11. Caporaso N, Romano M, Marmo R, de Sio I, Morisco F, Minerva A, et al. Hepatitis C virus infection is an additive risk factor for development of hepatocellular carcinoma in patients with cirrhosis. J Hepatol 1991;12:367–71.
12. Nishioka K, Watanabe J, Furuta S, Tanaka E, Iino S, Suzuki H, et al. A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. Cancer (Phila) 1991;67:429–33.
13. Liang TJ, Jeffers LJ, Reddy KR, De Medina M, Parker IT, Cheinquer H, et al. Viral pathogenesis of hepatocellular carcinoma in the United States. Hepatology 1993;18:1326–33.
14. Okuda K. Hepatitis C virus and hepatocellular carcinoma. In: Okuda K, Tabor E, editors. Liver cancer. New York: Churchill Livingstone; 1997. p. 39–50.
15. Ikeda K, Saitoh S, Koide I, Arase Y, Tsubota A, Chayama K, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis. Hepatology 1993;18:47–53.
16. Chiba T, Matsuzaki Y, Abei M, Shoda J, Aikawa T, Tanaka N, et al. Multivariate analysis of risk factors for hepatocellular carcinoma in patients with hepatitis C virus-related liver cirrhosis. J Gastroenterol 1996;31:552–8.
17. Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, Guettier C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut 2000;47:131–6.
18. 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. Cancer (Phila) 2000;89:53–9.
19. Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, et al. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. Ann Intern Med 2005;142:105–14.
20. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–801.
21. Yohida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma. Ann Intern Med 1999;131:174–81.
22. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups, Poynard T, Bedossa P, Oplon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–32.
23. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:730–9.
24. Ohishi W, Kitamoto M, Aikata H, Kamada K, Kawakami Y, Ishihara 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;8:894–900.
25. Liver cancer study group of Japan. Survey and follow-up study of primary liver cancer in Japan: Report 14. Acta Hepatol Jpn 2000;40:799–811.
26. Hamada H, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, et al. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. Cancer (Phila) 2002;95:331–9.
27. Tanaka N, Chiba T, Matsuzaki Y, Osuga T, Aikawa T, Mitamura K. High prevalence of hepatitis B and C viral markers in Japanese patients with hepatocellular carcinoma. Gastroenterol Jpn 1993;28:547–53.
28. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 1998;27:1394–402.
29. The Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. Version 4. Tokyo: Kanehara; 2000.
30. Ono E, Shiratori Y, Okudaira T, Imamura M, Teratani T, Kanai F, et al. Platelet count reflects stage of chronic hepatitis C. Hepatol Res 1999;15:192–200.
31. Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. J Viral Hepat 1997;4:199–208.
32. Forns X, Ampurdanes S, Lloert JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986–92.
33. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–26.
34. 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.
35. Kaul V, Friedenberg FK, Braitman LE, Anis U, Zaeri N, Fazili J, et al. Development and validation of a model to diagnose cirrhosis in patients with hepatitis C. Am J Gastroenterol 2002;97:2623–8.
36. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 1994;19:1513–20.
37. Silini E, Botteli R, Asti M, Bruno S, Candusso ME, Brambilla S, et al. Hepatitis C virus genotype and risk of hepatocellular carcinoma in cirrhosis. Gastroenterology 1996;111:199–205.
38. Haydon GH, Jarvis LM, Simmonds P, Harrison DJ, Garden OJ, Hayes PC. Association between chronic hepatitis C infection and hepatocellular carcinoma in a Scottish population. Gut 1997;40:128–32.
39. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. Cancer (Phila) 1999;85:2132–7.
40. Benson D, Mitchell N, Dix D. On the role of aging in carcinogenesis. Mutat Res 1996;356:209–16.
41. Aikata H, Takaishi H, Kawakami Y, Takahashi S, Kitamoto M, Nakanishi T, et al. Telomere reduction in human liver tissues with age and chronic inflammation. Exp Cell Res 2000;256:578–82.
42. Isokawa O, Suda T, Aoyagi Y, Kawai H, Yokota T, Takahashi T, et al. Reduction of telomeric repeats as a possible predictor for development of hepatocellular carcinoma. Hepatology 1999;30:408–12.

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HEPATOLOGY

Etiology and outcome of acute liver failure: Retrospective analysis of 50 patients treated at a single center

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Key words
acute liver failure, artificial liver support system, continuous hemodiafiltration, liver transplantation, plasma exchange, prognosis, survival rate.

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Abstract

Background and Aim: Acute liver failure (ALF) remains a devastating disease carrying considerable mortality. Since deceased donor liver transplantation is rarely performed in Japan, the artificial liver support system (ALS) and living donor liver transplantation (LDLT) are the main modalities used for treatment of ALF. The aim of this study was to analyze the outcome of ALF patients and to evaluate therapies for ALF according to etiology.

Methods: Fifty consecutive patients with ALF were treated between January 1990 and December 2006. Prior to 1997, patients received ALS only. After 1997, ALS and/or LDLT were applied. LDLT was performed in 10 patients.

Results: Four of 15 (27%) pre-1997 ALF patients survived, and 16 of 35 (46%) post-1997 ALF patients survived, including eight who underwent LDLT. The causes of ALF were acute hepatitis B virus (HBV) infection in 18%, severe acute exacerbation (SAE) of chronic HBV infection in 18%, autoimmune hepatitis (AIH) in 8%, and cryptogenic hepatitis in 44%. In total, 67% of the patients with ALF caused by acute HBV infection and AIH were cured without LDLT; only 11% of patients with ALF caused by SAE of HBV and 24% of cryptogenic hepatitis were successfully treated without LDLT. Notably, 80% of patients with cryptogenic hepatitis who underwent LDLT survived.

Conclusion: Since 1997, the survival rate of ALF patients has increased, mainly due to the introduction of LDLT. Liver transplantation should be performed especially in patients with ALF caused by SAE of HBV and cryptogenic hepatitis.

Introduction

Acute liver failure (ALF) is a syndrome characterized by severe liver dysfunction and hepatic encephalopathy. Various conditions can precipitate ALF, such as viral infections, drug reactions, and autoimmune hepatitis (AIH). Although advances in intensive care, medical management, and artificial liver support systems (ALS) have improved the outcome, the prognosis of ALF remains poor, with a reported survival rate of only 10–30%[1,2] in those patients who do not undergo liver transplantation (LT). However, it has been reported that the long-term survival rate of ALF patients who undergo LT increases to 70–90%.[3] Therefore, in many countries, LT is accepted as the only effective therapy for ALF.[4,5]

In Japan, deceased donor liver transplantation (DDLT) has not been performed generally for emotional, social, religious, and historical reasons. The first law for orthotropic transplantation was approved in Japan in 1998. However, only 2–4 DDLT per year have been performed. Therefore, living donor liver transplantation (LDLT) is the primary type of liver transplantation and has been performed in Japan both for children[6,7] and adults.[8–10] However, some ALF patients who need LDLT do not undergo it because of the lack of suitable donors. In such circumstances, intensive care primarily based on ALS has been developed and is still the first choice and main treatment for ALF in Japan. Plasma exchange (PE) and continuous hemodiafiltration (CHDF) therapy is the mainstream of ALS, with the aim of removing toxins and supplying blood components, such as clotting factors. CHDF is used with PE to remove mid-molecular-weight substances and correct hypernatremia, metabolic alkalosis, and reduced colloid osmotic pressure.[11] Based on the above circumstances, ALS is highly developed in Japan compared to other countries.[12,13] However, the effectiveness of ALS for ALF patients is still controversial.

The present study was designed to further evaluate the ALS and LDLT for patients with ALF. For this purpose, we retrospectively analyzed the prognosis of 50 consecutive patients with ALF who were treated at a single center, according to the type of therapy and etiology of ALF.

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Methods

Patients

Between January 1990 and December 2006, 50 consecutive patients with ALF were treated at our hospital. The diagnosis of ALF was based on the presence of grade II or worse hepatic encephalopathy with plasma prothrombin activity of ≤40%. Based on the time elapsed from the onset of illness to the development of grade II or worse encephalopathy, ALF was classified into hyperacute type (within 7 days), acute type (8–28 days), and subacute type (4–12 weeks).

The following parameters were recorded: age, sex, type of ALF, date from onset to admission, etiology, with or without LDLT, survival or death. Serum samples from almost all ALF patients were analyzed for markers of hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), and Epstein–Barr virus (EBV). HEV was not routinely tested unless there was a history of positive exposure.

The patients included 33 men and 17 women with a mean age 46.3 ± 15.9 years (±SD, Table 1). The type of ALF was hyperacute in 14, acute in 21, and subacute in 15 patients. For patients who subsequently died of ALF, the time from onset to death was 43.7 ± 40.1 days. At admission, the mean serum total bilirubin was 16.3 ± 11.1 mg/dL, direct bilirubin 11.6 ± 7.8 mg/dL, prothrombin activity 22.1 ± 14.1%, alanine aminotransferase 2176 ± 2833 IU/L, cholinesterase 169.2 ± 75.0 IU/L, serum albumin 3.1 ± 0.53 mg/dL, leukocyte count 9572 ± 4957/μL, platelet count 13.2 ± 9.5 × 10^4/μL, blood urea nitrogen 16.2 ± 12.8 mg/dL, and serum creatinine 1.45 ± 1.71 mg/dL. There were no significant differences between the pretreatment features of patients who later received intensive care versus those who underwent LDLT (data not shown).

Diagnosis of underlying disease

HAV infection was diagnosed based on a positive result for the serum immunoglobulin M (IgM) anti-HAV antibody (detected by radioimmunoassay [RIA] or enzyme immunoassay [EIA]). Acute HBV infection was diagnosed based on a positive result for hepatitis B surface antigen (HBsAg), IgM anti-hepatitis B core (HBc) antibody, or HBV–DNA (IgM anti-HBc detected by RIA or EIA). Severe acute exacerbation (SAE) of chronic HBV infection was diagnosed based on a history of HBsAg positivity for more than 6 months and a high titer of immunoglobulin G (IgG) anti-HBc antibody. HEV infection was diagnosed based on a positive result for serum IgM anti-HEV antibody, IgG anti-HEV antibody, and HEV-RNA (by reverse transcription polymerase chain reaction). AIH was diagnosed according to the criteria of the International Autoimmune Hepatitis Scoring System.[14] Other hepatitis markers were also measured, including anti-HCV antibody, HCV-RNA, anti-CMV antibody, and anti-EBV antibody. For patients negative for all the above markers and with no history of drinking or taking medicine, the cause of ALF was considered cryptogenic.

Table 1 Clinical features of ALF patients on admission

Age	46.3 ± 15.9
Sex (male/female)	33/17
Type (hyperacute/acute/subacute)	14/21/15
Time from onset to admission (days)	18.5 ± 16.0
Time from onset to death (days)	43.7 ± 40.1
Serum total bilirubin (mg/dL)	16.3 ± 11.1
Serum direct bilirubin (mg/dL)	11.6 ± 7.8
D/T ratio (= D-BIL/T-BIL)	0.62 ± 0.10
Prothrombin time activity (%)	22.1 ± 14.1
Serum alanine transaminase (IU/L)	2175.7 ± 2833.1
Cholinesterase (IU/L)	169.2 ± 75.0
Serum albumin (mg/dL)	3.1 ± 0.53
Leukocyte count (/μL)	9572.4 ± 4957.1
Platelet count (×10^4/μl)	13.2 ± 9.5
Blood urea nitrogen (mg/dL)	16.2 ± 12.8
Serum creatinine (mg/dL)	1.45 ± 1.71

ALF, acute liver failure; D/T, serum direct bilirubin/serum total bilirubin; D-BIL, direct bilirubin; T-BIL, total bilirubin. (Mean ± SD).

Treatment

Intensive care support was provided immediately after admission, which included ALS (PE and CHDF), immunosuppressive therapy, and antiviral therapy. Immunosuppressive therapy comprised cyclosporine A (CyA) for patients with ALF, and corticosteroids for AIH patients. Antiviral therapy consisted of interferon (IFN) for HBV-related cases, and nucleoside analogs, such as lamivudine (LAM), for the HBV cases admitted after 2000. In our hospital, patients with ALF, in whom recovery was considered highly improbable, received LDLT since 1997, irrespective of intensive care. Since 1997, it has been our practice to explain the possibility of LDLT at admission to the families of ALF patients in whom no contraindications for the procedure were considered. If the family considered LDLT seriously and a suitable donor existed, we prepared for LDLT in addition to intensive care.

Eligibility for LDLT

The decision to list patients for LDLT was based on the criteria of the Acute Liver Failure Study Group of Japan.[15] In brief, when the ALF patient satisfied more than two items out of the following five items at the time of development of encephalopathy, she/he was registered for liver transplantation: (i) age >45 years; (ii) time from onset to encephalopathy >11 days; (iii) prothrombin time (PT) activity <10%; (iv) serum total bilirubin >18.0 mg/dL; and (v) serum direct bilirubin/serum total bilirubin ratio <0.67. However, satisfaction of the following two items at 5 days after development of encephalopathy resulted in cancellation of the registration: (i) recovery of encephalopathy within one degree or more than two degrees; and (ii) PT activity >50%.

Statistical analysis

Data were analyzed using SPSS 11.0 (SPSS, Chicago, IL, USA). The examined variables and age were expressed as mean ± SD. We compared the outcome of patients with ALF according to etiology and treatment (with or without LDLT). Survival rates according to each condition were compared using Fisher's exact test. Statistical significance was considered when the *P*-value was less than 0.05.

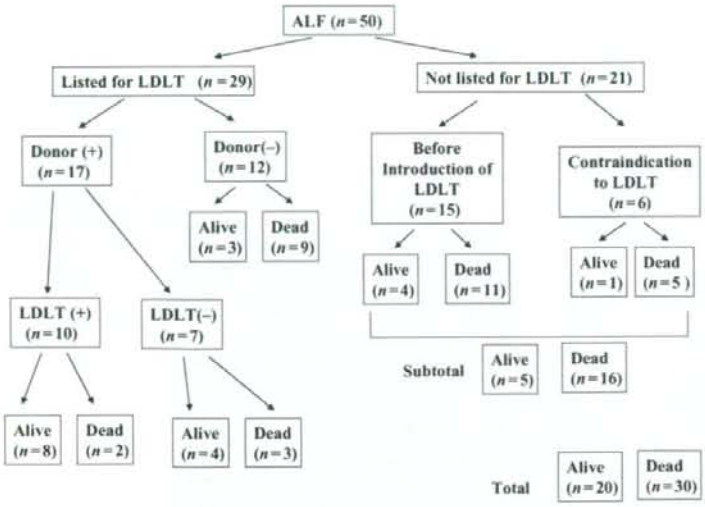

Figure 1 Outcome of 50 patients with acute liver failure (ALF). Of these patients, 29 were listed for living donor liver transplantation (LDLT); 17 had suitable donors, and 10 underwent LDLT. Survival rate of patients who underwent LDLT was 80%, whereas the total survival rate of patients with ALF was 40%.

Results

Clinical outcome

Of the 50 patients, 29 were scheduled for LDLT based on the criteria of the Acute Liver Failure Study Group of Japan (Fig. 1). However, no suitable donor could be found for 12 patients, among whom only three (25%) patients survived without LDLT. The other 17 patients had suitable living donors. Ten patients underwent LDLT, and eight (80%) of them survived. One patient died of inconformity of blood type and acute rejection 20 days after LDLT. Another died of sepsis caused by bile duct infection 40 days after LDLT. Of the 17 patients who had suitable donors, LDLT was not performed in seven patients. Among them, four survived with intensive care support. The other three patients passed into a critical condition during the waiting period and ultimately died. Twenty-one patients were not scheduled for LDLT. Among them, 15 were hospitalized before 1997 when LDLT was not introduced at our hospital. The other six had contraindication to LDLT (two were in a critical condition, two were too old to be recipients, and the other two suffered from malignant lymphoma). Of the 21 non-scheduled patients, only five (24%) survived with intensive care. Thus, 20 of the 50 (40%) patients were alive at the end of the study.

Impact of LDLT on the survival rate

Since 1997, LDLT has been available in our hospital for patients with ALF who had no expectation of recovery regardless of the intensive care, including ALS. We compared the survival rate of ALF patients treated before 1997 with that after 1997 (Fig. 2). Before 1997, four of 15 (27%) ALF patients survived with intensive care. After 1997, 10 of all 35 ALF patients underwent LDLT. Eight of these 10 (80%) patients survived. However, of the 35 ALF patients admitted after 1997, only eight (32%) survived without

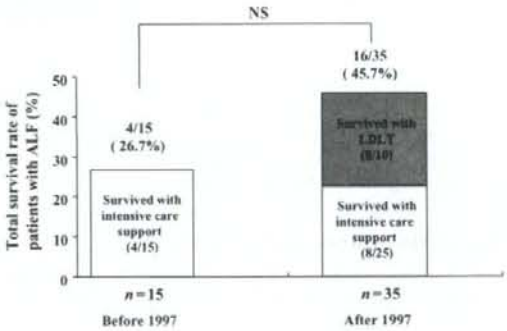

Figure 2 Survival rate of acute liver failure (ALF) patients. Comparison of survival rates in patients admitted before and after 1997. Survival rate of patients with ALF improved after 1997, and the improvement in outcome was due to living donor liver transplantation (LDLT).

LDLT. Since CHDF was developed in the late 1990s and LAM was introduced in 2001 in Japan, it was expected that the survival rate of ALF patients treated by intensive care would improve after 1997. However, it was almost the same as pre-1997. After 1997, the survival rate of ALF patients increased through the introduction of LDLT.

The baseline characteristics of the ALF patients according to etiology are listed in Table 2. The cause of ALF was acute HBV infection in nine, SAE of chronic HBV infection in nine, AIH in four, medication in three, HAV in one, HEV in one, alcohol in one, and cryptogenic hepatitis in 22 patients. The age of the patients with ALF caused by acute HBV (36.4 ± 14.8) and medication (26.3 ± 1.2) was less than the median age of all ALF patients (46.3 ± 15.9). The proportion of women was significantly higher

Table 2 Clinical features of ALF patients according to etiology on admission

Etiology	*n*	Age (years)	Sex (male/female)	Type (hyperacute/acute/subacute)	Serious cases†
HAV	1	57	1/0	0/1/0	1
Acute HBV	9	36.4 ± 14.8	5/4	6/3/0	1
SAE	9	52.8 ± 11.4	7/2	1/4/4	6
HEV	1	65	1/0	0/0/1	1
Drugs	3	26.3 ± 1.2	1/2	2/0/1	1
Alcohol	1	54	1/0	0/1/0	1
AIH	4	55.8 ± 19.3	0/4	0/2/2	4
Cryptogenic hepatitis	22	47.0 ± 15.7	17/5	5/10/7	12
Total	50	46.3 ± 15.9	33/17	14/21/15	27

†Estimated to die by Muto's formula. AIH, autoimmune hepatitis; ALF, acute liver failure; HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus; SAE, severe acute exacerbation of chronic hepatitis B infection.

Table 3 Therapy and survival rate of patients with ALF according to etiology

Etiology	*n*	ALS	Immunosuppressive therapy	Antiviral therapy	LDLT	Survival rate
HAV	1	1	0	1	0	0/1 (0)
Acute HBV	9	9	6	5	3	7/9 (78)
SAE	9	7	5	3	0	1/9 (11)
HEV	1	1	1	0	0	0/1 (0)
Drugs	3	3	0	0	1	1/3 (33)
Alcohol	1	0	1	0	0	0/1 (0)
AIH	4	4	4	0	1	3/4 (75)
Cryptogenic hepatitis	22	20	15	2	5	8/22 (36)
Total	50	45	32	11	10	20/50 (40)

Antiviral therapy: interferon or nucleoside analogs, such as lamivudine. Immunosuppressive therapy: cyclosporine A- or prednisolone-containing therapy. AIH, autoimmune hepatitis; ALF, acute liver failure; ALS, artificial liver support; HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus; LDLT, living donor liver transplantation; SAE, severe acute exacerbation of chronic hepatitis B infection. ALS is based on plasma exchange and continuous hemodiafiltration.

in the patients with AIH (100%) compared to the median proportion of women among all ALF patients (34%). The rate of hyperacute-type ALF was higher among patients with ALF caused by acute HBV infection (67%) and medication (67%) than the median rate of hyperacute-type ALF (28%).

Therapeutic modality and survival rate according to etiology

The therapeutic modality chosen for each patient and survival rate according to etiology are shown in Table 3. As the initial therapy, ALS was provided in most cases (90%, 45/50 cases). Immunosuppressants, such as bolus steroids and/or cyclosporine, were administrated in 64% (32/50). Since steroids are most effective for AIH cases, immunosuppressive therapy was selected in all cases of AIH. Immunosuppressive therapy was provided for 60% of viral hepatitis cases (HAV, acute HBV, SAE, and HEV; 12/20 cases) and 68% of cryptogenic hepatitis cases (15/22 cases). With regard to antiviral therapy, IFN was administrated in four cases (HAV, 1 case acute HBV, 1 case; and cryptogenic ALF, 2 cases) and LAM was administrated in eight cases (acute HBV, 5 cases; and SAE of chronic HBV infection, 3 cases). IFN was administered in two patients with cryptogenic hepatitis because they were suspected to suffer from viral hepatitis on admission. LDLT was performed in 10 patients among whom three had acute HBV, one was drug induced, one had AIH, and five were cryptogenic.

We compared the prognosis of patients according to the etiology of ALF, excluding patients with ALF caused by HAV, HEV, medication, and alcohol because they were only a few. The survival rates of patients with ALF caused by acute HBV infection or AIH were relatively high (78% and 75%, respectively). The survival rates of patients with ALF caused by SAE of chronic HBV infection and cryptogenic cause were significantly low (11% and 36%, respectively). Moreover, we compared the prognosis with or without LDLT in these four groups (acute HBV, SAE, AIH, cryptogenic hepatitis) (Fig. 3). In the case of acute HBV and AIH, there was no significant difference between the survival rates with and without LDLT. However, in the case of cryptogenic cause, the survival rate with LDLT was significantly higher than that without LDLT ($P = 0.03$). In the case of acute exacerbation of chronic HBV infection, the survival rate without LDLT was low (11%) and no case underwent LDLT. This is because most of their near relatives could not donate to the LDLT even if they wanted to as they were also HBV positive.

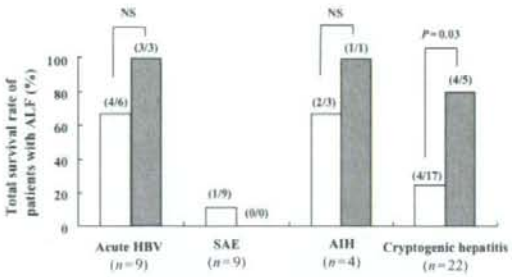

Figure 3 Survival rate of acute liver failure (ALF) patients according to etiology. Living donor liver transplantation (LDLT) was not performed in patients with ALF caused by severe acute exacerbation of chronic hepatitis B infection (SAE). In patients with ALF caused by acute hepatitis B virus (HBV) and autoimmune hepatitis (AIH), the prognosis was relatively good, irrespective of LDLT. In patients with ALF caused by cryptogenic cause, the survival rate without LDLT was significantly lower than that with LDLT. NS, not significant. □, Survival rate without LDLT; ■, survival rate with LDLT.

Survival rate according to etiology limited to serious cases

The survival rate according to etiology is shown in Table 4. The probability values for a prognostic index for patients with ALF ("risk of death") were determined by the above-mentioned formula, and 27 patients were estimated to die. The survival rate was 14% (3/22) for those without LDLT, but 80% (4/5) for those with LDLT ($P = 0.0089$). We compared the prognosis of patients according to the etiology, excluding patients with ALF caused by HAV, acute HBV, HEV, medication, and alcohol because of their small number. The survival rate of patients with ALF caused by AIH (75%) was significantly higher than those of ALF patients caused by SAE of chronic HBV infection and cryptogenic cause (17% and 25%, respectively). Moreover, we compared the prognosis with or without LDLT in these three groups (SAE, AIH, and cryptogenic hepatitis) (Fig. 4). In the case of SAE and AIH, there was no significant difference between the survival rates with and without LDLT. However, in the case of cryptogenic cause, the survival rate with LDLT was significantly higher than that without LDLT ($P = 0.004$). These results indicate that the usefulness of LDLT for patients with ALF caused by cryptogenic cause is more remarkable in serious cases.

Discussion

Since Starzl *et al.* performed the first liver transplantation in 1963, the techniques of liver transplantation have improved, and its efficacy and safety have been well established. Nowadays, there is no doubt that liver transplantation is the most effective therapy for ALF.[16] However, in Japan, liver transplantation is not always the first choice treatment modality for ALF. This is because DDLT is not generally performed in this country. Although the first law for DDLT in Japan was approved in 1997, only a few DDLT are performed annually. Instead, LDLT is the major transplantation procedure in Japan; the first LDLT was performed in 1989.[17] In the late 1990s, many base hospitals, including university hospitals, started to perform LDLT and the procedure has been widely performed across Japan since 2000. Until then, intensive care primarily based on ALS was the only treatment and was developed aggressively. PE and CHDF combination therapy is the mainstream of ALS. PE was designed to remove toxins and to supply important components, such as coagulation factors, while CHDF, in combination with PE, was designed to remove mid-range molecular-weight substances and reduce the chance of adverse effects, such as hepatic coma.[11]

To verify the impact of LDLT and intensive care with ALS, we compared the survival rate of ALF patients treated before and after 1997, when the first LDLT for ALF was performed at our hospital. While LDLT increased the overall survival rate of ALF after 1997, the survival rate of ALF with intensive care was similar for pre- and post-1997 (Fig. 2). These results mean that: (i) some patients can be cured by intensive care alone; (ii) there is a limitation to the increase in the survival rate with intensive care alone despite biotechnological advances; and (iii) some patients can be cured only by LDLT. This outcome may depend on the etiology of ALF.

The cause of ALF seems to be different in different countries. The most common cause of ALF is paracetamol poisoning in England, cryptogenic hepatitis in the USA, and HEV or HBV in India.[18] In Japan, HBV is the most common cause of ALF, which is similar to our present data. The reason for the larger proportion of patients with ALF caused by HBV infection in Japan relative to Europe and the USA is the higher prevalence rate of HBV infection in Japan. The use of immunoglobulin vaccines against HBV and HBV in newborns of HBV-positive mothers has effectively reduced the vertical transmission of HBV and decreased the number of new HBV carriers.[19] However, the number of HBV carriers with genotype A and horizontal transmission has increased,[20] suggesting that ALF due to HBV infection remains high.

Patients with ALF caused by SAE of chronic HBV infection showed fatal prognosis. While LAM has a potent antiviral activity against HBV, its effect appears only 1–2 months after administration in some cases. Therefore, it is necessary to use another agent with LAM for a more rapid treatment of hepatitis. Previously, we used CyA in patients with ALF, including SAE of chronic HBV infection. It seemed that the combination treatment of LAM and CyA is effective in preventing the progression of SAE of chronic HBV infection to ALF (data not shown). However, once SAE of chronic HBV infection progresses to ALF, the prognosis is extremely poor. Due to the use of LDLT in Japan, the donor needs to be a close relative of the ALF patient. According to the recommendation of the Japan Liver Transplantation Society, the donor needs to be a close relative of the ALF patient in Japan. Hence LDLT is often difficult because suitable candidates for donors virtually do not exist. In our cases, the prognosis of patients with ALF caused by SAE of HBV was also very poor. Of nine cases, no case underwent LDLT and only one (11%) survived with intensive care alone. Therefore, at present, in patients with SAE of chronic HBV infection, progression to ALF should be prevented with the use of nucleoside analogs, such as LAM or entecavir and immunosuppressants, such as CyA or steroids.

The outcome of patients with ALF caused by acute HBV infection seems to be satisfactory, with an overall survival rate of 78%, and 67% with intensive care alone. In the case of acute HBV infection, HBV is promptly eliminated by the immune response.

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Table 4 Survival rate of serious† ALF patients according to etiology

Etiology	Total	LDLT	Survival rate without LDLT	Survival rate with LDLT	Total survival rate
HAV	1	0	0/1 (0)	–	0/1 (0)
Acute HBV	1	0	0/1 (0)	–	0/1 (0)
SAE	6	0	1/6 (17)	–	1/6 (17)
HEV	1	0	0/1 (0)	–	0/1 (0)
Drugs	1	1	–	0/1 (0)	0/1 (0)
Alcohol	1	0	0/1 (0)	–	0/1 (0)
AIH	4	1	2/3 (67)	1/1 (100)	3/4 (75)
Cryptogenic hepatitis	12	3	0/9 (0)	3/3 (100)*	3/12 (25)
Total	27	5	3/22 (14)	4/5 (80)**	7/27 (26)

*$P = 0.004$ versus without LDLT; **$P = 0.0089$ versus without LDLT. †Estimated to die by Muto's formula (log it[λ] = 0.0649 × prothrombin time + 0.0357 × age − 2.81 × direct/indirect bilirubin + 0.703 × log total bilirubin + 1.04 × [O − C] [O − C]; acute form =0, subacute form =1.0, death rate (pi) =1/1 + $e^{-\lambda}$). When limited to patients with serious acute liver failure (ALF), the prognosis of cryptogenic cases with LDLT was significantly better than that of patients without LDLT. AIH, autoimmune hepatitis; HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus; LDLT, living donor liver transplantation; SAE, severe acute exacerbation of chronic hepatitis B infection.

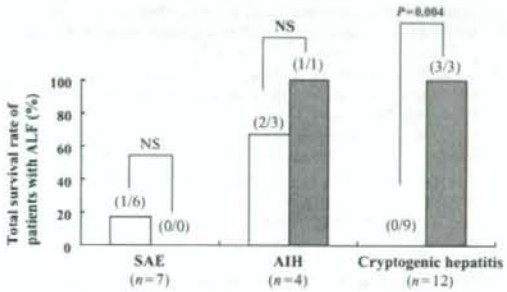

Figure 4 Survival rate of acute liver failure (ALF) patients who were estimated to die according to etiology. When limited to patients with serious ALF, the prognosis of cryptogenic cases with living donor liver transplantation (LDLT) was significantly better than that without LDLT. AIH, autoimmune hepatitis; NS, not significant; SAE, severe acute exacerbation of chronic hepatitis B infection. □, Survival rate without LDLT; ■, survival rate with LDLT.

Therefore, such patients can be rescued if sufficient intensive care, such as ALS, is provided until HBV is eliminated. At our hospital, patients with ALF caused by acute HBV infection have been treated with CyA for hepatitis. Moreover, we use LAM simultaneously because some patients have persistent liver damage or it is difficult to distinguish from SAE of chronic HBV infection. Two patients who died in this study developed acute complications. Although one of them was scheduled to undergo LDLT, he developed acute respiratory distress syndrome and brain edema before LDLT. Some patients with ALF caused by acute HBV infection deteriorate quickly and thus it is necessary to treat them, keeping the necessity of LDLT in mind from the first day of hospitalization.

In the USA, the prognosis of patients with ALF caused by AIH is reported to be poor without liver transplantation.[21] In Japan, it is reported that the short-term prognosis of patients with fulminant-type AIH may be good, although patients whose serum total bilirubin levels worsen during days 8–15 should be considered for liver transplantation.[22] However, at our hospital, the outcome of patients with ALF caused by AIH was relatively good, with or without LDLT. These findings suggest that steroid therapy plays a role in improving the prognosis of AIH patients.

While the prognosis of patients with ALF caused by acute HBV infection or AIH is relatively good, the outcome of patients with ALF caused by cryptogenic cause seems to be poor.[23] Our data also support this conclusion. It should be noted that cryptogenic hepatitis might encompass diverse etiologies, such as acute HBV infection that had already been undetectable on admission, rare viral infection but hepatitis virus, or unknown hepatitis virus that should be identified in the future. Unless the exact cause of ALF is identified, it is difficult to treat the hepatitis itself. This would be the reason why the survival rate of fulminant cryptogenic hepatitis without LT is still poor.

Taken together, for ALF to which effective therapy exists, such as AIH and acute HBV, ALS without LDLT was effective, while for ALF to which no effective therapy exists, such as cryptogenic hepatitis and SAE, it is difficult to cure.

In this study, we adopted the criteria of the Acute Liver Failure Study Group of Japan in order to assess whether ALF patients would die or not. Since King's College criteria is one of the most reliable formula for the prediction of ALF patients' survival, King's College criteria was tentatively adopted for the prognosis estimation.

The positive predictive value and negative predictive value of King's College criteria for the all ALF patients were 84.8% and 41.2%, respectively. The positive predictive value and negative predictive value of the criteria of the Acute Liver Failure Study Group of Japan for the all ALF patients were 80% and 60%, respectively. Generally, King's College criteria have a high positive predictive value (around 80% in paracetamol-induced ALF, and 70–>90% in non-paracetamol cases) and a low predictive value (70–90% in paracetamol-induced ALF, and 25–50% in non-paracetamol cases).[24] From these results, the criteria of the Acute Liver Failure Study Group of Japan is not inferior to the King's College criteria in positive and negative predictive values for ALF in our study.

In conclusion, our experience has shown that LDLT affects the prognosis of ALF patients. Specifically, at our hospital, 10 of 35

(28.6%) patients with ALF underwent LDLT. Their survival rate (80%) was significantly higher than that of ALF patients who received intensive care only (30%). The difference in the survival rate was clearer in the case of patients with ALF caused by cryptogenic hepatitis. For patients who could not be cured with ALS alone, the decision regarding liver transplantation has to be made as soon as possible.

References

1 Dhiman RK, Seth AK, Jain S, Chawla YK, Dilawari JB. Prognostic evaluation of early indicators in fulminant hepatic failure by multivariate analysis. *Dig. Dis. Sci.* 1998; **43**: 1311–16.
2 Tygstrup N, Ranek L. Fulminant hepatic failure. *Clin. Gastroenterol.* 1981; **10**: 191–208.
3 Farmer DG, Anselmo DM, Ghobrial RM *et al.* Liver transplantation for fulminant hepatic failure: experience with more than 200 patients over a 17-year period. *Ann. Surg.* 2003; **237**: 666–76.
4 Schiodt FV, Atillasoy E, Shakil AO *et al.* Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transpl. Surg.* 1999; **5**: 29–34.
5 Caraceni P, Van Thiel DH. Acute liver failure. *Lancet* 1995; **21**: 163–9.
6 Kiuchi T, Kasahara M, Uryuhara K *et al.* Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation* 1999; **67**: 321–7.
7 Makuuchi M, Kawasaki S, Noguchi T *et al.* Donor hepatectomy for living related partial liver transplantation. *Surgery* 1993; **113**: 395–402.
8 Kawasaki S, Makuuchi M, Matsunami H *et al.* Living related liver transplantation in adults. *Ann. Surg.* 1998; **227**: 269–74.
9 Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. *Transplantation* 2000: 258–64.
10 Shimada M, Fujii M, Morine Y, Imura S, Ikemoto T, Ishibashi H. Living-donor liver transplantation: present status and future perspective. *J. Med. Invest.* 2005; **52**: 22–32.
11 Sadahiro T, Hirasawa H, Oda S *et al.* Usefulness of plasma exchange plus continuous hemodiafiltration to reduce adverse effects associated with plasma exchange in patients with acute liver failure. *Crit. Care Med.* 2001; **29**: 1386–92.
12 Sekido H, Matsuo K, Takeda K *et al.* Usefulness of artificial liver support for pretransplant patients with fulminant hepatic failure. *Transplant. Proc.* 2004; **36**: 2355–6.
13 Yonekawa C, Nakae H, Tajimi K, Asanuma Y. Effectiveness of combining plasma exchange and continuous hemodiafiltration in patients with postoperative liver failure. *Artif. Organs* 2005; **29**: 324–8.
14 Alvarez F, Berg PA, Bianchi FB *et al.* International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* 1999; **31**: 929–38.
15 Ishiki Y. Criteria of liver transplantation for acute liver failure. *Acta Hepatol. Jpn* 1996; **37**: 760–1.
16 Bernal W, Wendon J. Liver transplantation in adults with acute liver failure. *J. Hepatol.* 2004; **40**: 192–7.
17 Kaneko M, Kosaka Y, Asano M *et al.* A case report of anesthetic management of orthotopic liver transplantation from a living donor to his son. *Masui* 1991; **40**: 454–67.
18 Acharya SK, Panda SK, Saxena A, Gupta SD. Acute hepatic failure in India: a perspective from the East. *J. Gastroenterol. Hepatol.* 2000; **15**: 473–9.
19 Noto H, Terao T, Ryou S *et al.* Combined passive and active immunoprophylaxis for preventing perinatal transmission of the hepatitis B virus carrier state in Shizuoka, Japan during 1980–1994. *J. Gastroenterol. Hepatol.* 2003; **18**: 943–9.
20 Suzuki Y, Kobayashi M, Ikeda K *et al.* Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J. Med. Virol.* 2005; **76**: 33–9.
21 Ostapowicz G, Fontana RJ, Schiodt FV *et al.* Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann. Intern. Med.* 2002; **137**: 947–54.
22 Miyake Y, Iwasaki Y, Terada R, Onishi T, Okamoto R, Sakai N. Clinical characteristics of fulminant-type autoimmune hepatitis: an analysis of eleven cases. *Aliment. Pharmacol. Ther.* 2006; **23**: 1347–53.
23 Ellis AJ, Saleh M, Smith H, Portmann B, Gimson A, Williams R. Late-onset hepatic failure: clinical features, serology and outcome following transplantation. *J. Hepatol.* 1995; **23**: 363–72.
24 Renner EL. How to decide when to list a patient with acute liver failure for liver transplantation? Clichy or King's College criteria, or something else? *J. Hepatol.* 2007; **46**: 554–7.

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

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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

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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

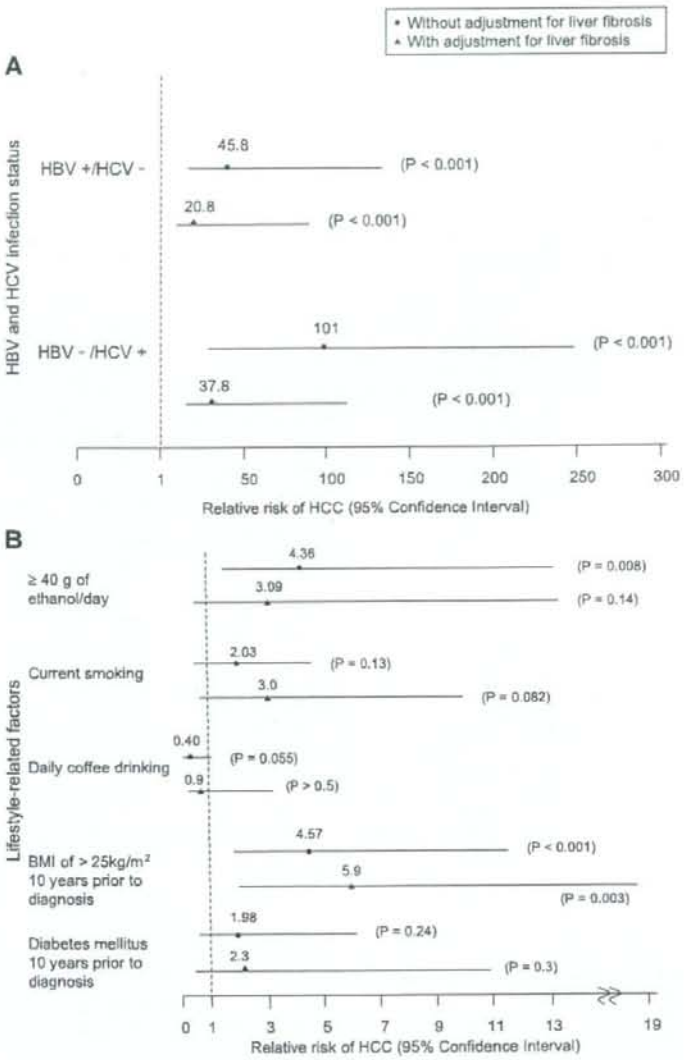

Figure 1. Multivariate relative risk for hepatocellular carcinoma for individual risk factors, with and without adjustment for variables associated with severity of liver fibrosis. Each relative risk was analyzed with and without adjustment for ln(type IV collagen) and platelet count, using HBV and HCV 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.

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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.

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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

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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]

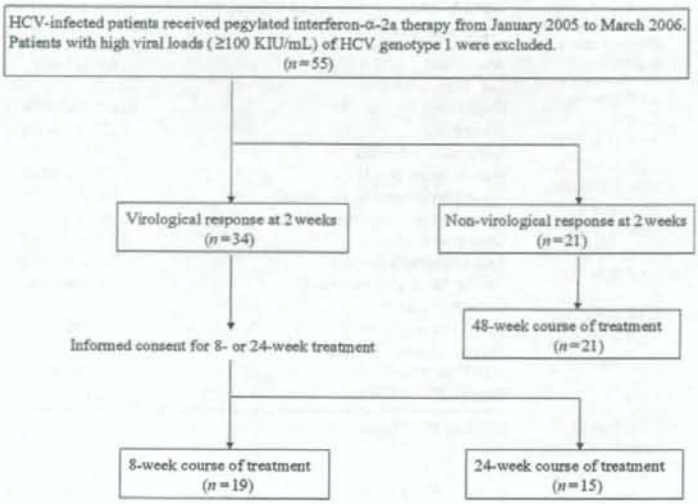

Figure 1 Flow diagram of the clinical trial. HCV, hepatitis C virus.

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.

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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-