronic hepatitis

Of 693 patients, 133 patients underwent hepatic resection within 6 months from first diagnosis. The resected specimens were fixed in 10% formalin neutral buffered solution, embedded in paraffin, and processed for staining with hematoxylin and eosin. Staging of fibrosis and grading of liver disease activity was assessed by a pathologist on a blinded basis according to the criteria of Desmet et al.[36]

Statistical analysis

Values are expressed as median and 75th and 25th percentiles. Statistical analysis was performed using the Mann–Whitney *U* test and Spearman's nonparametric test. Univariate and multivariate logistic regression using the Statistical Package for Social Science (SPSS) version 6.1 for Windows (SPSS Japan, Tokyo, Japan) was performed to evaluate the values of the patients' clinical and laboratory features associated with age at initial diagnosis of HCC. *P* values less than 0.05 were considered to indicate statistical significance.

Results

Characteristics of patients with HCV-related HCC

Table 1 summarizes the characteristics of HCC patients with HCV, according to age at initial diagnosis. Male/female ratio was significantly higher in the younger group than the elderly group ($P < 0.001$). The median age of male patients (66 years old) was lower than that of female patients (69 years old) ($P < 0.001$). The median of platelet count was 8.6 (25^{th}, 6.1; 75^{th}, 13.3) and 10.0 (25^{th}, 7.3; 25^{th}, 13.4) $\times 10^4/mm^3$ in the younger and the elderly groups, respectively. Platelet count of the elderly group was significantly higher than that of the younger group ($P = 0.005$). The proportion of patients whose platelet count was $\geq 10 \times 10^4/mm^3$, considered as having a noncirrhotic liver, was greater in the elderly group than the younger group ($P = 0.003$). When we compared the platelet count of the younger group and that of the elderly group according to sex, the median of platelet count was 9.2 (25^{th}, 6.2; 75^{th}, 14.2) and 10.5 (25^{th}, 7.8; 25^{th}, 13.7) $\times 10^4/mm^3$ in the younger and the elderly male groups, respectively. The median of platelet count was 7.9 (25^{th}, 5.9; 75^{th}, 10.6) and 8.8 (25^{th}, 7.0; 25^{th}, 12.4) $\times 10^4/mm^3$ in the younger and the elderly female groups, respectively. Platelet count of the elderly group was significantly higher than that of the younger group for both sexes (males, $P = 0.023$; females, $P = 0.017$). The

Table 1. Characteristics of all patients with hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC), according to age at diagnosis

Age at diagnosis	Younger group (<70 years)	Elderly group (≥70 years)	P^a
Sex (male/female)	325/115	149/104	<0.001
Platelet count ($\times 10^4/mm^3$)[b]	8.6	10.0	0.005
(<10/≥10) $\times 10^4/mm^3$	266/174	124/129	0.003
Child-Pugh classification (A or non-cirrhosis/B/C)	271/149/20	184/69/0	0.001
Stage (I/II/III/IV)	88/170/116/66	49/109/73/22	NS
Alpha-fetoprotein (ng/ml)[b]	44.6	46.8	NS
History of blood transfusion (+/–)	100/340	55/198	NS
History of habitual alcohol consumption (Excessive/habitual/none)	64/193/183	23/95/135	0.001
Anti-HBc (+/–)	100/340	67/186	NS
Diabetes mellitus (with/without)	96/344	60/193	NS

NS, not significant
[a] Comparison between patients less than 70 years of age and those older than 70 years
[b] Values are median

Table 2. Significant variables in association with development of HCV-related HCC under 70 years old using univariate logistic analysis

Variables	Odds ratio	95% confidence interval	*P* value
Habitual alcohol consumption*	2.363	1.351–4.133	0.003
Male*	1.973	1.420–2.740	<0.001
Child-Pugh class B or C*	1.663	1.187–2.329	0.003
Platelet count $<10 \times 10^4/mm^3$*	1.575	1.153–2.152	0.004

*Significant variables in multivariate analysis

Child-Pugh class was A or non-cirrhosis, B and C in 62%, 34%, and 4% of the younger group, and 73%, 27%, and 0% of the elderly group, respectively. Child Pugh class were significantly better in the elderly group than the younger group ($P = 0.001$). No significant difference was observed in stage grouping of HCC between the two groups. The median AFP level was 44.6 and 46.8 ng/ml for the younger and the elderly group, respectively. No significant difference was observed in serum AFP level between the two groups. History of blood transfusion was reported by 23% and 22% of the younger and the elderly group, respectively; it was not a significant difference. With regard to alcohol consumption, 14%, 44%, and 42% of the younger group and 9%, 38%, and 53% of the elderly group had history of excessive, habitual, and no drinking, respectively. Significant differences were observed in history of habitual alcohol consumption between the younger and the elderly group ($P = 0.001$). No significant difference was observed in positive rate of anti-HBc and the rate of diabetes mellitus.

Variables associated with age <70 years at diagnosis of HCC

Sex, platelet count, Child-Pugh class, history of blood transfusion, history of habitual alcohol consumption, anti-HBc and diabetes mellitus were selected as independent variables in logistic regression analyses, with the age <70 years at diagnosis as dependent variables. In a univariate analysis, habitual alcohol consumption, male sex, Child-Pugh class B or C, and platelet count $<10 \times 10^4/mm^3$ were significantly correlated with age <70 years at diagnosis (Table 2). In a stepwise multivariate analysis, habitual alcohol consumption (odds ratio, 1.968, 95% confidence interval, 1.107–3.500; $P = 0.021$), male sex (odds ratio, 1.933, 95% confidence interval, 1.373–2.722; $P < 0.001$), platelet count $<10 \times 10^4/mm^3$ (odds ratio, 1.587, 95% confidence interval, 1.144–2.200; $P = 0.006$) and Child-Pugh class B or C (odds ratio, 1.532, 95% confidence interval, 1.080–2.174, $P = 0.017$) were significant variables associated with age <70 years at diagnosis in HCV-related HCC patients.

Table 3. Fibrosis stage and activity grade of liver histology

	<70 years	≥70 years	*P*[a]
Male/female	67/23	29/14	
Fibrosis stage			0.009
F0–1	4 (4%)	2 (5%)	
F2	13 (14%)	14 (38%)	
F3	26 (27%)	8 (22%)	
F4	53 (55%)	13 (35%)	
Activity grade			0.047
A0–1	6 (6%)	5 (13%)	
A2	79 (82%)	31 (84%)	
A3	11 (12%)	1 (3%)	

Data are numbers and percentages (in parentheses) of patients
[a]Comparison between patients less than 70 years of age and those older than 70 years

Fibrosis stage and activity grade

Of the 693 patients, 133 underwent hepatic resection. Their fibrosis staging and activity grading of the liver were evaluated (Table 3). Fibrosis stage F0–1, F2, F3, and F4 were identified in 4%, 14%, 27%, and 55% of the younger group, and in 5%, 38%, 22%, and 35% of the elderly group, respectively. The fibrosis stage of liver histology was significantly lower in the elderly group ($P = 0.009$). The activity grades A0–1, A2, and A3 were recognized in 6%, 82%, and 12% of the younger group, and 13%, 84%, and 3% of the elderly group, respectively. The activity grade of liver histology was significantly lower in the elderly group ($P = 0.047$). Among 133 patients who underwent hepatic resection, both fibrosis stage and activity grade were significantly lower in the elderly patients.

Characteristics of pure HCV-related HCC patients

Table 4 summarizes the characteristics of pure HCV-related HCC patients. Male/female ratio was significantly higher in the younger group than the elderly group ($P = 0.004$). The median age of male patients (67 years) was lower than that of female patients (69 years) ($P = 0.001$). The median platelet count was 8.7 (25^{th}, 6.1; 75^{th}, 12.4) and 9.9 (25^{th}, 7.0; 75^{th}, 13.1) $\times 10^4/mm^3$ in the younger and the elderly group, respectively. The pro-

Table 4. Characteristics of patients with pure HCV-related HCC, according to age at diagnosis

Age at diagnosis	Younger group (<70 years)	Elderly group (≥70 years)	P^a
Sex (male/female)	138/68	67/64	0.004
Platelet count ($\times 10^4/mm^3$)[b]	8.7	9.9	NS
($<10/\geq 10$) $\times 10^4/mm^3$	134/72	69/62	0.024
Child-Pugh classification (A or non-cirrhosis/B/C)	123/72/11	83/48/0	NS
Stage (I/II/III/IV)	48/69/55/34	23/63/33/12	NS
Alpha-fetoprotein (ng/ml)[b]	45.2	60.2	NS
Genotype (1b/2a or 2b or 3a)[c]	127/18	83/24	0.035
History of blood transfusion (+/−)	47/159	32/99	NS
Diabetes mellitus (with/without)	43/163	35/96	NS

NS, not significant
[a] Comparison between patients less than 70 years of age and those older than 70 years
[b] Values are median
[c] Of the patients, 75 were not tested (51 were <70 years, 24 were ≥70 years)

Table 5. Significant variables in association with development of pure HCV-related HCC under 70 years old using univariate logistic analysis

Variables	Odds ratio	95% confidence interval	*P* value
Male[a]	1.924	1.195–3.098	0.007
Platelet count $<10 \times 10^4/mm^3$[a]	1.672	1.070–2.614	0.024
Genotype 1	2.040	1.043–3.990	0.037

[a] Significant variables in multivariate analysis

portion of patients whose platelet count was $\geq 10 \times 10^4/mm^3$ was greater in the elderly group than the younger group ($P = 0.024$). Child-Pugh class also seemed to indicate better liver function in the elderly group than that of the younger group, but it was not statistically significant. Stage grouping of HCC, serum AFP level, history of blood transfusion, and rate of diabetes mellitus showed no significant difference between the younger and the elderly group. The proportion of patients with HCV genotype 1 was greater in the younger group than the elderly group ($P = 0.035$). Next, we selected sex, platelet count, Child-Pugh class, genotype, history of blood transfusion, and diabetes mellitus as independent variables in logistic regression analyses, with the age <70 years at diagnosis as dependent variable. In a univariate analysis, male sex, platelet count $<10 \times 10^4/mm^3$, and genotype 1 were significantly correlated with age <70 years at diagnosis (Table 5). In a stepwise multivariate analysis, male (odds ratio, 2.035, 95% confidence interval, 1.208–3.427; $P = 0.008$) and platelet count $<10 \times 10^4/mm^3$ (odds ratio, 2.105, 95% confidence interval, 1.242–3.570; $P = 0.006$) were significant variables associated with age <70 years at diagnosis in pure HCV-related HCC patients.

Latency period to development of HCC

Of the 337 patients, 79 patients received blood transfusion (BT) and none of these transfusions was provided after the diagnosis of chronic liver disease. These patients were presumed infected with HCV by BT, and thus we considered the interval between BT and diagnosis of HCC as the latency period to development of HCC. Figure 1 shows that the duration to development of HCC was shorter in patients received BT at an older age than those transfused at a younger age. Interestingly, platelet counts of many patients who received BT at an older age were $\geq 10 \times 10^4/mm^3$, which meant that many patients who received BT at an older age develop HCC more rapidly when they had a noncirrhotic liver, in other words, elderly patients might tend to develop HCC despite their liver fibrosis stage and activity grade. Then, we selected sex, platelet count, Child-Pugh class, genotype, diabetes mellitus, and age at BT as independent variables in logistic regression analyses, with the duration to development of HCC <25 years as dependent variable. In a univariate analysis, age when received BT ≥35 years, platelet count $\geq 10 \times 10^4/mm^3$, and Child-Pugh class A were significantly correlated with duration to development of HCC <25 years (Table 6). In a stepwise multivariate analysis, age when received BT ≥35 years (odds ratio, 43.666, 95% confidence interval, 4.950–385.231; $P = 0.001$) and platelet count $\geq 10 \times 10^4/mm^3$ (odds ratio, 6.962, 95% confidence interval, 1.156–41.931; $P = 0.034$) were significant variables associated with the duration to development of HCC <25 years.