13. Jones SA, Rose-John S: The role of soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6 complex. Biochim Biophys Acta 1592:251, 2002.
14. Heinrich PC, Graeve L, Rose-John S, Schneider-Mergener J, Dittrich E, Erren A, Gerhartz C, Hemann U, Lutticken C, Wegenka U: Membrane-bound and soluble interleukin-6 receptor: studies on structure, regulation of expression, and signal transduction. Ann N Y Acad Sci 762:222, 1995.
15. Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM: The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J 15:43, 2001.
16. Kallen KJ: The role of transsignalling via the agonistic soluble IL-6 receptor in human diseases. Biochim Biophys Acta 1592:323, 2002.
17. Jostock T, Mullberg J, Ozbek S, Atreya R, Blinn G, Voltz N, Fischer M, Neurath MF, Rose-John S: Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur J Biochem 268:160, 2001.
18. Rehermann B, Trautwein C, Boker KH, Manns MP: Interleukin-6 in liver diseases. J Hepatol 15:277, 1992.
19. Bedossa P, Poynard T: An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24:289, 1996.
20. Yamada Y, Kirillova I, Peschon JJ, Fausto N: Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 94:1441, 1997.
21. Fausto N: Liver regeneration. J Hepatol 32(1 Suppl):19, 2000.
22. Lapinski TW: The levels of IL-1beta, IL-4 and IL-6 in the serum and the liver tissue of chronic HCV-infected patients. Arch Immunol Ther Exp 49:311, 2001.
23. Malaguarnera M, Di Fazio I, Romeo MA, Restuccia S, Laurino A, Trovato BA: Elevation of interleukin 6 levels in patients with chronic hepatitis due to hepatitis C virus. J Gastroenterol 32:211, 1997.
24. Frieling JT, van Hamersvelt HW, Wijdenes J, Hendriks T, Sauerwein RW, van Der Linden CJ: Circulating concentrations of soluble interleukin 6 receptors gp80 and gp130 in chronic renal failure and effects of renal replacement therapy. Am J Nephrol 19:571, 1999.
25. Dandona P, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 25:4, 2004.
26. Genesca J, Marti R, Gonzalez A, Torregrosa M, Segura R: Soluble interleukin-6 receptor levels in liver cirrhosis. Am J Gastroenterol 94:3074, 1999.
27. Rose-John S. Interleukin-6 biology is coordinated by membrane bound and soluble receptors. Acta Biochim Pol 50:603, 2003.
28. Montero-Julian FA: The soluble IL-6 receptors: serum levels and biological function. Cell Mol Biol 47:583, 2001.
29. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R: Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274:1379, 1996.
30. Maione D, Di Carlo E, Li W, Musiani P, Modesti A, Peters M, Rose-John S, Della Rocca C, Tripodi M, Lazzaro D, Taub R, Savino R, Ciliberto G: Coexpression of IL-6 and soluble IL-6R causes nodular regenerative hyperplasia and adenomas of the liver. EMBO J 17:5588, 1998.
31. Schirmacher P, Peters M, Ciliberto G, Blessing M, Lotz J, Meyer zum Buschenfelde KH, Rose-John S: Hepatocellular hyperplasia, plasmacytoma formation, and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double-transgenic mice. Am J Pathol 153:639, 1998.

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HEPATOLOGY

Clinical features of hepatocellular carcinoma that occur after sustained virological response to interferon for chronic hepatitis C

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Abstract

Background and Aim: This study investigated the clinical features of hepatocellular carcinoma in patients with sustained virological response to interferon for hepatitis C viral (HCV) infection.

Methods: A total of 7715 patients with HCV infection were treated with interferon and followed up for more than 1 year after withdrawal of interferon in 64 Japanese hospitals and clinics between July 1988 and August 2001. Sustained virological response was obtained in 2515 (32.6%) patients. Of these 2515 patients, clinical data were collected for 38 patients in whom hepatocellular carcinoma developed. Sustained virological response was defined as HCV RNA negativity more than 6 months after the termination of interferon.

Results: All patients were HCV RNA negative at the time of diagnosis of hepatocellular carcinoma. The median period until the detection of hepatocellular carcinoma was 4.7 years (range 1.4–9.0 years). There were significant improvements in hepatic function including serum albumin, aspartate aminotransferase, alanine aminotransferase, indocyanine green test, platelet count and histological activity grade in comparison with those before interferon therapy and at the onset of hepatocellular carcinoma. The maximum tumor size in patients without medical follow-up for 1 year or more (median: 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median: 25 mm) ($P = 0.002$).

Conclusions: The present findings emphasize the importance of regular medical follow up of patients with HCV infection, as even patients showing a sustained virological response to interferon and in whom hepatic function has improved have the potential to develop hepatocellular carcinoma.
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Key words: follow-up, hepatitis C virus, hepatocellular carcinoma, interferon, sustained virological response.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of most prevalent malignant tumors worldwide, and its incidence is increasing. Most cases are attributable to chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection.[1,2] In Japan, epidemiological studies have shown that HCV is more common than HBV as the causative agent of HCC.[3,4] Because HCV infection is related to the development of cirrhosis and HCC, it was assumed that eradication of this infection would provide the most effective means of preventing HCV-related complications, including HCC.

Currently, interferon (IFN), peginterferon or combination therapy with ribavirin, are the available drugs that are effective for terminating HCV infection. IFN

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can induce a long-term favorable response and eradication of HCV RNA from serum after treatment cessation, although the response rate is not fully satisfactory.[5–8] Furthermore, patients with HCV appear to derive a definitive benefit in terms of prevention of progression to cirrhosis and the development of HCC.[7–15] However, even in some patients from whom HCV infection has been eliminated by IFN therapy, HCC can still be detected.[11–27] In these patients, the clinical features of developing HCC have not been fully investigated,[20] although they have been documented in individuals or small numbers of cases.[21–27]

The present study therefore attempted to elucidate the clinical features of HCC, especially the serial changes occurring in the period from before IFN therapy to the detection of HCC. A multicenter, collective study was conducted in the setting of hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, in Japan, as it was felt that a study conducted in a single institution would provide inadequate numbers of sustained responders who developed HCC.

METHODS

Patients

This study was conducted at major hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, Japan. A patient cohort in whom HCC had been detected among sustained responders to IFN therapy for chronic hepatitis C was collected by means of data collection instruments. All of the patients included had tested positive for HCV RNA before IFN therapy, and were followed up after withdrawal of IFN therapy for more than 1 year prior to the end of August 2001. Sustained virological response (SVR) was defined as HCV RNA negativity for more than 6 months after termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely that the cancer had been present at the end of IFN treatment.

In Japan at the time of the study, the standard schedule was 6–10 MU IFN-α every day for the first 2–4 weeks and then three times a week for the following 20–22 weeks, or 6 MU IFN-β every day for 6–8 weeks. Patients treated with peginterferon or combination therapy with ribavirin were not included because these therapies had not been approved by the Ministry of Health, Labor and Welfare in Japan at the time of the study.

At the first data collection, hospitals were approached and information on the number of patients who had undergone IFN therapy for chronic hepatitis C and who had been followed up for more than 1 year after the termination of IFN therapy, the number of SVR patients among them, and the number of patients in whom HCC had developed among the SVR patients was requested; 64 hospitals responded, listed in Appendix I.

In the second data collection, carried out on SVR patients in whom HCC had developed, clinical data were requested for each patient from before IFN therapy and at detection of HCC.

Data collected

To elucidate the clinical features of HCC that developed in SVR patients, host-related, treatment-related and tumor-related variables before IFN therapy and at detection of HCC were investigated (Table 1). Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet *et al.*,[28] where staging is defined as: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis); and grading is defined as: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

Follow-up ended with the last recorded visit before 31 August 2001. The period until the detection of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities such as ultrasonography or computed tomography. The medical follow-up period for the detection of HCC after SVR was defined as the interval

Table 1 Clinical features of 38 patients with chronic hepatitis C in whom hepatocellular carcinoma (HCC) developed after sustained response to interferon

Clinical feature	Before interferon	At detection of HCC	*P*-value
Host-related variables			
Age (years) [median (range)]	60 (36–71)	64 (38–77)	<0.0001
<60 [*n* (%)]	16 (42%)	4 (11%)	
>60 [*n* (%)]	22 (58%)	34 (89%)	
Sex [*n* (%)]			
Men	34 (89%)	—	—
Alcohol abuse [*n* (%)]†			
Positive	2 (5%)	—	—
Viral load before interferon (copies/mL) [*n* (%)]			
>10^6	2 (13%)	—	—

Table 1 *Continued*

Clinical feature	Before interferon	At detection of HCC	*P*-value
Serological group before interferon [*n* (%)]			
Group 1	6 (33%)	—	—
Group 2	12 (67%)	—	—
Hepatic function [median (range)]			
Platelet ($\times 10^4$/mm^3)	11.6 (6.6–31.0)	16.5 (7.3–31.0)	<0.0001
Total bilirubin (mg/dL)	0.7 (0.3–1.5)	0.7 (0.3–16.8)	0.32
Albumin (g/dL)	4.2 (3.3–5.0)	4.4 (3.2–5.2)	0.10
Aspartate aminotransferase (IU/L)	78 (29–288)	29 (14–159)	<0.0001
Alanine aminotransferase (IU/L)	109 (24–295)	23 (8–178)	<0.0001
Prothrombin time	81 (49–124)	89 (68–137)	0.03
Indocyanine green R_{15} (%)	15.0 (5.0–45.0)	10.6 (3.1–27.4)	0.0009
Histologic fibrosis staging [*n* (%)]			
F0	0 (0%)	1 (6%)	
F1	9 (26%)	3 (19%)	
F2	10 (29%)	8 (50%)	
F3	10 (29%)	2 (13%)	
F4	6 (17%)	2 (13%)	0.11
Histologic activity grade [*n* (%)]			
A0	0 (0%)	6 (38%)	
A1	7 (23%)	8 (50%)	
A2	17 (57%)	2 (13%)	
A3	6 (20%)	0 (0%)	0.001
Treatment-related variables			
Treatment periods (weeks) [median (range)]	24 (2–31)	—	—
Interferon type [*n* (%)]			
α	36 (95%)	—	—
β	2 (5%)	—	—
Total amount of interferon [median (range)]	480 (126–846)	—	—
Prior interferon therapy [*n* (%)]			
Positive	2 (5%)	—	—
Tumor-related variables			
Number of tumors [*n* (%)]			
Solitary	—	31 (82%)	—
Multiple (range)	—	7 (18%)	—
Maximum tumor size (mm)			
Median		30 (12–150)	
≤30 [*n* (%)]	—	21 (57%)	—
>30 [*n* (%)]	—	16 (43%)	—
Alpha-fetoprotein (ng/mL) [*n* (%)]			
>20	4 (16%)	15 (41%)	0.07
PIVKA-II (AU/mL) [*n* (%)]			
>0.063	0 (0%)	13 (43%)	0.01
Differentiation of HCC [*n* (%)]			
Well-differentiated	—	11 (44%)	—
Moderately differentiated	—	11 (44%)	—
Poorly differentiated	—	2 (8%)	—
Combined type	—	1 (4%)	—
Period until development of HCC (years) [median (range)]	—	4.7 (1.4–9.0)	—
Period of medical follow-up (months) [median (range)]	—	3 (0.5–57)	—
First treatment for HCC[‡] [*n* (%)]			
Resection	—	16 (43%)	—
Local ablation	—	10 (27%)	—
Transarterial treatment	—	11 (30%)	—

PIVKA-II, protein induced by vitamin K absence or antagonist-II; R_{15}, indocyanine green retention rate at 15 min.
[†]Ethanol intake ≥80 g/day for ≥5 years. [‡]One patient has not yet undergone treatment for HCC.

during which checks for HCC were performed using tumor markers and/or imaging modalities.

Differences between data obtained before IFN therapy and at detection of HCC were evaluated using the Wilcoxon signed-rank test. All *P*-values presented in this report are of the two-tailed type. Differences at $P < 0.05$ were considered statistically significant. All analyses were conducted using SPSS 8.0 J (SPSS Inc. Chicago, IL, USA).

RESULTS

In the first data collection, a total of 7715 patients with chronic hepatitis C were identified who had been treated with IFN and followed up for more than 1 year after the termination of IFN therapy from July 1988 to August 2001 in 64 hospitals and clinics. A SVR was obtained in 2515 patients (32.6%), among whom HCC was detected in 42 (1.7%) from 24 hospitals (38%).

In the second data collection, clinical data were received for 41 patients from 23 hospitals. Of these patients, three were excluded from the analysis because of detection of HCC within 1 year after IFN therapy (one patient), concomitant hepatitis B virus infection (one patient), and a history of treatment for HCC before IFN therapy (one patient). Accordingly, the study subjects comprised 38 patients who had developed HCC after SVR to IFN therapy for chronic hepatitis C. The profiles of the patients are shown in Fig. 1.

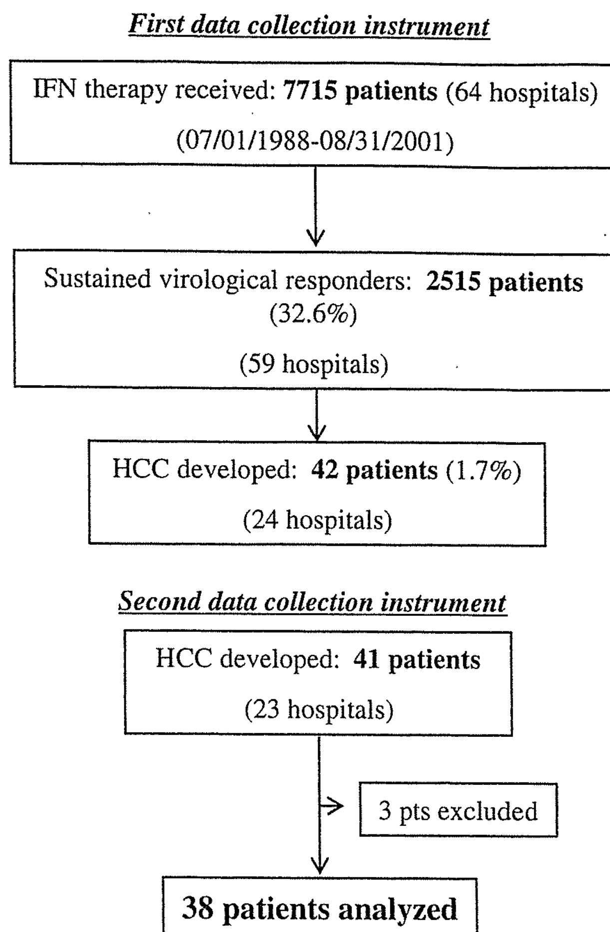

Figure 1 Profile of patients and data collection. One hospital did not respond to second data collection request. IFN, interferon; HCC, hepatocellular carcinoma.

Table 1 summarizes the clinical features of the 38 HCV patients in whom HCC developed after SVR to IFN therapy. All of the patients were HCV RNA negative at the time of HCC detection, when their median age was 64 (range 38–77) years, and 34 of the patients (89%) were ≥60 years of age. Thirty-four patients (89%) were men (sex ratio 8.5:1). When data from before IFN therapy and at the detection of HCC were compared, there were significant improvements in platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and indocyanine green retention rate at 15 min (ICG R_{15}). In the 16 patients who underwent liver biopsy before IFN therapy and at the time of HCC detection, serial changes in histological fibrosis staging and activity grade were observed (Fig. 2). Histological activity grade improved significantly after IFN therapy ($P = 0.004$). However, there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$).

With regard to the HCC that developed, 31 patients (82%) had a solitary tumor and 22 patients (57%) had a tumor <3 cm in diameter. The median period until the detection of HCC was 4.7 years (range 1.4–9.0 years), and there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy (Fig. 3). The median period of medical follow-up after the termination of IFN therapy was 3 months (range 0.5–57 months), and eight patients were not followed up for 1 year or more. The maximum tumor size in these patients (median 60 mm; range 40–150 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm; range 12–51 mm) ($P = 0.002$). Of the 38 patients, 16 underwent hepatic resection for HCC.

DISCUSSION

Chronic hepatitis C is a progressive disease that is related to the development of cirrhosis and HCC. IFN, peginterferon, or combination therapy with ribavirin are widely used as standard treatments for chronic hepatitis C, the therapeutic scope being viral clearance and resolution of hepatic inflammation.[5–8] In theory, if successful in this respect, these treatments should have the additional effect of preventing HCC. Sustained eradication of HCV by IFN therapy has been shown to improve hepatic fibrosis as well as hepatic inflammation, and to suppress the occurrence of HCC.[5–15] However, there have been several reported cases of HCC that developed after successful IFN therapy.[11–27] The clinical features of HCC and the mechanisms of carcinogenesis have not yet been fully elucidated because development of HCC is very rare in sustained responders to IFN therapy.[20–27] Therefore, a multicenter study was set up to collect and analyze the clinical data for

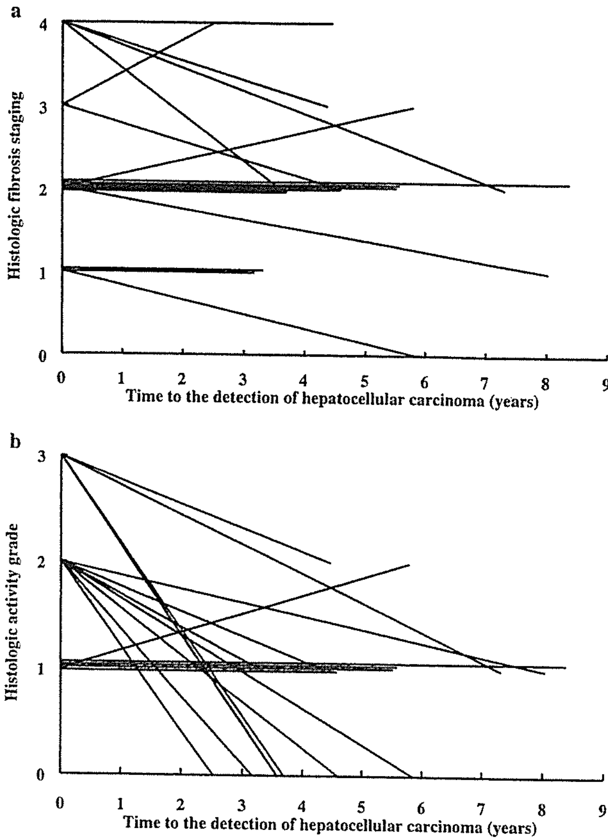

Figure 2 Serial changes in (a) histological fibrosis staging and (b) histological activity grading for each patient when compared before interferon therapy and at detection of hepatocellular carcinoma.

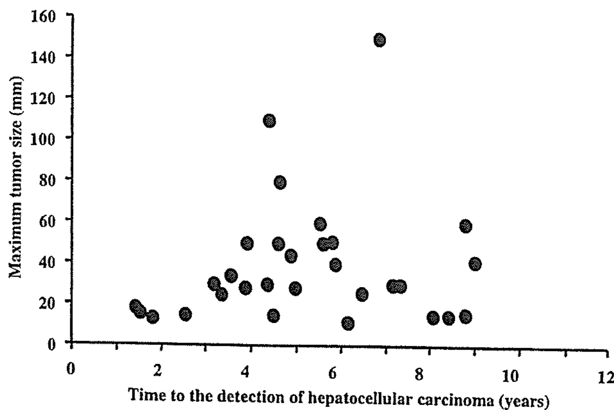

Figure 3 Maximum tumor size and time until detection of hepatocellular carcinoma.

patients who showed a SVR to IFN therapy for chronic hepatitis C and in whom HCC subsequently developed.

In this study, a total of 7715 patients with chronic hepatitis C received IFN therapy, and among them, a SVR was obtained in 2515 (32.6%). Among the patients with SVR who developed HCC, clinical data were collected for 38 patients. In regards to the clinical features of the HCC that developed in these patients, the percentage of those who were ≥60 years of age at the time of HCC detection (89%), and the percentage of men (89%) (sex ratio 8.5:1) were both high. In these patients, platelet count, albumin, AST, ALT, indocyanine green R_{15} and histological activity grade also improved significantly after IFN therapy ($P < 0.05$), although there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$). Therefore, it was obvious that IFN therapy improved hepatic inflammation and hepatic function, as suggested by the results of other studies.[7–15] However, the other clinical features could not be clarified in this study, because we had no data from controls with which to compare the clinical variables of HCC that developed in patients showing SVR to IFN therapy. Potential control groups might include HCV patients with HCC who did not receive IFN therapy, or HCV patients with HCC who received IFN therapy but did not show a sustained response.[20–23] Additional comparative studies will be required in order to sufficiently elucidate the clinical features of HCC developing after SVR to IFN.

In the present study, there were 38 patients who developed HCC after successful IFN therapy, with a median period of 4.7 years (range 1.4–9.0 years) until detection of HCC. Moreover, the maximum tumor size in patients without medical follow-up for 1 year or more (median 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm) ($P = 0.002$). As other studies have also indicated,[20,21] these findings suggest that the risk of HCC in sustained responders is not completely eliminated and that careful medical follow-up is important even after successful IFN therapy, which makes it difficult to determine the optimal follow-up period after SVR. If HCC had been detected at an earlier stage by regular follow-up, these patients could have been offered potentially curative treatment such as hepatic resection; such patients generally have good hepatic function after elimination of HCV. Moreover, it has also been reported that recurrence after curative treatment of HCC in SVR patients is less frequent than in non-SVR patients.[22,23] However, the enormous health care costs associated with screening all SVR patients for many years should be borne in mind. Therefore, it is also essential to identify the risk factors for development of HCC[20] and to establish the follow-up strategies in SVR patients.

Why does HCC develop even in patients showing a SVR to IFN therapy? HCV is a positive, single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that integration of HCV nucleic acid sequences into the host genome, like that occurring in HBV infection, seems unlikely.[29] Therefore, HCV itself is probably not the causative factor of HCC after SVR. One assumption is that preexisting microscopic tumor foci that are not detected by diagnostic imaging are responsible for the appearance of HCC after SVR to IFN therapy, although in this study patients were excluded if HCC was detected within 1 year after the termination of IFN therapy. However, in the present series, there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy. Although the rapidity of tumor growth may depend on individual tumor characteristics, considering

the late onset of small HCC in these patients, de novo HCC development after eradication of HCV should not be ignored. This has also been reported by Toyoda *et al.* on the basis of analysis that calculated the doubling time of HCC that occurred after SVR[24] and a long-term follow-up study of SVR patients.[21] It is conceivable that long-standing chronic liver inflammation and liver regeneration may provide the basis for tumor development. Carcinogenesis may not be a single-step event, but a complex, multi-step process, although the mechanisms are still unknown. Future studies should be aimed at defining the basic oncogenic mechanisms by which SVR patients develop HCC. Moreover, exploring the underlying mechanisms for the development of HCC in SVR patients may help identify new strategies for prevention of HCC.

In conclusion, even patients showing a SVR to IFN treatment of chronic hepatitis C and in whom hepatic function improves have the potential to develop HCC. The results of this study underline the importance of periodic medical follow-up for these patients.

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REFERENCES

1 Colombo M, Kuo G, Choo QL *et al.* Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; **2**: 1006–8.
2 Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. *Lancet* 1990; **335**: 873–4.
3 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671–5.
4 Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995; **21**: 650–5.
5 Causse X, Godinot H, Chevallier M *et al.* Comparison of 1 or 3 MU of interferon α-2b and placebo in patients with chronic non-A, non-B hepatitis. *Gastroenterology* 1991; **101**: 497–502.
6 Davis GL, Balart LA, Schiff ER *et al.* Treatment of chronic hepatitis C with recombinant interferon alpha. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. *N. Engl. J. Med.* 1989; **321**: 1501–6.
7 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346**: 1051–5.
8 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann. Intern. Med.* 1999; **131**: 174–81.
9 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; **29**: 1124–30.
10 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J. Hepatol.* 1999; **30**: 653–9.
11 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann. Intern. Med.* 2000; **132**: 517–24.
12 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27**: 1394–402.
13 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann. Intern. Med.* 1998; **129**: 94–9.
14 Yoshida H, Arakawa Y, Sata M *et al.* Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology* 2002; **123**: 483–91.
15 Yoshida H, Tateishi R, Arakawa Y *et al.* Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. *Gut* 2004; **53**: 425–30.
16 Hirashima N, Mizokami M, Orito E *et al.* Development of hepatocellular carcinoma in a patient with chronic hepatitis C infection after a complete and sustained response to interferon-alpha. *J. Gastroenterol. Hepatol.* 1996; **11**: 955–8.
17 Tong MJ, Lai LP, Murakami-Mori K. Development of hepatocellular carcinoma after clearance of hepatitis C virus with interferon therapy. *West. J. Med.* 1997; **167**: 103–5.
18 Miyano S, Togashi H, Shinzawa H *et al.* Occurrence of hepatocellular carcinoma 4.5 years after successful treatment with virus clearance for chronic hepatitis C. *J. Gastroenterol. Hepatol.* 1999; **14**: 928–30.
19 Yamada M, Ichikawa M, Matsubara A, Ishiguro Y, Yamada M, Yokoi S. Development of small hepatocellular carcinoma 80 months after clearance of hepatitis C virus with interferon therapy. *Eur. J. Gastroenterol. Hepatol.* 2000; **12**: 1029–32.
20 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; **101**: 1616–22.
21 Toyoda H, Kumada T, Tokuda A *et al.* Long-term follow-up of sustained responders to interferon therapy,

in patients with chronic hepatitis C. Yon-Ken HCV-HCC Follow-up Study Group. *J. Viral Hepatol.* 2000; 7: 414–19.

22 Toyoda H, Kumada T, Hayashi K *et al.* Characteristics and prognosis of hepatocellular carcinoma detected in sustained responders to interferon therapy for chronic hepatitis C. Yon-Ken HCV-HCC Follow-up Study Group. *Cancer Detect. Prev.* 2003; **27**: 498–502.

23 Ikeda K, Kobayashi M, Saitoh S *et al.* Recurrence rate and prognosis of patients with hepatocellular carcinoma that developed after elimination of hepatitis C virus RNA by interferon therapy. A closed cohort study including matched control patients. *Oncology* 2003; **65**: 204–10.

24 Toyoda H, Kumada T, Honda T *et al.* Analysis of hepatocellular carcinoma tumor growth detected in sustained responders to interferon in patients with chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2001; **16**: 1131–7.

25 Kashiwagi K, Furusyo N, Kubo N *et al.* A prospective comparison of the effect of interferon-alpha and interferon-beta treatment in patients with chronic hepatitis C on the incidence of hepatocellular carcinoma development. *J. Infect. Chemother.* 2003; **9**: 333–40.

26 Suzuki K, Ohkoshi S, Yano M *et al.* Sustained biochemical remission after interferon treatment may closely be related to the end of treatment biochemical response and associated with a lower incidence of hepatocarcinogenesis. *Liver Int.* 2003; **23**: 143–7.

27 Yoneyama K, Yamaguchi M, Kiuchi Y, Morizane T, Shibata M, Mitamura K. Analysis of background factors influencing long-term prognosis of patients with chronic hepatitis C treated with interferon. *Intervirology* 2002; **45**: 11–19.

28 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513–20.

29 Fong TL, Shindo M, Feinstone SM, Hoofnagle JH, Di Bisceglie AM. Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. *J. Clin. Invest.* 1991; **88**: 1058–60.

APPENDIX I

Participating hospitals and clinics

In addition to the hospitals of the study authors, data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Nippon Steel Yawata Memorial Hospital; Yame General Hospital; 1st Department of Internal Medicine, Ryukyu University School of Medicine; 2nd Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga University School of Medicine; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; 2nd Department of Internal Medicine, Nagasaki University School of Medicine; Yonabaru Central Hospital; 2nd Department of Internal Medicine, Miyazaki University School of Medicine; Fukuoka Red Cross Hospital; Kita-kyushu Medical Center Hospital; Munakata Medical Association Hospital; Naika Yamaguchi Urban Clinic; Yamaga Municipal General Hospital; Shin Kokura Hospital; Iizuka Hospital; Oita Prefectural Hospital; 3rd Department of Internal Medicine, University of Occupational and Environmental Health School of Medicine; Kokura National Hospital; Kumamoto Regional Medical Center; Shin Beppu Hospital; Oita National Hospital; Kami Goto Hospital; 1st Department of Internal Medicine, Oita University School of Medicine; National Kyushu Medical Center; 1st Department of Internal Medicine, Nagasaki University School of Medicine; Hirahara Naika Clinic; Kumamoto National Hospital; Saiseikai Fukuoka General Hospital; Arita Ichou Hospital; Labour Welfare Corporation Moji Rosai Hospital; 1st Department of Internal Medicine, Kumamoto University; Kumamoto Red Cross Hospital; National Kyushu Cancer Center Hospital; Kagoshima Kyousaikai Nanpuh Hospital; Iwao Hospital; Saga Social Insurance Hospital; Labour Welfare Corporation Nagasaki Rosai Hospital; Oita Red Cross Hospital; Koebaru Central Hospital; National Sanatorium Kumamoto South Hospital; 2nd Department of Internal Medicine, Oita Medical University; Labour Welfare Corporation Kumamoto Rosai Hospital; Amakusa Regional Medical Center; Miyuki Hospital; Saiseikai Futsukaichi Hospital; Hakujyuji Hospital; Hakuaikai Hospital.

＜短　報＞

C型慢性肝炎に対するPEG-IFNα2b＋ribavirin併用療法における早期治療効果予測 —血中HCV抗原によるモニタリング解析—

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緒言：難治性C型慢性肝炎の治療は，Pegylated IFN（Peg IFN）とRibavirin（RBV）併用療法が標準的治療法になってきているが，ウイルス学的著効（Sustained virological response：SVR）率は50％に過ぎない[1)2)]．

近年，SVR予測因子として治療直後から治療開始後4週間以内（第1相から第3相）のウイルス減少，治療開始後12週目・24週目のHCVRNA陰性化などウイルス動態が重要となってきている[3)~5)]．今回，我々は難治性C型慢性肝炎に対してPeg IFNとRBV併用療法（併用療法）を行い，治療経過中のHCV Core抗原（HCV抗原）を測定し，治療早期に効果予測が可能であるかを検討した．

対象と方法：当院において2005年1月から5月までに併用療法を行った症例のうち，書面で同意を得たC型慢性肝炎患者45例（Genotype 1b，アンプリコアHCVモニターv2.0オリジナル法：Amplicor-Mで100KIU/ml以上）を対象とした．併用療法12週目まで投与を行った症例の治療前，治療中3日目，1，2，4，12週目の凍結保存（－80℃）血清検体を解析に用いた．併用療法は，体重換算を行い，PEG-IFNα2b80～120μg/回・皮下注・週1回・48週間投与にRibavirin600～1000mg/日・経口・連日48週間投与を併用した．併用療法の治療効果は，治療12週目のHCVRNA定性陰性もしくはHCV抗原量2Log以上の減少例をEarly Virological Response（EVR），それ以外をNon Early Virological Response（NEVR）とした．両群間の有意差検定は，Mann-Whitney's U-testおよびFisher's exact testを用いて行った．HCV抗原量の測定（オーソHCV抗原IRMAテスト，オーソクリニカル・ダイアグノスティックス）は，血清中のHCVウイルス粒子からエンベロープを除去し，Core粒子を構成単位のCore抗原に分解する．同時に共存するHCVCore蛋白に対する抗体を失活させる．この処理済み検体をHCVコア領域に特異的なモノクローナル抗体を用いた2ステップサンドイッチ法にて補足する．これに^{125}I標識抗ペルオキシダーゼポリクローナル抗体を反応させ，放射線量（cpm）を計測する．HCV抗原の測定値は，7濃度のスタンダードのcpm値より作成した検量線よりHCV抗原量（fmol/L）を算出した．HCV抗原の測定結果は，20fmol/L以下をHCV抗原陰性，20fmol/L以上HCV抗原陽性と判定した．

成績：45例中EVRは36例（80％），NEVRは9例（20％）であった．EVR群の平均年齢は55.8±3.2歳，男女比は22：14，治療前Peg-IFN量92.5±14.1，治療前RBV量731±131，治療前HCV量は抗原で5387±4800fmol/L，Amplicor-Mで383±158KIU/mLであった．一方，NEVR群の平均年齢は57.2±6.7歳，男女比は4：5，治療前Peg-IFN量88.9±10.5，治療前RBV量689±105，治療前HCV量は抗原で4577±2064 fmol/L，Amplicor-Mで456±86KIU/mLであった．両群間に有意差は認めなかった．

併用療法での治療効果別，HCV抗原量の推移をFig. 1に示す．HCV抗原量は治療直後より3日目にかけて急激に減少し，3日目より1週目にかけて反跳上昇した．この時点において治療前HCV抗原量を100％として1週目HCV抗原量の50％減少率を検討するとEVR群では100％（36/36），NEVR群では33％（3/9）でNEVR群に比し，EVR群で有意にHCV抗原量の減少を示した（$p<0.01$）．さらに1週目から12週目にかけてもNEVR群に比し，EVR群の方が明らかにHCV抗原量低値で推移した（$p<0.01$）．治療開始12週目の効果予測の可能性をHCV抗原量治療2週目1000 fmol/Lを基準値として検討した．HCV抗原量治療2週目1000 fmol/L以上は，8例でうち6例（75％）が治療12週目HCV抗原陽性を示した．一方，HCV抗原量治療2週目1000fmol/L未満は，37例でうち35例（95％）が治療12週目HCV抗原陰性を示した．

考察：わが国でも医療保険の適用になって以来，PEG-IFN＋Ribavirin併用療法が盛んに施行されている．しかし，一方で副作用・費用対効果に対しても十分考慮し慎重に治療しなければいけない．この点からも治療開始早期における効果予測が重要となってくる．最近，HCV動態の解析が海外，国内において報告されている[3)~7)]．今回の検討では，最終治療効果判定までには至っていないも，治療開始12週目時点までの効果予測が治療早期HCV抗原量を測定することにより可能であった．とくに1週目のHCV抗原50％減少率で

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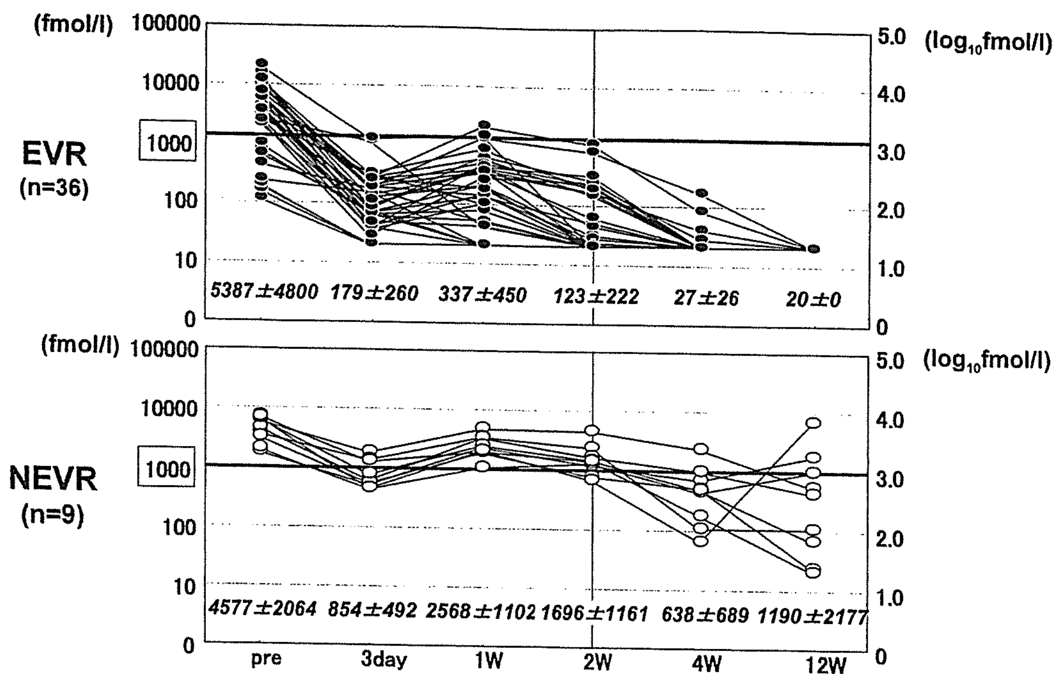

Fig. 1　Serum levels of HCV core antigen during combination therapy of PEG-IFNα2b and ribavirin

はEVR群とNEVR群間において減少量に有意な差が認められた．また，2週目のHCV抗原量からは基準値（1000fmol/L）を設定することにより12週目の効果予測が可能であった．このことから安価で簡便なHCV抗原測定法は，HCVRNA測定法と同様に早期治療効果予測に有用であることが示唆された．

索引用語：HCV抗原，HCV動態

文献：1）Manns MP, et al. Lancet 2001; 358: 958—965
2）Fried MW, et al. N Engl J Med 2002; 347: 975—982
3）Neumann AU, et al. J Infect Dis 2000; 182: 28—35
4）田中榮二，他．新薬と臨床　2001；50：865—875
5）Herrmann E. Hepatology 2003; 37: 1351—1358
6）朝比奈靖浩，八橋　弘．DDW Japan Luncheon Seminar記録集　2004；1—18　7）松永誠治朗，他．医学と薬学　2004；52：119—125

英文要旨

An early prognosis for the treatment of Chronic Hepatitis C patients with Pegylated Interferon-α2b plus Ribavirin
—Monitoring analysis by the serum HCV core antigen values—

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The capability of an early prognosis for the treatment of Chronic Hepatitis C (CH-C) patients with genotype 1b and high viral loads with Pegylated Interferon-α2b plus Ribavirin was evaluated by the levels of serum HCV core antigens. Serum samples were drawn before the treatment, 3 days or 1, 2, 4, and 12 weeks after the treatment. Out of 45 cases with CH-C, 36 cases (80%) and 9 cases (20%) were early virological response (EVR) and non-early virological response (NEVR), respectvery.

The serum levels of core antigen in EVR were significantly lower than those in NEVR from the third day to the 12th week after the treatment. The levels of core antigen less than 1000fmol/L in the second week could predict a better prognosis in the 12th week. The measurement of serum HCV core antigen was useful for the early prognosis of the treatment in CH-C patients with genotype 1b and high viral loads with Pegylated Interferon-α2b plus Ribavirin.

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T1653 Mutation in the Box α Increases the Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B Virus Genotype C Infection

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Background. Most patients with chronic hepatitis B virus infection become carriers of inactive virus after hepatitis B e antigen seroconversion; however, a subgroup of patients have persistent abnormal transaminase levels and develop hepatocellular carcinoma after seroconversion.

Methods. In an age-matched case-control study, 40 carriers of inactive virus (mean age ± standard deviation [SD], 50.9 ± 11.1 years), 40 patients with chronic hepatitis (mean age ± SD, 50.2 ± 8.9 years), and 40 patients with hepatocellular carcinoma (mean age ± SD, 50.7 ± 9.4 years) who were infected with hepatitis B virus genotype C and had test results positive for antibody to hepatitis B e antigen were analyzed.

Results. The prevalence of T1653 in the box α was significantly higher among patients with hepatocellular carcinoma than among carriers of inactive virus who did not have hepatocellular carcinoma (70% vs. 25%; $P < .0001$) or chronic hepatitis (70% vs. 35%; $P = .003$). Mutations in the basic core promoter region (T1762/A1764) were frequently found in all groups, regardless of clinical status (in 77.5% of carriers of inactive virus, 77.5% of patients with chronic hepatitis, and 90% of patients with hepatocellular carcinoma). In the multivariate analysis, the presence of T1653, an alanine aminotransferase level of ⩾37 U/L, and a platelet count of $<18 \times 10^4$ platelets/mm^3 were independent predictive values for hepatocellular carcinoma (odds ratio [95% confidence interval], 5.05 [1.56–16.35], 12.56 [3.05–51.77], and 11.5 [3.47–38.21], respectively). High α-fetoprotein level was the only independent predictive value for T1653 in patients with hepatocellular carcinoma (odds ratio, 12.67; 95% confidence interval, 1.19–134.17]). Among patients with test results positive for antibody to hepatitis B e antigen who had hepatocellular carcinoma and were infected with different genotypes of hepatitis B virus, the prevalence of T1653 was 40%, 15%, 25%, 25%, 67%, and 23% in patients infected with hepatitis B virus genotypes Aa, Ae, Ba, Bj, C, and D, respectively ($P < .05$ for genotype C vs. genotypes Ae, Ba, Bj, or D).

Conclusions. Our data indicate that the addition of T1653 mutation in the box α to the basic core promoter mutation increases the risk of hepatocellular carcinoma in patients with hepatitis B virus genotype C.

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third leading cause of cancer-related death in the world, with an estimated prevalence of >500,000 cases worldwide per year [1]. It is now accepted that hepatitis B virus (HBV) has a carcinogenic potential in humans. Several mutations in the HBV genome have been reported to occur during the course of persistent viral infection, and there has been increasing evidence of an association between these molecular alterations and the development of HCC in patients with HBV infection.

During persistent HBV infection, carriers frequently undergo seroconversion from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe). Most patients who acquire chronic HBV infection with HBV genotype C (which is a common genotype in East

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Asian countries) by perinatal transmission become carriers of inactive virus after seroconversion. A subgroup of patients have persistent abnormal serum transaminase levels and develop HCC in the anti-HBe–positive phase. Many of these patients have active viral replication and are infected with several mutant viruses. The association between different clinical events after seroconversion and specific HBV genomic mutations has not been clearly defined.

Mutations in the basic core promoter (BCP) region at nucleotides (nt) 1762/1764 (T1762/A1764) and mutation in the precore (preC) region at nt 1896 (A1896) are associated with seroconversion and persistent viral replication. It is noteworthy that both BCP and preC mutations are often found in patients with advanced liver disease, (e.g., HCC) [2–8]. The T1762/A1764 mutation alters HBeAg production at the transcription level, and the A1896 in the preC region terminates translation of the precursor protein, abrogates HBeAg production, and results in seroconversion. A1896 was also reported previously to be associated with severe forms of chronic liver disease [7,8].

HBV has been classified into 8 major genotypes with use of the complete nucleotide sequence of the viral genome [10]. HBV genotypes not only have distinct geographical distributions [7, 11, 12] but also have different clinical manifestations and responses to therapy (e.g., IFN therapy). Furthermore, HBeAg positivity and levels of HBV DNA, which are controlled by specific mutations, differ between HBV genotypes (e.g., the BCP double mutation is more prevalent among strains of HBV genotype C, followed by HBV genotype A, and the A1896 mutation is frequently found in HBV genotypes B and D) [13–16].

There have been many studies involving viral mutations associated with clinical features, but most previous studies have ignored age, sex, HBeAg status, and HBV genotypes. In Japan, most patients with HCC experience seroconversion (i.e., they are anti-HBe positive) and have HBV genotype C; therefore, we performed an age-matched case-control study among anti-HBe–positive patients infected with HBV genotype C (including carriers of inactive virus, patients with chronic hepatitis, and patients with HCC) to determine the specific HBV genome mutations associated with disease progression.

PATIENTS AND METHODS

Serum samples. Serum samples were obtained from 211 patients from different regional areas worldwide. A total of 120 patients from Japan who were infected with HBV genotype C (40 carriers of inactive virus, 40 patients with chronic hepatitis, and 40 patients with HCC) were matched with control subjects according to age and HBe status. Control serum samples were obtained from patients with HCC who were positive for anti-HBe and who were infected with HBV genotype Aa (10 subjects), Ae (13), Ba (20), Bj (20), C (15), and D (13). Control subjects were from Hong Kong (19 subjects), Japan (36), and the United States (36). The majority of patients infected with HBV genotypes Aa, Ba, Bj, and C were Asian, and the majority of patients infected with HBV genotypes Ae and D were white and black. None of the subjects had serological test results positive for markers of infection with hepatitis C virus or HIV-1.

The study protocol was approved by ethics committees of the participating institutions in accordance with the 1975 Helsinki declaration. Informed consent was obtained from each patient.

Serological assays for HBV markers. HBeAg and anti-HBe were detected by chemiluminescent EIA (Lumipulse *f*, Fujirebio). HBV genotypes were determined by the restriction fragment–length polymorphism method on the S gene sequence amplified by PCR [29] and ELISA with monoclonal antibodies directed to distinct epitopes on the preS2 region products [18], with use of commercial kits (HBV genotype EIA; Institute of Immunology). The genotypes were also confirmed with use of a phylogenetic tree analysis. α-Fetoprotein and serum protein induced by the absence of vitamin K (antagonist II) were examined with use of chemiluminescent EIA.

Amplification and sequencing of the core promoter and the precore region plus core gene. HBV DNA sequences bearing the core promoter and preC or core regions were amplified by PCR with heminested primers by the method described elsewhere [19]. Thereafter, PCR products were sequenced directly with Prism Big Dye (Applied Biosystems) in the ABI 3100 DNA automated sequencer (Applied Biosystems). Accession numbers for all strains are AB236515–AB236634.

Case-control study. A carrier of inactive virus was defined as an HBsAg-positive individual with normal alanine aminotransferase (ALT) levels for a 2-year period (with at least 4 evaluations at 3-month intervals) and without the presence of portal hypertension. Chronic hepatitis was defined as persistent elevation of ALT levels ($>1.5\times$ upper limit of normal [35 U/L]) during a 6-month period (with at least 3 evaluations at 2-month intervals) without a decrease in platelet count or albumin level, and hypersplenism (splenomegaly on ultrasonographic examination). Twenty-one patients were confirmed to have chronic hepatitis by means of a fine-needle biopsy of the liver. Staging and grading (expressed as mean value $\pm$ SD [95% CI]) were 1.24 ± 0.64 (0.99–1.58) and 1.36 ± 0.58 (1.07–1.59), respectively, as previously described [30]. None had received antiviral treatment during the follow-up period. Of 40 patients with HCC, 23 patients received a diagnosis of HCC on the basis of a pathologic examination, and 17 patients received a diagnosis of HCC on the basis of results of abdominal ultrasonography, angiography, CT, or MRI, as well as an elevated serum α-fetoprotein level ($\geqslant$400 ng/mL).

Statistical evaluation. Data were expressed as mean $\pm$

SD. Statistical analyses were performed using χ^2 test and Fisher's exact test for categorical variables. Mann-Whitney U test or 1-way analysis of variance were used for continuous variables, as appropriate. Mantel-Haenszel χ^2 test was used to analyze the trend of frequencies of viral mutations. Multivariate analyses with logistic regression were used to determine the independent factors associated with HCC and T1653. Differences were considered to be significant for P values <.05. The statistical analysis software used was Stata software, version 8.0 (StataCorp).

RESULTS

Table 1 compares ALT level, platelet count, and HBV DNA level, as well as mutations in the box α (enhancer II), core promoter, and preC region, among 40 carriers of inactive virus, 40 patients with chronic hepatitis, and 40 patients with HCC who were infected with HBV genotype C in an age-matched case-control study. ALT and HBV DNA levels were significantly lower among carriers of inactive virus than among patients with chronic hepatitis or patients with HCC ($P<.0001$ and $P = .001$, respectively). Platelet count was lower among patients with HCC than among carriers of inactive virus or patients with chronic hepatitis ($P<.0001$).

The frequency of the T1653 mutation in the box α was significantly higher among patients with HCC (70%) than among carriers of inactive virus (25%) or patients with chronic hepatitis (35%; $P<.0001$) (table 1). Of interest, the T1653 mutation had an opposite correlation with the M1753 mutation. The prevalence of T1762/A1764 was high in all clinical status groups, with no statistically significant difference between groups (table 1). The trend of the frequency of T1653, increasing from carriers of inactive virus to patients with chronic hepatitis to patients with HCC, was analyzed by Mantel-Haenszel χ^2 test (OR, 2.48; 95% CI, 1.59–3.85; $P = .0001$) (figure 1). The trend of the frequency of T1762/A1764 was not statistically significant ($P = .1502$) (figure 1).

The attributable risk of multiple factors, including sex, HBV DNA level, ALT level, platelet count, and the presence of the T1653, M1753, T1762/A1764, and A1896 mutations for HCC in the HBV carriers was determined by multiple logistic regression analysis (table 2). There was a statistically significant association between development of HCC and ALT level >37 U/L (OR, 12.56; 95% CI, 0.55–6.21; $P<.0001$) and platelet count $<18 \times 10^4$ platelets/mm^3 (OR, 11.5; 95% CI, 3.47–38.21; $P<.0001$). The T1653 mutation was still significantly associated with the development of HCC (OR, 5.05; 95% CI, 1.56–16.35; $P = .007$).

The attributable risk of multiple factors, including HBV DNA level, ALT level, platelet count, α-fetoprotein level, protein in-

Table 1. Demographic, clinical, and virologic characteristics of patients infected with hepatitis B virus (HBV) genotype C who were matched for age and hepatitis B e antigen (HBeAg) status.

	Clinical status			
Variable	Carriage of inactive virus (n = 40)	Chronic hepatitis (n = 40)	Hepatocellular carcinoma (n = 40)	P
Male sex	31 (77.5)	37 (92.5)	36 (90)	.171
Age, years	50.9 ± 11.1	50.2 ± 8.9	50.7 ± 9.4	Matched
HBeAg positive	0 (0)	0 (0)	0 (0)	Matched
Anti-HBeAg positive	40 (100)	40 (100)	40 (100)	Matched
HBV genotype C	40 (100)	40 (100)	40 (100)	Matched
Alanine transaminase level, U/L[a]	20.8 ± 7.6	102 ± 108.7	83.2 ± 84.8	.0001
Platelet count, $\times 10^4$ platelets/mm^3 [b]	20.7 ± 3.1	17.4 ± 4.1	12.8 ± 5.7	.0001
HBV DNA level, LGE/mL[c]	4.3 ± 0.8	5.9 ± 1.5	5.4 ± 1.5	<.0001
Mutation in the box α: T1653[d]	10 (25)	14 (35)	28 (70)	<.0001
Mutation in the core promoter				
M1753	10 (25)	6 (15)	9 (22.5)	.609
T1762/A1764	31 (77.5)	31 (77.5)	36 (90)	.289
Mutation in the precore region: A1896	25 (62.5)	26 (65)	25 (62.5)	1.0

NOTE. Data are no. (%) of patients or mean value ± SD. Anti-HBeAg, antibody to HBeAg; LGE, log genome equivalents.

[a] $P<.0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .002$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

[b] $P<.0001$ for patients with hepatocellular carcinoma vs. carriers of inactive virus or patients with chronic hepatitis; $P = .002$ for carriers of inactive virus vs. patients with chronic hepatitis.

[c] $P<.0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .001$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

[d] $P<.0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .001$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

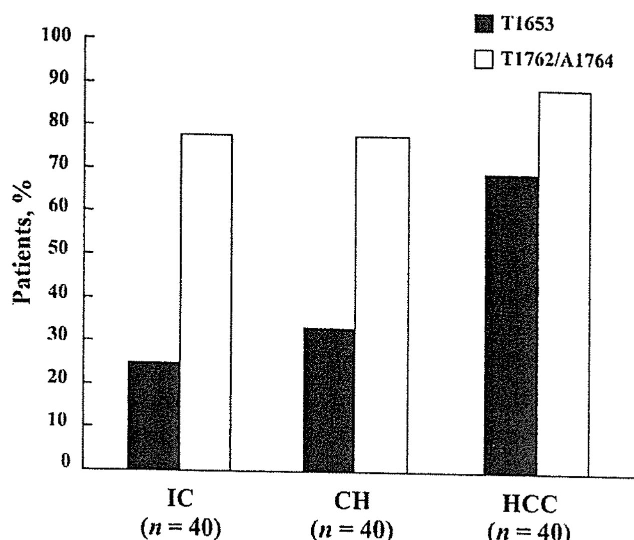

Figure 1. Prevalence of T1653 box α and T1762/A1764 basic core promoter mutations among patients with chronic hepatitis B virus infection, stratified by clinical status. The trend of the frequency of the T1653 mutation was analyzed by Mantel-Haenszel χ^2 test. The OR estimate is an approximation of the OR for carriers of inactive virus (IC), patients with chronic hepatitis (CH), and patients with hepatocellular carcinoma (HCC) having a strain with the mutation (OR, 2.48; 95% CI, 1.59–3.85; $P = .0001$). The trend of the frequency of the T1762/A1764 mutation was not statistically significant according to the Mantel-Haenszel χ^2 test ($P = .1502$).

duced by the absence of vitamin K (antagonist II) level, for T1653 in patients with HCC with HBV genotype C infection was determined by multiple logistic regression analysis (table 3). An α-fetoprotein level >300 ng/mL was the only independent predictive value for the presence of the T1653 mutation in patients with HCC with HBV genotype C infection (OR, 12.67; 95% CI, 1.19–134.17; $P = .035$).

Table 4 compares sex, age, and mutations in the box α, core promoter, and preC region among patients infected with HBV genotypes Aa (10 patients), Ae (13), Ba (20), Bj (20), C (15), and D (13) with the same variables among patients with HCC. Mean age was significantly higher among patients with HBV genotype Bj infection, compared with patients with HBV genotype Ba, genotype C, and genotype D infection ($P < .05$). The prevalence of T1653 among patients with HBV genotype C infection (66.7%) was significantly higher than it was among patients infected with other genotypes (15%–25%; $P < .05$), excluding patients infected with HBV genotype Aa. The prevalence of T1762/A1764 among patients with HBV genotype Ba infection (85%) and HBV genotype C infection (86.7%) was also significantly higher than it was among patients infected with other genotypes (20%–50%; $P < .05$). The prevalence of A1896 among patients with HBV genotype Aa infection and HBV genotype Ae infection was significantly lower than it was among patients infected with other genotypes ($P < .05$).

DISCUSSION

Many previous studies have reported that the clinical course of chronic HBV infection may be modified by several specific viral mutations [5, 20, 21], although the significance of such specific mutations in patients with chronic hepatitis B remains controversial. Because most studies have not controlled for different variables, such as age, HBV genotype, and HBe status, it is unknown whether the mutations were associated with disease progression, greater age of the patient, the specific HBV genotype or subtype, or HBe status. In this study, to exclude any biases, we performed an age-matched case-control study involving only anti-HBe–positive patients infected with HBV genotype C.

In the present case-control study, the prevalence of T1653 was found to be significantly higher among patients with HCC, compared with carriers of inactive virus and patients with chronic hepatitis with HBV genotype C infection; however, the prevalence of T1762/A1764 was high in all clinical status groups. During the anti-HBe–positive phase of infection, T1653 was more reliable than T1762/A1764 as a predicting factor for

Table 2. Multivariate analysis of variables with independent predictive value for development of hepatocellular carcinoma among a group of 120 patients with hepatitis B virus infection.

Variable	OR (95% CI)	*P*
Sex		
Female	1	
Male	5.06 (0.85–30.15)	.075
HBV DNA level		
<4.8 LGE/mL	1	
≥4.8 LGE/mL	0.34 (0.09–1.21)	.096
Alanine transaminase level		
<37 U/L	1	
≥37 U/L	12.56 (3.05–51.77)	.0001[a]
Platelet count		
≥18 × 10^4 platelets/mm^3	1	
<18 × 10^4 platelets/mm^3	11.51 (3.47–38.21)	.0001[a]
T1653 mutation		
No	1	
Yes	5.05 (1.56–16.35)	.007[a]
M1753 mutation		
No	1	
Yes	1.23 (0.31–5.04)	.770
T1762/A1764 mutation		
No	1	
Yes	2.67 (0.57–12.54)	.214
A1896 mutation		
No	1	
Yes	0.96 (0.29–3.11)	.943

NOTE. Each OR was adjusted for age and other variables in the analysis. LGE, log genome equivalents.
[a] Statistically significant.

Table 3. Multivariate analysis of variables with independent predictive value for the presence of the T1653 mutation among 40 patients with hepatocellular carcinoma.

Variable	OR (95% CI)	P
HBV DNA level		
<4.9 LGE/mL	1	
⩾4.9 LGE/mL	0.89 (0.16–4.79)	.899
ALT level		
<53 U/L	1	
⩾53 U/L	1.72 (0.29–9.96)	.541
Platelet count		
⩾12 × 10^4 platelets/mm^3	1	
<12 × 10^4 platelets/mm^3	1.39 (0.28–7.02)	.683
α-Fetoprotein level		
<300 ng/mL	1	
⩾300 ng/mL	12.67 (1.19–134.17)	.035[a]
PIVKA-2 level		
<50 mAU/mL	1	
⩾50 mAU/mL	0.25 (0.05–1.43)	.120

NOTE. Each OR was adjusted for age and other variables in the table. PIVKA-2, protein induced by the absence of vitamin K (antagonist II).

[a] Statistically significant.

the development of HCC. In fact, in the multivariate analysis, the presence of T1762/A1764 was not an independent predictor of HCC, but ALT level >37 U/L, platelet count <18 × 10^4 platelets/mm^3, and the presence of T1653 were independent predictors of HCC. The T1653 mutation had also been reported by Takahashi et al. [17]; they reported that this specific mutation was prevalent among Japanese patients with HCC, although their study was not a case-control study. These results do not deny that T1762/A1764 is associated with hepatocarcinogenesis, because poor prognosis associated with HBV genotype C infection, compared to that associated with HBV genotype B (Ba and Bj) infection, correlated with a high prevalence of T1762/A1764 [2, 9, 16], indicating that the BCP double mutation is associated with a high potential for hepatocarcinogenesis. The appearance of the T1653 mutation after the occurrence of the T1762/A1764 mutation (the T1762/A1764 mutation usually occurs earlier than the T1653 mutation) could indicate that the virulence of HBV is increasing, which could result in the development of HCC. In the multivariate analysis, however, HBV DNA level was no longer a predicting factor for HCC. One of the reasons for this is that the HBV DNA data used in this study were obtained at the time of diagnosis of HCC. A recent prospective study from Taiwan has indicated that high HBV DNA levels at baseline and infection with HBC genotype C were independent predictors for HCC, but the mean viral load at the time of diagnosis of HCC was significantly lower than at baseline [27]. Although our data could not indicate an association between HBV DNA level and hepatocarcinogenesis, if we could measure the HBV DNA level before diagnosis of HCC, it might found to be be a predicting factor for HCC. Furthermore, an examination of the characteristics of patients with HCC who had the T1653 mutation showed that an elevated α-fetoprotein level (⩾300 ng/mL) was the only predictor for the development of HCC in patients with the T1653 mutation. It has been reported that α-fetoprotein level is useful not only for diagnosis but also as a prognostic indicator for patients with HCC [22, 23], suggesting that the T1653 mutation might be associated with poor prognosis for patients with HCC.

The prevalence of several mutations among patients with HCC differed from that among patients with different HBV genotypes (Aa, Ae, Ba, Bj, C, and D) (table 4). The prevalence

Table 4. Demographic and virological characteristics of patients with hepatocellular carcinoma who were positive for antibody to hepatitis B e antigen (anti-HBe), by hepatitis B virus (HBV) genotype.

	HBV genotype						
Variable	Aa (*n* = 10)	Ae (*n* = 13)	Ba (*n* = 20)	Bj (*n* = 20)	C (*n* = 15)	D (*n* = 13)	P
Male	10 (100)	12 (92.3)	18 (90)	15 (75)	15 (100)	13 (100)	.10
Age, years[a]	54.4 ± 7.7	55.3 ± 4.4	54.4 ± 14.8	64.9 ± 9.6	47.9 ± 7.6	53.5 ± 8.3	.0002
HBeAg positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Matched
Anti-HBe positive	10 (100)	13 (100)	20 (100)	20 (100)	15 (100)	13 (100)	Matched
Mutation in the box α: T1653[b]	4 (40)	2 (15.4)	5 (25)	5 (25)	10 (66.7)	3 (23.1)	.039
Mutations in the core promoter region							
M1753	3 (30)	3 (23.1)	5 (25)	4 (20)	2 (13.3)	1 (7.7)	.759
T1762/A1764[c]	5 (50)	6 (46.2)	17 (85)	4 (20)	13 (86.7)	5 (38.5)	<.0001
Mutation in the precore region: A1896[d]	0 (0)	0/13 (0)	9/20 (45)	15/20 (75)	9/15 (60)	8/13 (61.5)	<.0001

NOTE. Data are no. (%) of patients or mean value ± SD. HBeAg, hepatitis B e antigen.

[a] $P<.05$ for Bj vs. Ba or D; $P<.0001$ for Bj vs. C.

[b] $P<.05$ for C vs. Ba or Bj or D; $P<.01$ for Ae vs. C.

[c] $P<.05$ for Ae vs. Ba or C; $P<.01$ for D vs. Ba or C; $P<.0001$ for Bj vs Ba or C.

[d] $P<.05$ for Ba vs. Aa or Ae; $P<.005$ for Aa vs. C or D and for Ae vs. Ba or C or D; $P<.0001$ for Bj vs Aa or Ae.

of T1653 was the highest among patients with HBV genotype C infection, followed by those with HBV genotype Aa infection, although the number of patient with HBV genotype Aa infection was too small for any conclusions to be drawn. The prevalence of T1762/A1764 was higher among patients with HBV genotype Ba and HBV genotype C infection than among patients infected with other genotypes. HBV genotype Ba has a sequence that closely resembles that of HBV genotype C in the core promoter region, because it is recombinant HBV between HBV genotype Bj and HBV genotype C from nucleotides 1740 to 2485. Although A1896 was not found in HBV genotype Aa and HBV genotype Ae, as has been reported elsewhere [15], HBV genotype Aa had some specific mutations upstream of the preC initiation codon and encapsidation signal site. Therefore, several HBV genotype–specific mutations would be associated with different mechanisms on seroconversion or HBV replication for each genotype or subtype.

Buckwold et al. [24] reported that T1762/A1764 can no longer bind liver-enriched transcription factors and that the transcription of precore RNA and the expression of HBeAg were reduced. Thereafter, Li et al. [25] reported that this mutation not only removed the nuclear receptor–binding site but also created a hepatic nuclear factor 1 transcription factor–binding site. As for a factor correlated with BCP, the core upstream regulatory sequence, which has a strong stimulation effect on the BCP, was reported. In an earlier article by Yu et al. [28], the box α elements (nucleotides 1646–1668) individually stimulated promoter activity >100-fold. The T1653 mutation converts the box α binding site for CCAAT/enhancer-binding protein and related factors into the perfect palindromic sequence 1648-TCTTATATAAGA, which might enhance binding affinity and core promoter/enhancer II activity. Therefore, it is possible that the mutation in the box α influenced the HBe production and viral replication through the BCP activity. In addition, the T1653 mutation corresponds to an amino acid change from histidine to tyrosine at aa 94 of the X protein, so this alteration of X protein might be hepatocarcionogenesis. Gunther et al. [26] analyzed T1653, T1762, and A1764 mutations in the context of an in vitro study involving wild-type HBV (genotype D, AF043594), and they reported that the preC mRNA and HBeAg secretion was reduced, but the amount of progeny virus DNA in the cells and in the culture medium increased only marginally (if at all), as determined by Southern blot analysis. However, because the genotype was different from that in our study (genotype D vs. genotype C) and the mutant type included not only T1653, T1762, and A1764 mutations but also other mutations in the core promoter, it is possible that some other mutation influenced the results in the earlier study.

In conclusion, the addition of the T1653 mutation in the box α to the BCP mutation increases the risk of HCC in patients with HBV genotype C infection, suggesting that HBV with both the T1653 mutation and the BCP double mutation in patients with chronic hepatitis B should be eradicated by antiviral therapy. Functional analyses of HBV strains with the T1653 mutation are needed in vitro and in vivo.

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References

1. Parkin DM. International variation. Oncogene **2004**; 23:6329–40.
2. Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology **2003**; 124:327–34.
3. Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. Hepatology **1999**; 29:946–53.
4. Blackberg J, Kidd-Ljunggren K. Mutations within the hepatitis B virus genome among chronic hepatitis B patients with hepatocellular carcinoma. J Med Virol **2003**; 71:18–23.
5. Lindh M, Gustavson C, Mardberg K, Norkrans G, Dhillon AP, Horal P. Mutation of nucleotide 1,762 in the core promoter region during hepatitis B e seroconversion and its relation to liver damage in hepatitis B e antigen carriers. J Med Virol **1998**; 55:185–90.
6. Laskus T, Rakela J, Nowicki MJ, Persing DH. Hepatitis B virus core promoter sequence analysis in fulminant and chronic hepatitis B. Gastroenterology **1995**; 109:1618–23.
7. Kobayashi M, Arase Y, Ikeda K, et al. Precore wild-type hepatitis B virus with G1896 in the resolution of persistent hepatitis B virus infection. Intervirology **2003**; 46:157–63.
8. Raimondo G, Schneider R, Stemler M, Smedile V, Rodino G, Will H. A new hepatitis B virus variant in a chronic carrier with multiple episodes of viral reactivation and acute hepatitis. Virology **1990**; 179: 64–8.
9. Sugauchi F, Orito E, Ichida T, et al. Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. Gastroenterology **2003**; 124:925–32.
10. Okamoto H, Tsuda F, Sakugawa H, et al. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol **1988**; 69:2575–83.
11. Magnius LO, Norder H. Subtypes, genotypes, and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. Intervirology **1995**; 38:24–34.
12. Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology **2001**; 34:590–4.
13. Lindh M, Horal P, Dhillon AP, Norkrans G. Hepatitis B virus DNA levels, precore mutations, genotypes and histological activity in chronic hepatitis. J Viral Hepat **2000**; 7:258–67.
14. Lindh M, Hannoun C, Dhillon AP, Norkrans G, Horal P. Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. J Infect Dis **1999**; 179: 775–82.
15. Tanaka Y, Hasegawa I, Kato T, et al. A case-control study for differences

among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. Hepatology **2004**; 40:747–55.

16. Orito E, Mizokami M, Sakugawa H, et al. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology **2001**; 33:218–23.
17. Takahashi K, Akahane Y, Hino K, Ohta Y, Mishiro S. Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. Arch Virol **1998**; 143:2313–26.
18. Usuda S, Okamoto H, Tanaka T, et al. Differentiation of hepatitis B virus genotypes D and E by ELISA using monoclonal antibodies to epitopes on the preS2-region product. J Virol Methods **2000**; 87:81–9.
19. Sugauchi F, Mizokami M, Orito E, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. J Gen Virol **2001**; 82:883–92.
20. Chu CM, Yeh CT, Lee CS, Sheen IS, Liaw YF. Precore stop mutant in HBeAg-positive patients with chronic hepatitis B: clinical characteristics and correlation with the course of HBeAg-to-anti-HBe seroconversion. J Clin Microbiol **2002**; 40:16–21.
21. Huy TT, Ushijima H, Quang VX, et al. Characteristics of core promoter and precore stop codon mutants of hepatitis B virus in Vietnam. J Med Virol **2004**; 74:228–36.
22. Tangkijvanich P, Anukulkarnkusol N, Suwangool P, et al. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. J Clin Gastroenterol **2000**; 31:302–8.
23. Izumi R, Shimizu K, Kiriyama M, et al. Alpha-fetoprotein production by hepatocellular carcinoma is prognostic of poor patient survival. J Surg Oncol **1992**; 49:151–5.
24. Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. J Virol **1996**; 70:5845–51.
25. Li J, Buckwold VE, Hon MW, Ou JH. Mechanism of suppression of hepatitis B virus precore RNA transcription by a frequent double mutation. J Virol **1999**; 73:1239–44.
26. Gunther S, Piwon N, Will H. Wild-type levels of pregenomic RNA and replication but reduced pre-C RNA and e-antigen synthesis of hepatitis B virus with C(1653) → T, A(1762) → T and G(1764) → A mutations in the core promoter. J Gen Virol **1998**; 79:375–80.
27. Yu MW, Yeh SH, Chen PJ, et al. Hepatitis B virus genotype and DNA level and hapatocellular carcinoma: a prospective study in men. J Natl Cancer Inst **2005**; 97:265–72.
28. Yuh CH, Chang YL, Ting LP. Transcriptional regulation of precore and pregenomic RNAs of hepatitis B virus. J Virol **1992**; 66:4073–84.
29. Mizokami M, Nakano T, Orito E, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett **1999**; 450:66–71.
30. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology **1994**; 19:1513–20.

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Case report

Juvenile hepatocellular carcinoma with congestive liver cirrhosis

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A case of juvenile hepatocellular carcinoma (HCC) with congestive liver cirrhosis is reported. The patient was a 21-year-old woman. She had been diagnosed as having transposition of the great arteries, type 2, in 1978. She underwent the Mustard operation, but suffered from chronic heart failure. In 1995, she experienced abdominal pain and underwent examination. The laboratory data were normal, except for elevated total bilirubin (5.2 mg/dl). Blood examinations were performed at frequent intervals, and the total bilirubin level fluctuated between 0.9 and 8.1 mg/dl over the next 4 years, but the transaminase level remained normal. In 1999, she experienced abdominal pain again and was admitted to our hospital. Computed tomography showed four space-occupying lesions in the liver; 45 mm, 20 mm, 12 mm, and 10 mm in size. She was diagnosed as having HCC, and transcatheter arterial chemoembolization and percutaneous ethanol injection therapy were performed. Histology of the cancerous and the noncancerous liver tissue revealed HCC, moderately differentiated type, in cirrhotic liver with congestion. This patient had no background factors of liver disease, except for liver congestion, associated with the chronic heart failure. Because most patients with cardiac cirrhosis die of cardiac disease, only a small number of these patients develop liver failure. However, the incidence of HCC in patients with congestive liver disease is likely to increase in the future, as survival time is prolonged with the advances in treatment for chronic heart failure. Therefore, patients with congestive liver disease should be followed, taking into account the possibility of HCC.

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Key words: congestive liver cirrhosis, cardiac cirrhosis, juvenile hepatocellular carcinoma

Introduction

Congestive liver disease results from gross outflow block to the hepatic vein. It is caused by the Budd-Chiari syndrome (BCS), or by congestive heart failure. There are reports of some patients in whom BCS is complicated by hepatocellular carcinoma (HCC). However, there have been no previous reports of HCC occurring with congestive heart failure. According to the Fourteenth Report (1996–1997) of the Liver Cancer Study Group of Japan, of HCC patients in Japan, the rate of juvenile incidence, under age 40 years, is reported to be as low as 1.1%. The most common background factor of juvenile HCC in Japan is continuous infection with hepatitis B virus (HBV). The positivity rate of hepatitis B surface antigen (HBsAg) in juvenile HCC is 66.7%–91.0%.[1–4] We encountered juvenile HCC caused by congestive liver cirrhosis due to chronic heart failure. The patient had no background factors for liver disease except for liver congestion. We report this case and also discuss the relationship between congestive liver cirrhosis and HCC.

Case report

In 1978, the patient, as a newborn female baby, was admitted to Osaka University Hospital with cyanosis. Examination by cardiovascular angiography led to the diagnosis of transposition of the great arteries type 2. She underwent the Mustard operation in 1979, but

continued to suffer from pulmonary hypertension and right-sided heart failure.

In 1995, she complained of right hypochondrial pain. The laboratory data were normal, except for the total bilirubin level: aspartate aminotransferase (AST), 25 U/l; alanine aminotransferase (ALT), 18 U/l; albumin, 3.5 g/dl; total bilirubin, 5.2 mg/dl; and direct bilirubin, 1.3 mg/dl. Ultrasonography and computed tomography (CT) also showed normal findings. She was followed at our hospital, and regularly underwent blood examinations (average interval, 7.6 days). The results showed that the total bilirubin level was elevated (2.9 mg/dl on average), but the transaminase level was normal (Fig. 1).

Fig. 1. Blood examination data. The patient was examined at regular intervals from 1995 to 1999. The total bilirubin level was elevated, while the ALT level was normal. *T-Bil*, total bilirubin; *ALT*, alanine aminotransferase

In September 1999, she experienced right hypochondrial pain again and was admitted to our hospital. Ultrasonography revealed a hyperechoic lesion, 50 mm in size, in Couinaud's segment 8 (S8) of the liver.

Physical examination on admission revealed jaundice of the blepharoconjunctival tissue, jugular vein swelling, and cyanosis of the nail bed. Auscultation revealed Levine III/VI degree continuous cardiac murmur in the third intercostal space on the left side of the sternum. Lung fields were clear. She had an enlarged liver that was palpable 4 cm below the right costal margin, with tenderness. There was no neurological abnormality. Laboratory data included the following: AST, 11 U/l; ALT, 8 U/l; albumin, 3.3 g/dl; total bilirubin, 3.9 mg/dl; direct bilirubin, 0.8 mg/dl; α-fetoprotein (AFP), 88 ng/ml; and protein-induced vitamin-K absence-II (PIVKA-II), 704 μg/ml (Table 1). All hepatitis viral markers were negative. She did not habitually consume alcohol or smoke. The findings were negative for both antinuclear antibody and anti-DNA antibody. Antimitochondrial antibody (AMA) was positive, but the AMA titer was low. Because ferric and cuprous metabolic markers were atypical, both hemochromatosis and Wilson's disease were ruled out.

Chest X-ray film showed cardiac dilatation and bulging of the right diaphragm (Fig. 2). The cardiothoracic ratio was 60%. Ultrasonography showed hyperechoic lesions in S8, S7, and S3 of the liver. Unenhanced CT showed space-occupying lesions (SOLs), as low-density areas, in S8, S7/8, and S3 of the liver. These lesions were enhanced in the early phase of incremental CT (Fig. 3abc). In the portal phase, the liver parenchyma showed

Table 1. Laboratory data on admission

Hematology		**Biochemical examination**		**Infection**	
WBC	3840/μl	Na	142 mEq/l	HBsAg	(−) <0.5 U/ml
RBC	540 × 10^4/μl	K	3.5 mEq/l	Anti-HBs	(−) <10.0 mU/ml
Hb	10.8 g/dl	Cl	110 mEq/l	Anti-HBc IgM	(−) 0.1 CI
Ht	38.4%	BUN	13 mg/dl	Anti-HBc IgG	19.3%
Plt	11.4 × 10^4/μl	Creatinine	0.5 mg/dl	Dilution (×200)	(−)
		Ca	4.4 mEq/l	HBV-DNA polymerase	0 cpm
Coagulation test		AST	11 U/l	Anti-HAV IgM	(−)
PT-%	40%	ALT	8 U/l	Anti-HCV	(−)
PT-INR	1.80	ALP	184 U/l	RPR	(−)
APTT	43 s	γ-GTP	48 U/l	ATLA	(−)
Fibrinogen	179 mg/dl	LDH	279 U/l	HIV	(−)
FDP	2.95 μg/ml	T-Bilirubin	3.9 mg/dl		
		D-Bilirubin	0.8 mg/dl	**Others**	
Tumor markers		I-Bilirubin	3.1 mg/dl	ANA	(−)
AFP	88 ng/ml	CRP	<0.2 mg/dl	Anti-DNA antibody	(−)
PIVKA-II	704 μg/ml	T-Protein	6.4 g/dl	AMA	×20
CEA	1 ng/ml	Albumin	3.3 g/dl	IgM	104 U/ml
CA19-9	28 U/ml	T-Cholesterol	89 mg/dl	Fe	23 μg/dl
		NH_3	99 μg/dl	Ferritin	10 ng/dl
				Cu	127 μg/dl
				Ceruloplasmin	29 mg/dl

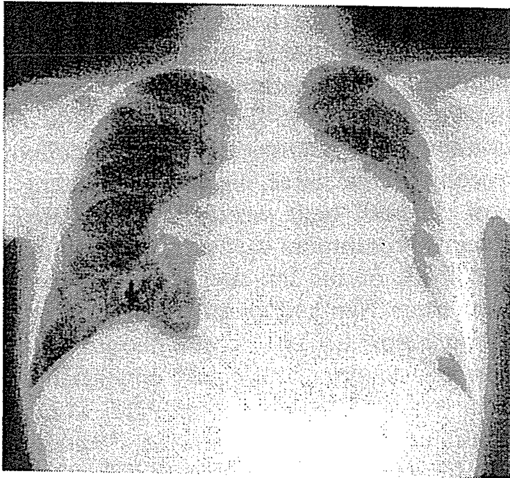

Fig. 2. Chest X-ray. Cardiac dilatation and bulging of the right diaphragm can be seen (*arrow*)

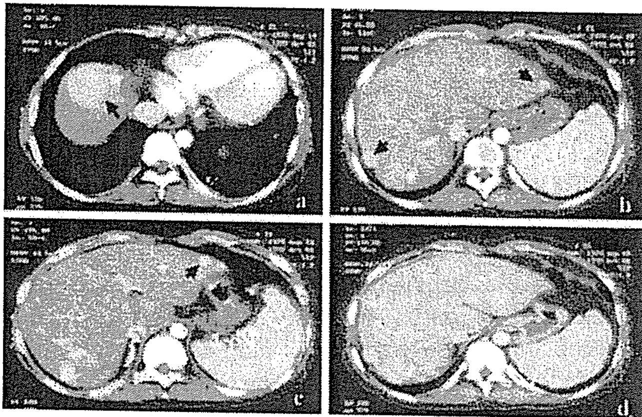

Fig. 3a–d. Abdominal computed tomography (CT) scans. **a** Space-occupying lesion (SOL), 45 mm in size, in S8 of the liver, which was enhanced in the early phase (*arrow*). **b** SOLs, one, 20 mm in size, in S7/8 of the liver, and one, 10 mm in size, in S3 of the liver, which were enhanced in the early phase (*arrows*). **c** SOL, 12 mm in size, in S3 of the liver, which was enhanced in the early phase (*arrow*). **d** In the portal phase, the liver parenchyma was spottily enhanced

spotty enhancement (Fig. 3d). Magnetic resonance imaging (MRI) showed the same SOLs as those described above. Echocardiography revealed hypokinesis and hyposystole of the left ventricle, congestion, and pulmonary hypertension (Fig. 4). Selective angiography of the celiac artery demonstrated hypervascular lesions in S8, S7, and S3 of the liver.

The patient was diagnosed as having HCC, based on these findings. To treat the HCC, transcatheter arterial chemoembolization with emulsion of epirubicin (Farumorubicin; Kyowa Hakko Kogyo, Tokyo, Japan) and Lipiodol (Lipiodol TACE) and percutaneous ethanol injection therapy (PEIT) were performed. After the Lipiodol TACE and PEIT, subsequent examination of AFP and PIVKA-II showed normalized values.

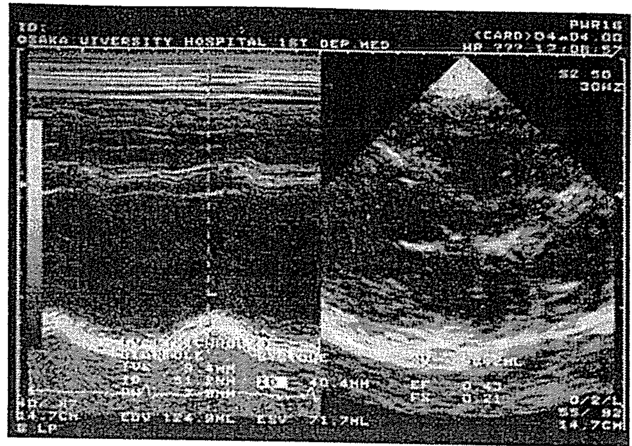

Fig. 4. Echocardiography showed hypokinesis and hyposystole of the left ventricle, congestion, and pulmonary hypertension. LVDd (left ventricular end-diastolic diameter), 53 mm; LVDs (left ventricular end-systolic diameter), 38 mm; *IVST* (interventricular septum thickness), 9.4 mm; *PWT* (posterior wall thickness), 7.8 mm; *EF* (ejection fraction), 43%; *FS* (fractional shortening), 21%; ΔPG (pressure gradient); 38 mm

To investigate the etiology of HCC, needle biopsy of the cancerous and noncancerous liver tissues was performed under ultrasonographic guidance. Histologically, the cancerous liver tissue had large nuclei (Fig. 5a) and was diagnosed as HCC of moderately differentiated type. In the noncancerous liver tissue, fibrous thickening was observed in the region of the central vein (Fig. 5b). The cancerous and the noncancerous liver tissues did not show staining for HBsAg or hepatitis B core antigen (HBcAg). Histological findings suggested that chronic passive congestion was the main cause of the liver cirrhosis.

After discharge from the hospital, the patient was reviewed at regular intervals until November 2000. AFP and PIVKA-II levels remained normal, and further ultrasonography and CT showed no evidence of HCC recurrence during the follow-up period at our hospital.

Discussion

HCC generally arises from chronic hepatitis and cirrhosis. Known etiological factors are hepatitis viral infection; alcoholic liver disease; autoimmune liver diseases, such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis; and metabolic liver diseases, such as hemochromatosis and Wilson's disease. Among these etiological factors, the most important is chronic hepatitis viral infection. In a recent