–80°C until analysis. All patients underwent hepatic resection for histologically confirmed HCC between January 1996 and December 2003. There were 189 men and 51 women, with a mean age of 63.9 years (range, 25 to 83 years).

Liver specimens were fixed in 10% formalin for 48 h, then cut into 0.5-cm-thick slices through the maximum diameter of the tumor for histologic analysis. The system of the Liver Cancer Study Group of Japan[15] was used to categorize histologic findings and tumor stage. When clusters of cancer cells were present in the portal vein, the specimen was classified according to the degree of portal vein thrombus (vp1 was defined as tumor thrombus distal to the second branch of portal vein; vp2, as tumor thrombus in the second portal vein branch; and vp3 as tumor thrombus located in the first portal vein branch). The grade and stage of chronic active hepatitis in the noncancerous liver were scored using the histologic activity index (HAI),[16] which was calculated from four parameters: periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis. All patients were followed from surgery until either death or the endpoint of this study (December 31, 2003). This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institution. Informed consent was obtained from each patient.

Measurement of tumor markers

Serum CYFRA 21-1 was measured using an electrochemiluminescent immunoassay (ECLIA). The assay, using an Elecsys 2010 analyzer (Roche Diagnostics, Basel, Switzerland) is based on the ability of an electrochemically luminescent molecule, a tris (2,2'-bipyridyl) ruthenium (II) complex, to be repeatedly excited by tripropylamine. The system can be applied to both competitive and sandwich-format immunoassays. CYFRA 21-1 was recognized by two mouse monoclonal antibodies, a biotinylated monoclonal cytokeratin 19-specific antibody (Ks 19-1) and a monoclonal cytokeratin 19-specific antibody (BM 19-21), directed against two different epitopes of a fragment of cytokeratin 19. In the first incubation, Ks 19-1 and BM 19-21 labeled with the ruthenium complex were allowed to react, forming a sandwich complex. The next incubation was done after the addition of streptavidin-coated microparticles, so the sandwich complex could bind to the particulate solid phase via the interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell, where the microparticles were captured magnetically upon the surface of the electrode. Unbound reactants were removed with a phosphate-tripropylamine buffer. Application of voltage to the electrode then induced chemiluminescent emission, measured by a photomultiplier. For serum CYFRA 21-1, a cutoff value of 3.0 $ng \cdot ml^{-1}$ was derived from the finding of 95% specificity for benign liver disease, in our previous report.[10] Serum DCP (cutoff value, 40 $mAU \cdot ml^{-1}$) was also determined by an ECLIA. AFP (cutoff value, 20 $ng \cdot ml^{-1}$) was measured using a chemiluminescent immunoassay (CLIA).

Statistical analysis

For correlations between CYFRA 21-1 and other tumor markers, Pearson's correlation coefficient was used, with –0.4 to 0.4 considered as having no correlation. Data values are given as medians, with 25th and 75th percentiles, of marker concentrations. Kruskal-Wallis one-way analysis of variance was performed initially for multiple-comparison tests. When this analysis was significant, pairs of groups were compared using the Mann-Whitney *U*-test. Survival rates were calculated by the Kaplan-Meier method, and survival differences were tested in a univariate manner, using the log-rank test.

Results

In patients with HCC, the median concentration of CYFRA 21-1 was 1.9 $ng \cdot ml^{-1}$ (interquartile range, 1.4 to 2.8 $ng \cdot ml^{-1}$). The sensitivities of CYFRA 21-1, AFP, and DCP were 18.8%, 53.8%, and 52.5%, respectively (Table 1). No correlations between the individual tumor markers were found (coefficients of correlation, CYFRA vs AFP, 0.031; CYFRA vs DCP, –0.049; AFP vs DCP, 0.014). Therefore, combination assays using CYFRA 21-1, AFP, and DCP were performed (Table 1). A positive rate higher than 80% was obtained when AFP was combined with DCP, suggesting that combinations using CYFRA 21-1 were useless for diagnosing HCC.

Table 1. Positive rates with combination assays using CYFRA 21-1, alpha-fetoprotein (AFP), and des-gamma-carboxy prothrombin (DCP)

CYFRA	AFP	DCP	CYFRA + AFP	CYFRA + DCP	AFP + DCP	CYFRA + AFP + DCP
18.8% (45/240)	53.8% (129/240)	52.5% (126/240)	62.5% (150/240)	60.8% (146/240)	80.4% (193/240)	83.8% (201/240)

Table 2. Serum CYFRA 21-1 concentrations ($ng \cdot ml^{-1}$) in 240 patients with hepatocellular carcinoma

	Number of patients	Serum CYFRA 21-1 concentration; median (interquartile range)	*P* value
Age (years)			
<65	103	1.6 (1.1–2.6)	0.0554[a]
≥65	137	2.0 (1.5–3.0)	
Sex			
Male	189	1.9 (1.3–2.8)	0.6808[a]
Female	51	1.8 (1.5–3.0)	
HCV-Ab			
Present	169	1.9 (1.3–2.8)	0.7475[a]
Absent	71	1.6 (1.4–2.6)	
HBs-Ag			
Present	37	1.6 (1.2–2.2)	0.0868[a]
Absent	203	1.9 (1.4–2.8)	
HAI score			
Grade 0–1	70	1.8 (1.3–3.2)	0.1020[a]
2–4	170	1.8 (1.1–2.3)	
Stage 0–3	122	2.0 (1.3–2.9)	0.3233[a]
4 (Cirrhosis)	118	1.7 (1.4–2.5)	
Tumor size (cm)			
<5.0	181	1.8 (1.3–2.5)	0.0079[a]
≥5.0	59	2.4 (1.4–3.4)	
Portal vein tumor thrombus			
Present	80	2.2 (1.5–3.3)	0.0024[a]
Absent	160	1.7 (1.2–2.7)	
Number of tumors			
Single	130	1.9 (1.3–2.8)	0.6596[a]
Multiple	110	1.8 (1.4–2.8)	
Tumor differentiation			
Well	17	1.8 (1.6–2.4)	0.4983[b]
Moderate	142	1.8 (1.3–2.8)	
Poor	81	2.1 (1.4–3.0)	
TNM Stage			
I	25	1.9 (1.2–2.1)	0.0813[b]
II	89	1.7 (1.1–2.8)	
III	79	1.8 (1.4–2.7)	
IV	47	2.1 (1.5–3.5)	

[a] Mann-Whitney *U*-test
[b] Kruskal-Wallis test

The serum CYFRA 21-1 titer did not differ with regard to age, sex, type of hepatitis viral infection, or degree of inflammation and fibrosis in the noncancerous portion (Table 2). A significant difference in CYFRA 21-1 concentration (median and interquartile range) was noted between patients with tumors greater than 5 cm (2.4; 1.4 to 3.4 $ng \cdot ml^{-1}$) versus smaller ones (1.8; 1.3 to 2.5 $ng \cdot ml^{-1}$; Mann-Whitney *U*-test; $P = 0.0079$). Concentrations of CYFRA 21-1 were significantly higher in patients with portal vein thrombus (2.2; 1.5 to 3.3 $ng \cdot ml^{-1}$) than in those without portal vein thrombus (1.7; 1.2 to 2.7 $ng \cdot ml^{-1}$; Mann-Whitney *U*-test; $P = 0.0024$). In patients with portal vein thrombus, the sensitivity and specificity for CYFRA 21-1 were 28.8% and 86.3%, respectively, whereas these parameters for DCP were 70% and 47.5%, respectively. The median and interquartile ranges of serum CYFRA 21-1 concentrations ($ng \cdot ml^{-1}$) for patients with vp0, vp1, vp2, and vp3, respectively were 1.7 (1.2 to 2.7), 2.1 (1.5 to 2.7), 2.7 (1.3 to 3.4), and 3.4 (2.2 to 4.6) (Fig. 1). Although CYFRA 21-1 demonstrated low sensitivity in patients with portal vein thrombus, serum CYFRA 21-1 was significantly elevated with progression from vp0 to vp3 (Kruskal-Wallis test; $P = 0.0040$). The sensitivities of CYFRA 21-1 for vp1, vp2, and vp3, respectively, were 19.0%, 50.0%, and 58.3%. Serum CYFRA 21-1 did not differ according to tumor differentiation or number of tumors. Although tumor size and portal vein thrombus were related to serum CYFRA 21-1, no significant difference was evident among CYFRA 21-1 concentrations at any TNM stage (Kruskal-Wallis test; $P = 0.0813$). However, CYFRA 21-1 concentrations for stage IV tumor (2.1; 1.5 to 3.5 $ng \cdot ml^{-1}$) were significantly higher than concentrations for tumors below stage III

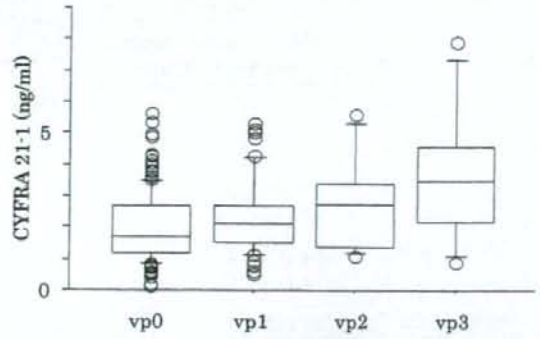

Fig. 1. Distribution of individual serum cytokeratin-19 fragment (*CYFRA*) 21-1 values, according to portal vein tumor thrombus, in patients with hepatocellular carcinoma. Data values are presented as upper and lower quartiles and ranges (*boxes*), median values (*horizontal lines*), and middle 90% distribution (*whisker lines*). *vp0*, no portal vein thrombus; *vp1*, tumor thrombus distal to the second branch of portal vein; *vp2*, tumor thrombus in the second portal vein branch; *vp3*, tumor thrombus located in the first portal vein branch

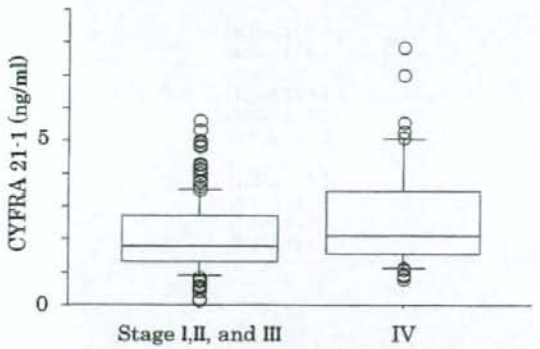

Fig. 2. Distribution of individual serum CYFRA 21-1 values, according to TNM stage, in patients with hepatocellular carcinoma. Data values are presented as upper and lower quartiles and ranges (*boxes*), median values (*horizontal lines*), and middle 90% distribution (*whisker lines*)

(1.8; 1.3 to 2.7 ng·ml^{-1}; Mann-Whitney *U*-test; $P = 0.0075$; Fig. 2).

Tumor-free survival rates for patients with high (exceeding 3.0 ng·ml^{-1}) and low concentrations of CYFRA 21-1 were 53% and 43% respectively, at 3 years. There was no significant difference in tumor-free survival according to serum CYFRA 21-1 levels (log-rank test; $P = 0.3400$). Even when the tumor-free survival rate was calculated for each subgroup stratified by TNM stage, patients with high concentrations of CYFRA 21-1 showed tumor-free survival comparable to that in those with low concentrations of CYFRA 21-1.

Discussion

Characteristic combinations of CK polypeptides in epithelia differ depending on the organ or type of differentiation, suggesting that the CK pattern in cells is associated with their biologic function.[17] In normal human liver, hepatocytes contain CK8 and CK18, while bile duct cells have CK7 and CK19.[18-20] Because CK patterns are preserved during neoplastic transformation, HCC would be expected to express CK8 and CK18, but not CK7 or CK19.[17-19] However, CK19 expression has been reported in some HCC tumors, even in those with typical HCC growth patterns and morphologic appearance.[20-22] The CYFRA 21-1 assay was developed to measure a soluble fragment of CK19 in serum, one of the most sensitive tumor markers for non-small-cell lung cancer.[23-29] This test is useful for the clinical monitoring of treatment and as a possible predictor of survival in patients with non-small-cell lung cancer.[29-33] In primary liver cancer, serum CYFRA 21-1 showed higher sensitivity for ICC than established markers, including CEA and CA 19-9, and reflected differences in tumor burden.[10] These findings indicated that CYFRA 21-1 could be a reliable diagnostic and monitoring tool for ICC. In contrast, the clinical usefulness of serum CYFRA 21-1 in HCC is still unknown, although some investigators have reported high concentrations of CYFRA 21-1 in some patients with HCC.[10-12]

Nagai et al.[12] reported that CYFRA 21-1 might be useful for diagnosing HCC, because CYFRA 21-1 was elevated above the reference range (2.5 ng·ml^{-1}) in 33 of 70 patients with HCC. In the present study, however, serum CYFRA 21-1 was elevated in only 45 of 240 patients with HCC, a sensitivity of 18.8%. In addition, the positive rates of combinations using AFP and DCP were much higher than those of combinations using CYFRA 21-1 plus AFP or DCP. These results suggested that serum CYFRA 21-1 has limited diagnostic value in HCC.

In this study, patients with tumors measuring more than 5 cm had significantly higher concentrations of CYFRA 21-1 than patients with smaller tumors. Previous studies also reported that CYFRA 21-1 was elevated with increased tumor size and number.[11,13] In the present study, patients with portal vein tumor thrombus had significantly higher CYFRA 21-1 concentrations than those without thrombus. Furthermore, serum CYFRA 21-1 consistently increased with the progression of portal vein tumor thrombus. This increased CYFRA 21-1 concentration may have been due to the destruction of large numbers of tumor cells released into the bloodstream. CYFRA 21-1 concentrations in sera from a nude mouse model of HCC metastasis increased in parallel with tumor progression and were

markedly elevated when lung metastasis occurred.[34] Previous studies have identified that CKs, including CK19, play an important role in the regulation of cell migration and invasion.[35,36] MHCC97-H and MHCC97-L are well-established HCC cell lines, with high and low metastastic potential, respectively.[37] HCCLM5 cells, with progressively higher metastastic potential, were obtained from lung metastasis selections of MHCC97-H.[38] Ding et al.[34] have suggested that CK19 expression is related to the metastatic potential of HCC cells, because CK19 showed consistently increased expression from MHCC97-L to HCCLM5. Clinically, an immunohistochemical study of 102 human HCC specimens revealed that patients with CK19-positive HCC more frequently showed metastatic nodules or vascular tumor thrombus than patients with CK19-negative HCC.[34]

CYFRA 21-1 has prognostic value, especially in non-small-cell lung cancer.[29–33] Wu et al.[22] found that prognosis was poorer in patients with biliary differentiation marker (AE1-AE3, CK19)-positive HCC than in those without. We have previously reported that CK19 expression in HCC was associated with early postoperative recurrence due to intra- and extrahepatic metastasis, even after curative resection.[21] However, in the present study, serum CYFRA 21-1 was not related to postoperative prognosis in patients with HCC, even though patients with advanced HCC showed higher concentrations of CYFRA 21-1.

In conclusion, although high concentrations of CYFRA 21-1 were often detected in patients with a tumor greater than 5 cm in diameter or tumor thrombus in the major portal vein, CYFRA 21-1 is not a useful diagnostic tool for hepatocellular carcinoma, because of its low sensitivity.

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SURGERY TODAY

Hepatocellular Carcinoma Arising from Nonalcoholic Steatohepatitis: Report of Two Cases

SEIKAN HAI[1], SHOJI KUBO[1], TAICHI SHUTO[1], HIROMU TANAKA[1], SHIGEKAZU TAKEMURA[1], TAKATSUGU YAMAMOTO[1], AKISHIGE KANAZAWA[1], MASAO OGAWA[1], KAZUHIRO HIROHASHI[1], and KENICHI WAKASA[2]

[1]Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
[2]Department of Clinical Pathology, Osaka City University Hospital, Osaka, Japan

Abstract
Sporadic cases of hepatocellular carcinoma (HCC) originating from nonalcoholic steatohepatitis (NASH) have recently been reported. Thus, we investigated the prevalence of NASH in patients with HCC. A review of the clinical records of 481 patients who underwent liver resection for HCC in our department between January 1991 and December 2003 revealed only two (0.4%) patients with HCC associated with NASH. Both of these patients had noninsulin-dependent diabetes mellitus, and neither had a history of alcohol consumption or blood transfusion. All serologic markers for hepatitis B and C viruses were negative. Histological examination of the noncancerous hepatic tissue revealed NASH with moderate hepatic fibrosis in one patient and cirrhosis in the other. Thus, clinical follow-up and screening for HCC should be done for patients with hepatic fibrosis caused by NASH, even though this form of hepatitis is an uncommon cause of HCC.

Key words Nonalcoholic steatohepatitis · Hepatocellular carcinoma

Introduction

In 1980, Ludwig et al.[1] coined the term "nonalcoholic steatohepatitis" (NASH) to describe the morphologic pattern of liver injury seen in obese, diabetic women who denied alcohol abuse but whose hepatic histology was consistent with alcoholic hepatitis. Since then the epidemiology, clinical features, histology, pathogenesis, and natural history of this disease have been studied extensively. Originally, it was thought that patients with NASH were asymptomatic and that the disease was nonprogressive.[1,2] However, in some patients NASH has been seen to progress to fibrosis and ultimately, to hepatic cirrhosis,[2–5] hepatocellular carcinoma (HCC),[2,6–9] and hepatic failure.[2,4] Nevertheless, HCC is still thought to be rarely associated with NASH. We found only ten cases reported in the English literature. Among 481 patients who underwent liver resection for HCC in our department between January 1991 and December 2003, 2 had underlying NASH.

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Case Reports

Case 1

A 72-year-old Japanese man was referred to our hospital for evaluation and treatment of a tumor in the anterior segment of the liver, found by computed tomography (CT) at another hospital. The patient was being followed up for hypertension and noninsulin-dependent diabetes mellitus, diagnosed 6 years earlier. On admission, the patient was obese (body mass index: 30.4 kg/m^2). His blood sugar had been controlled at approximately 140 mg/dl (normal range, 70–105 mg/dl) with diet and exercise, and his hypertension had been treated with a calcium channel blocker. The patient denied any history of alcohol consumption or blood transfusion. All serologic markers for hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were negative. Laboratory examination revealed no abnormalities, except for an elevated serum concentration of triglyceride (424 mg/dl; normal range, 50–150 mg/dl). The serum concentration of α-fetoprotein was within the normal range (<20 ng/ml), but that of protein induced by vitamin K absence or antagonist II (PIVKA-II) was slightly elevated (58 mAU/ml; normal range, 0–40 mAU/ml).

Computed tomography showed a 4-cm low-density area in the anterior segment of the liver, which was

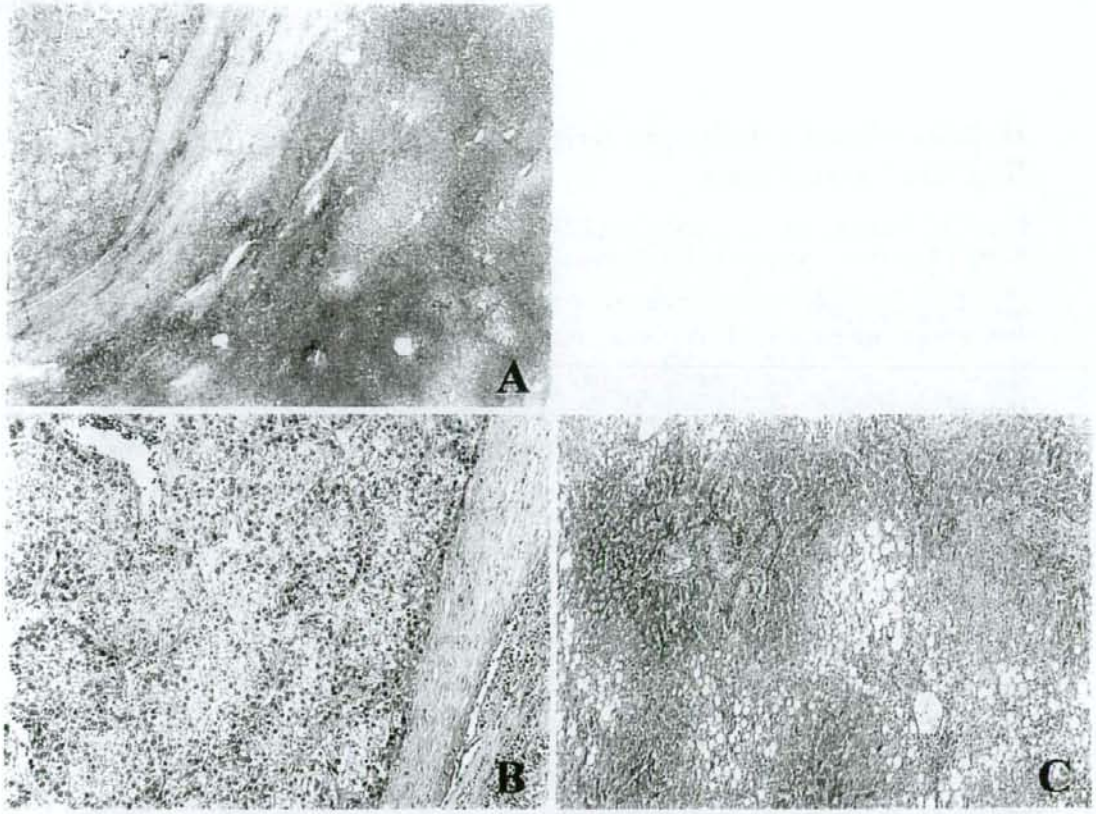

Fig. 1. Case 1. **A** Microscopic examination of the resected specimen revealed hepatocellular carcinoma with a thick fibrous capsule, and noncancerous hepatic tissue with mild fibrosis and macrovesicular fatty change. **B** The hepatocellular carcinoma had faint cytoplasm and a thick trabecular pattern with moderate differentiation. **C** Pathologic examination of the noncancerous hepatic tissue revealed macrovesicular fatty change in the pericentral venous area, and mild fibrosis in both the periportal and pericentral venous areas. Lymphocytic infiltration, proliferative cholangioles, and mild portal fibrosis in Glisson's sheath, as well as neutrophilic/monocytic infiltration in hepatic lobule, were seen (H&E stain; **A** ×20, **B** ×100, **C** ×50)

slightly enhanced during the early phase of contrast enhancement. The mass was stained with contrast medium during abdominal angiography (AAG). Abdominal ultrasonography showed a 4-cm hypoechoic mass within a bright liver. We made a diagnosis of HCC and performed right hepatic lobectomy. The tumor was diagnosed as a moderately differentiated HCC with macrovesicular fatty change and ballooning degeneration, by pathologic examination (Fig. 1A,B). Histologic examination of the noncancerous hepatic tissue revealed mild steatosis, mild periportal and perivenular fibrosis, and mild neutrophil and lymphocytic infiltration (Fig. 1C). These findings were consistent with NASH with moderate hepatic fibrosis but not cirrhosis.

Case 2

A 65-year-old Japanese man underwent a CT scan to investigate the cause of a convulsion. The CT scan showed a 6-cm, low density mass in the anterior segment of the liver, with enhancement during the early phase using intravenous contrast material. The lesion was stained with contrast medium on AAG. The serum concentration of PIVKA-II was remarkably elevated to 6240 mAU/ml. These findings indicated that the tumor was HCC, and the patient was referred to our hospital for treatment. On admission, he was not obese (body mass index: 22.9 kg/m^2). Laboratory examination disclosed abnormal elevations of aspartate transaminase

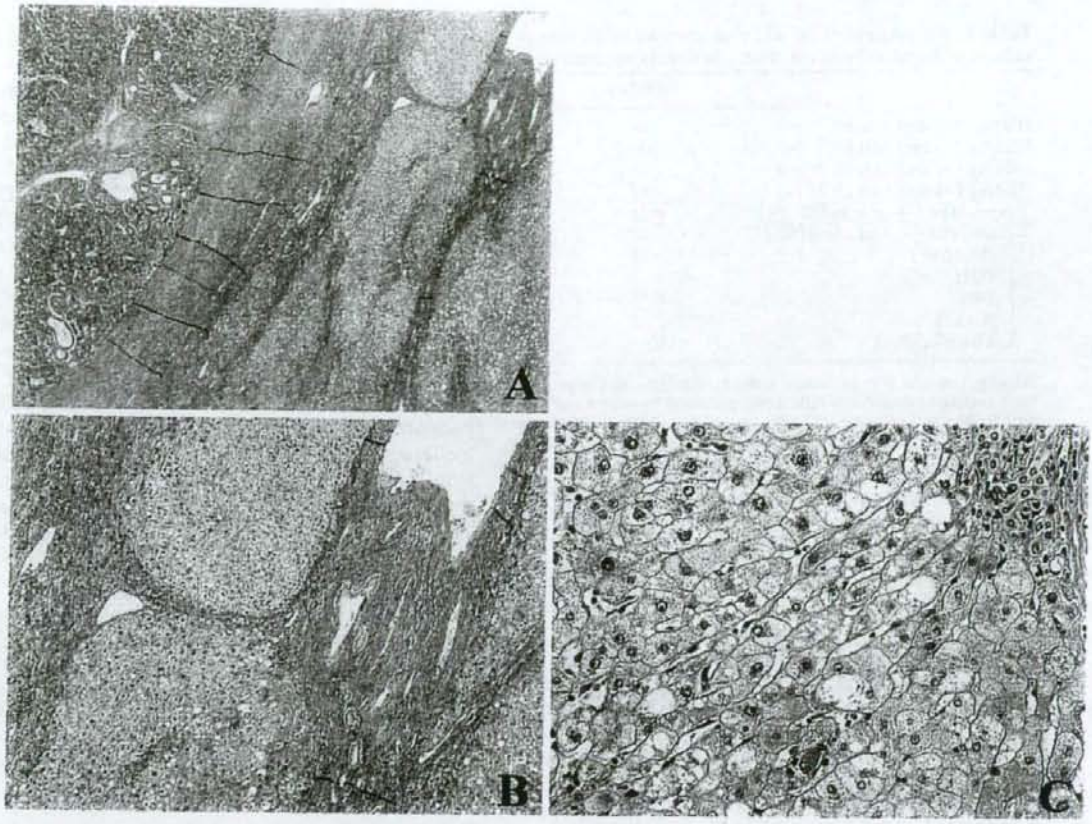

Fig. 2. Case 2. This hepatocellular carcinoma had a thick fibrous capsule and moderate differentiation. **A** Histologic examination of the noncancerous hepatic tissues revealed severe bridging fibrosis. **B** Monocytes and neutrophils infiltrated the portal areas and the hepatocytes showed fatty change associated with necrosis and inflammation. **C** In some noncancerous areas, macrovesicular fatty change, glycogenated nuclei, and Mallory bodies were seen within the hepatocytes (H&E stain; **A** ×20, **B** ×50, **C** ×200)

(63 IU/l; normal range, 12–40 IU/l), alanine transaminase (72 IU/l; normal range, 10–45 IU/l), γ-glutamyl transpeptidase (363 IU/l; normal range, 5–60 IU/l), blood sugar (160 mg/dl), and hemoglobin A1c (6.3%; normal range, 4.3%–5.8%). The patient denied any history of alcohol consumption or blood transfusion. The results of all serologic examinations for markers of HBV and HCV infection were negative.

We preformed subsegmental resection of the liver and the tumor, which had a thick fibrous capsule, was diagnosed pathologically as a moderately differentiated HCC (Fig. 2A). The adjoining noncancerous hepatic parenchyma showed portal infiltrates of monocytes and neutrophils, and the fatty changes of hepatocytes associated with necrosis and inflammation (Fig. 2B). In some noncancerous areas, macrovesicular fatty change, glycogenated nuclei, and Mallory bodies were seen within the hepatocytes (Fig. 2C). We also observed perisinusoidal fibrosis, consistent with portal fibrosis. These findings were suggestive of NASH with cirrhosis.

Discussion

The pathogenesis of HCC includes HBV, HCV, alcohol abuse, and dietary aflatoxin exposure. Most patients with HCC have been infected with HCV or HBV, or both, in areas where such viruses are prevalent,[10] and several studies have found that previous and occult HBV infection are etiologic factors for HCC.[11–14]

Table 1. Background of the 481 patients who underwent resection of hepatocellular carcinoma in our department

	Number	%
HBsAg (+) and anti-HCV (−)	66	13.7
HBsAg (−) and anti-HCV (+)	337	70.1
HBsAg (+) and anti-HCV (+)	10	2.1
HBsAg (−) and anti-HCV (−)	68	14.1
Anti-HBs (+) or anti-HBc (+)	41	8.5
Anti-HBs (−) and anti-HBc (−)	27	5.6
Alcohol	15	3.1
AIH	3	0.6
PBC	1	0.2
NASH	2	0.4
Miscellaneous	6	1.2

HBsAg, hepatitis B virus surface antigen; anti-HBs, anti-hepatitis B virus surface antibody; anti-HBc, anti-hepatitis B virus core antibody; anti-HCV, anti-hepatitis C virus antibody; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; NASH, nonalcoholic steatohepatitis

Several investigators have recently reported that HCC developed in patients with NASH, suggesting that NASH is also an etiologic factor in the pathogenesis of HCC.[2,6–9]

The prevalence of NASH varies geographically and has been estimated at 2%–3% in the general population of the United States,[15] but has been diagnosed pathologically in 7%–11% of patients undergoing liver biopsy in the United States and Canada.[1] On the other hand, only a few clinical series have been reported in Japan, and NASH was found in only 1% of 561 biopsy specimens.[16] This difference in prevalence may be related to differences in diet and lifestyle.[16] Although the prevalence of NASH in patients with HCC worldwide remains unclear, it seems to be very low in Japan, being only 0.4% in this series (Table 1), which may be related to the low prevalence of NASH in this country. In the United States, the percentage of patients with HCC who are not infected with HBV or HCV is higher than that in Japan.[10,17] Bugianesi et al.[18] reported that cryptogenic cirrhosis may sometimes be caused by NASH in patients with HCC. Thus, in the United States, the underlying cause of HCC in patients never infected with HBV or HCV, but who have cirrhosis, might be NASH because the incidence of HCC is relatively high in patients with NASH, especially once cirrhosis develops. According to one study, HCC was diagnosed in 6 of 82 patients with NASH, and in 3 of 13 patients with cirrhosis.[6]

The pathogenesis of HCC in NASH remains uncertain, although cirrhosis itself is considered a preneoplastic condition. Our case 2 had cirrhosis, whereas our case 1 had mild hepatic fibrosis but not cirrhosis. Therefore, even though cirrhosis caused by NASH is a risk factor for the development of HCC,[6,7] cirrhosis is not mandatory for the development of HCC in a patient with NASH.

According to our review of the literature, the average age of patients with HCC associated with NASH is higher than that of those with HCC associated with HCV or HBV infection,[19,20] having been reported as 66 years old,[7,9] with a median age of 67.5 years old,[6] and 68.5 years old (present cases). This suggests that HCC develops later or progresses more slowly in patients with NASH than in those infected with a hepatitis virus.

The risk factors for HCC in patients with NASH remain unclear, although cirrhosis has been reported to be closely related to its development.[6] Severe hepatic fibrosis was reported in 15%–50% of patients with NASH, and cirrhosis was present in 7%–16% of those patients.[1,2,4,21] Obesity, noninsulin-dependent diabetes mellitus, and hypertriglyceridemia are often associated with NASH.[21–24] Both our patients had at least one of these disorders. Angulo et al.[5] reported that advanced age (>45 years old), a serum aspartate transaminase/alanine transaminase ratio greater than 1.0, obesity, and noninsulin-dependent diabetes mellitus were independent predictive factors for advanced fibrosis. Thus, these factors may also affect the progress of NASH, resulting in the development of HCC. Although the prevalence of NASH in patients with HCC is low, clinical attention and screening for HCC is necessary in patients with hepatic fibrosis caused by NASH.

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Influence of interferon therapy on outcome after surgery for hepatitis C virus-related hepatocellular carcinoma

Takahiro Uenishi [a,d,*], Shuhei Nishiguchi [b], Akihiro Tamori [c], Takatsugu Yamamoto [d], Taichi Shuto [e], Kazuhiro Hirohashi [e], Shigekazu Takemura [a], Hiromu Tanaka [a], Shoji Kubo [a]

[a] *Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan*
[b] *Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Osaka, Japan*
[c] *Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Japan*
[d] *Department of Surgery, Ishikiri Seiki Hospital, Osaka, Japan*
[e] *Department of General Practice, Osaka City University Hospital, Osaka, Japan*

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Abstract

Influence of interferon (IFN) therapy on postoperative outcomes in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) is still inconclusive. Of 518 patients who underwent hepatic resection for HCV-related HCC, 312 patients with Japan integrated staging score 0–2 were included in this study. Of 50 patients underwent IFN therapy, 29 patients who obtained a normalized alanine aminotransferase (ALT) activity irrespective of disappearance of serum HCV RNA were classified as the response group, while 21 patients were classified as the non-response group because their ALT activities were not normalized and serum HCV RNA persisted. The non-IFN group included 262 patients who had not received IFN therapy. The tumor-free and the overall survival rates for patients in the response group were significantly higher than those in other groups. Only one patient in the response group died of HCC recurrence, and the proportion of deaths associated with liver disease (HCC recurrence or cirrhosis) was significantly lower in the response group than other two groups. IFN therapy can improve postoperative outcomes in patients with HCV-related HCC because of suppression of recurrence and preventing progress of cirrhosis, especially when IFN therapy has controlled their active hepatitis.

Keywords: Hepatocellular carcinoma; Hepatitis C virus; Interferon; Postoperative outcome

1. Introduction

Despite the development of various therapeutic modalities for hepatocellular carcinoma (HCC), surgical resection provided the best results of all those modalities [1–3]. However, the postoperative outcome for hepatitis C virus (HCV)-related HCC is still unsatisfactory because of a high rate of tumor recurrence and progression of the underlying chronic hepatitis or cirrhosis [4–8]. Interferon (IFN) therapy suppresses the development of HCC and prevents worsening of liver function, which increases the survival rate in patients with HCV infection [9–16]. In like manner, control of active hepatitis is important in patients who underwent a curative resection for HCV-related HCC, since the continuous active hepatitis due to persistent HCV infection is strongly associated with recurrence after surgery [4–7]. We reported that patients who detected HCC after IFN therapy are good candidates for hepatic resection because of rarity of postoperative recurrence [17], especially when previous IFN therapy has controlled their active hepatitis associated with HCV [18]. In our prospective randomized controlled study, postoperative long-term IFN-α therapy appears to decrease the incidence

* Corresponding author at: Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. Tel.: +81 6 6645 3841; fax: +81 6 6646 6057.
E-mail address: m6877710@msic.med.osaka-cu.ac.jp (T. Uenishi).

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of recurrence after curative resection of HCV-related HCC [19]. Moreover, postoperative IFN-α therapy may prolong a survival period after surgery in patients with HCV-related HCC by improving liver function and increasing the chance of radical treatment of recurrences [20]. This retrospective study was conducted to evaluate the influence of IFN therapy on postoperative outcome, including cause of death in patients with HCV-related HCC.

2. Patients and methods

2.1. Patients

Between April 1990 and December 2003, 518 patients with anti-HCV (enzyme-linked immunosorbent assay; International Reagents Corporation, Kobe, Japan), but not hepatitis B surface antigen (enzyme immunoassay; International Reagents Corporation, Kobe, Japan), underwent a curative hepatic resection for HCC in the Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Hospital. Curative surgery was defined as a complete removal of all macroscopic tumor masses and no histologic evidence of tumor cells along the parenchymal transection line. Of the 518 patients, 50 were performed IFN therapy (IFN group). Recently, the Japan integrated staging (JIS) score [21], which includes both the grade of cancer stage and grade of liver disease stage according to the Liver Cancer Study Group of Japan criteria [22], has been advocated. The JIS score is recognized as an international standard scale, since its effectiveness in stratifying patients and its prognostic predictive power have been demonstrated [23]. All 50 patients who received IFN therapy assigned a JIS score 0–2. Of 468 patients who had not received IFN therapy, the non-IFN group included 262 patients with a JIS score 0–2.

Of the 50 patients with IFN therapy, 32 patients underwent a hepatic resection for HCC that had been detected after IFN therapy. Twenty-four patients were received IFN therapy after surgery. Six patients underwent IFN therapy both before and after surgery because preoperative IFN therapy was not effective. Patients with postoperative IFN therapy received 6 MIU of IFN-α intramuscularly every day for 2 weeks, then three times weekly for 14 weeks, and finally twice weekly for 88 weeks (total dose, 1572 MIU). On the other hand, the kind, dosage, and duration of IFN administered before surgery were various, although most patients received IFN-α. The response to IFN therapy was classified on the basis of changes in the HCV RNA levels and serum alanine aminotransferase (ALT) activity during and immediately after IFN administration, and for at least 1 year after IFN therapy. The serum HCV RNA was assayed by a reverse transcriptase-nested polymerase chain reaction using primers derived from a conserved 5′-untranslated region of the viral genome [15] and viral load was measured by using a branched DNA probe method (Quantiplex HCV-RNA, Chiron Corp., Emeryille, CA). Nineteen patients obtained a sustained response that was defined as return of the ALT activity to within the reference range and no detectable serum HCV RNA for at least 1 year after IFN therapy. A biochemical response, which was defined as a normalized ALT activity for at least 1 year after IFN therapy with or without the transient disappearance of serum HCV RNA, was obtained in 10 patients. These 29 patients were defined as the response group, while the other 21 patients were defined as the non-response group because they had no decrease in their ALT activity and had persistent serum HCV RNA. The clinicopathologic findings and surgical outcomes were compared among these groups. This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institution. Informed consent was obtained from each patient.

2.2. Detection of recurrence

Serum alpha-fetoprotein and protein induced by Vitamin K absence and antagonist II were measured 1 month after surgery and every 3 months thereafter. Hepatic ultrasound scanning, computed tomography, or some combination of these tests was done 1 month after surgery and every 3 months thereafter. When a recurrence of the HCC was strongly suspected on the basis of tumor makers or imaging, selective hepatic angiography, ultrasound-guided biopsy, or both was conducted to establish a definitive diagnosis.

2.3. Histology

The system of the Liver Cancer Study Group of Japan [22] was used to categorize histological findings. The histologic grade of differentiation (well, moderate, or poor) of HCC was determined according to a modification of Edmondson and Steiner [22,24]. The grade (grade of active hepatitis) and stage (degree of hepatic fibrosis) in the non-cancerous portions were determined by the score of the histologic activity index [25,26], which was determined by four events, i.e., periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis.

2.4. Statistics

Differences in other clinicopathologic findings were analyzed by the Mann–Whitney *U*-test, Fisher exact test or χ^2-test. The survival rates were calculated by the Kaplan–Meier method, and were compared with the log-rank test. The tumor-free survival time was measured from the date of resection until the detection of a recurrent tumor or the end point of this study (31 December 2005) in patients without recurrence. The overall survival time was measured from the date of resection until death or the end point of this study in living patients. Multivariate analysis was performed using a Cox regression model with forward stepwise selection. The variables chosen (expect response of IFN therapy) were age (≥65 or <65 years), gender, aspartate aminotransferase (AST)

activity (≤40 or >40 IU/l), ALT activity (≤45 or >45 IU/l), total bilirubin (≤1.0 or >1.0 mg/dl), albumin concentration (<3.5 or ≥3.5 g/dl), platelet count (≥10 or <10 × 10^4 μl^{-1}), serum alpha-fetoprotein (≤20 or >20 ng/ml), the operative method (anatomic or limited resection), the largest diameter of the main tumor (<3.0 or ≥3.0 cm), the number of tumors (single or multiple), the presence of portal invasion, the degree of differentiation of the main tumor (well versus moderate or poor), the grading score (0–1 or 2–4), and the staging score (0–3 or 4).

3. Results

The clinical features, laboratory test results, and pathologic findings of the surgical specimens are summarized in Table 1. The mean age was significantly lower in the IFN group patients than in non-IFN group patients. Although the serum concentrations of albumin just before surgery were significantly higher in the IFN group than in the non-IFN group, there were no differences in the AST and ALT activities, the serum concentrations of total bilirubin, and the platelet counts between the IFN group and the non-IFN group. Tumor size of the IFN group was smaller than that of non-INF group, while no differences in other parameters such as the number of tumors, the degree of tumor differentiation, the proportion of macroscopic portal invasion, degree of inflammation and fibrosis in the noncancerous portion, or operative method did not differ among the two groups.

Although seven patients in non-IFN group were lost of follow-up, all living patients were followed for a minimum period of 6 months. The follow-up period from surgery until death or the end point of this study were 2107 ± 1046 days (median, 1918 days; range 205–4429 days) in the IFN group and 1733 ± 1121 days (median, 1492 days; range 28–5157 days) in the non-IFN group. The tumor-free survival rates for patients with IFN group and non-IFN group were 62 and 40% at 3 years; 42 and 21% at 5 years, respectively. The tumor-free survival rate was significantly higher in the IFN group ($p = 0.0008$). The tumor-free survival rates for patients with response group and non-response group were 73 and 48% at 3 years; 57 and 23% at 5 years, respectively (Fig. 1). The tumor-free survival rate of the response group was much higher than those of other groups ($p = 0.0002$). The tumor-free survival curve for non-response group was similar to that for non-IFN group. A log-rank test revealed that a high albumin

Table 1
Clinicalpathologic findings in patients with hepatocellular carcinoma

	IFN group ($n = 50$)	Non-IFN group ($n = 262$)	p-Value
Age (year)	62 (54, 68)	65 (55, 72)	0.0080[a]
Gender (M:F)	43:7	214:48	0.5477[b]
Child-Pugh score (A:B)	47:3	225:37	0.1154[b]
Albumin (g/dl)	3.8 (3.5, 4.4)	3.6 (3.2, 4.1)	<0.0001[a]
AST activity (IU/l)	56 (33, 107)	60 (35, 100)	0.6083[a]
ALT activity (IU/l)	79 (27, 130)	63 (31, 117)	0.1366[a]
Total bilirubin (mg/dl)	0.9 (0.5, 1.3)	0.8 (0.5, 1.3)	0.3909[a]
Platelet count (× 10^4 mm^{-3})	14.0 (7.3, 21.1)	13.2 (7.6, 22.2)	0.7784[a]
High AFP (>20 ng/ml)	21	129	0.3593[b]
Preoperative TAE	9	77	0.1203[b]
Type of resection			
Anatomic resection	20	108	>0.9999[b]
Limited resection	30	154	
Tumor size (cm)	2.2 (1.5, 4.0)	3.0 (1.5, 6.5)	0.0067[a]
Number of tumors			
Single	40	181	0.0874[b]
Multiple	10	81	
Microscopic portal invasion	12	56	0.7094[b]
Tumor differentiation			
Well	6	31	>0.9999[b]
Moderate or poor	44	231	
Histologic activity index score			
Grade			
0–1	11	62	0.8576[b]
2–4	39	200	
Stage			
0–3	29	130	
4 (cirrhosis)	21	132	

Data are presented as the median with the 10th and 90th percentiles indicated in parentheses. IFN, interferon; AST, aspartate aminotransferase activity; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; TAE, transcatheter arterial embolization.

[a] Mann–Whitney U-test.

[b] Fisher exact test.

Table 2
Causes of deaths in patients with hepatocellular carcinoma

	Responder ($n=29$)	Non-responder ($n=21$)	Non-IFN ($n=262$)	p-Value
Liver disease	1	7	145	<0.0001[a]
HCC recurrence	1	5	123	
Cirrhosis	0	2	22	
Perioperative death	0	0	4	
Unrelated liver disease	2	4	20	

[a] χ^2-test.

concentration ($p=0.0076$), and a single tumor ($p=0.0001$) were associated with a significantly higher tumor-free survival rate. By multivariate analysis, a single tumor (risk ratio, 0.60; $p=0.0006$) and being in the response group (risk ratio, 0.27; $p=0.0011$) were independent factors associated with a lower risk for postoperative recurrence.

The overall survival rates for patients with IFN group and non-IFN group were 94 and 76% at 3 years; 81 and 53% at 5 years, respectively. The overall survival rate was also significantly higher in the IFN group ($p<0.0001$). The overall survival rates for the response group and the non-response group were 100 and 86% at 3 years; 90 and 69% at 5 years, respectively (Fig. 2). Postoperative outcome in the response group was much better than those in other two groups ($p<0.0001$). The overall survival rate for the non-response group was also higher than that for the non-IFN group, although there was no significant difference between these groups. A log-rank test revealed that a high albumin concentration ($p=0.0005$) and a single tumor ($p=0.0115$) were associated with a significantly higher overall survival rate. Multivariate analysis showed that a high albumin concentration (risk ratio, 0.67; $p=0.0100$), a single tumor (risk ratio, 0.73; $p=0.0469$), and being in the response group (risk ratio, 0.16; $p=0.0053$) were independent variables for overall survival.

Among 262 patients in the non-IFN group, tumor recurrence was detected in 194 patients before the end of the study period. A tumor recurrence occurred in 16 of 21 patients

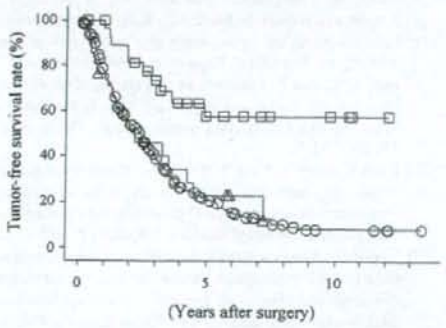

Fig. 1. Tumor-free survival rates after resection of hepatocellular carcinoma for patients with or without interferon therapy. Open circles: patients without previous interferon therapy ($n=262$); open squares: patients with response to interferon therapy ($n=29$); open triangles: patients with non-response to interferon therapy ($n=21$).

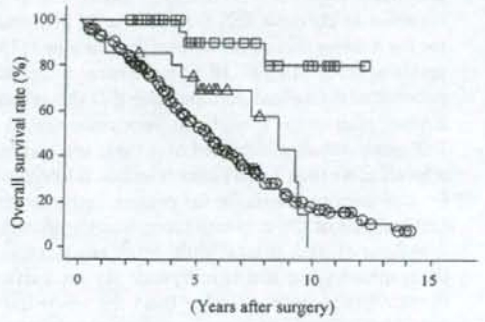

Fig. 2. Overall survival rates after resection of hepatocellular carcinoma for patients with or without interferon therapy. Open circles: patients without previous interferon therapy ($n=262$); open squares: patients with response to interferon therapy ($n=29$); open triangles: patients with non-response to interferon therapy ($n=21$).

in the non-response group and in 10 of 29 patients in the response group. During the follow-up period, 169 patients in the non-IFN group and 14 patients in the IFN group died. Of the 169 patients in the non-IFN group, 146 (86.4%) died of liver disease (HCC recurrence or cirrhosis). Eight (57.1%) of 14 deaths in the IFN group were due to liver disease, and the proportion of patients who died of liver disease was significantly lower in the IFN group than in the non-IFN group ($p=0.0137$). Among the response group, only one death occurred due to HCC recurrence and two deaths were due to causes unrelated to HCC recurrence or cirrhosis (Table 2). The proportion of deaths associated with liver disease was significantly lower in the response group than other two groups ($p<0.0001$).

4. Discussion

HCV infection is associated closely with HCC in many areas of the world [27]. Persistent active hepatitis and extensive fibrosis has important role in the development of HCV-related HCC [27–29]. Recent studies have indicated that HCC was less likely to develop in whom IFN therapy was effective at normalizing the serum ALT activity, even when HCV RNA did not disappear [9–16]. IFN therapy might suppress the development of HCC by causing remission of active hepatitis and improvement in hepatic fibrosis in patients infected with HCV. Postoperative recurrences of HCV-related HCC is

thought to result from the growth of intrahepatic metastases in the residual liver and metachronous, multicentric carcinogenesis due to continuous active hepatitis [4,8]. Previous reports identified that active hepatitis with a sustained increase in ALT activity is a risk factor for HCC recurrence [4–7]. We also reported that the presence of HCV viremia and an elevated AST activity are associated closely with recurrence after surgery [17]. Although HCC can occur even in sustained IFN responders [17,18,30], a sustained or biochemical response to previous IFN therapy was an independent factor for a lower risk of postoperative recurrence [18]. In our previous study [19], no HCC recurrences were detected in patients who received postoperative IFN therapy more than 2 years after surgery, while the recurrence rate in the non-INF group steadily increased over time, and recurrence that appears more than 2 years after resection is likely to be cause by new carcinogenesis. In the present study, the tumor-free survival rate of the response group was significantly higher than those of other groups. Multivariate analysis revealed that the response group had an independently lower risk for postoperative recurrence. On other hand, the tumor-free survival curve for non-response group was similar to that for non-IFN group, possibly indicating that a decrease in the ALT activity due to IFN therapy may prevent new carcinogenesis after surgery. These results suggested that IFN therapy may prevent recurrence due to new carcinogenesis after surgery in the same way that IFN therapy decreases the incidence of HCV-related HCC.

Recently, IFN therapy was found to prevent the worsening of compensated cirrhosis as well as to inhibit the development of HCC in patients with chronic active hepatitis C and compensated cirrhosis, increasing their survival rate [16]. Additionally, we reported that the suppression of recurrence and the improvement of liver function by postoperative IFN therapy prolonged the survival period after surgery for patients with HCV-related HCC [20]. In the present study, the overall survival rate for the IFN group was also higher than that for the non-IFN group. Moreover, the 3 and 5 years overall survival rates for the response group were 100 and 90%, respectively, and the overall survival rate for the response group was also much higher than those for the other two groups. Moreover, multivariate analysis revealed that the response group was independently associated with better prognosis. Especially when IFN therapy is effective against active HCV hepatitis, hepatic resection should be able to prolong survival period in patients with HCV-related HCC.

In the present study, 146 of 262 patients in the non-IFN group died of liver disease (HCC recurrence or cirrhosis) during the follow-up period, while nine deaths due to liver disease occurred in 50 patients who underwent IFN therapy. Especially in the response group, only one patient died of HCC recurrence, and the proportion of patients who died of liver disease was significantly lower in the response group than other two groups. Although the number of patients in this study was too small to confirm conclusion and this study was performed retrospectively, effective IFN therapy is likely to improve postoperative outcome of HCV-related HCC by suppression of recurrence and improvement of liver function.

In conclusion, patients who detected HCC after IFN therapy for HCV hepatitis may be good candidates for hepatic resection, especially when previous IFN therapy has controlled their active hepatitis. IFN therapy can improve postoperative outcomes in patients with HCV-related HCC because of suppression of recurrence and preventing progress of cirrhosis, especially when IFN therapy has controlled their active hepatitis.

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Original Paper

Bowel Injury Associated with Liver Surgery for Hepatocellular Carcinoma

Shogo Tanaka MD, Hiromu Tanaka MD, Shoji Kubo MD, Taichi Shuto MD
Shigekazu Takemura MD, Takatsugu Yamamoto MD, Takahiro Uenishi MD
Seikan Hai MD, Harushi Osugi MD, Kazuhiro Hirohashi MD
Department of Hepato-Biliary-Pancreatic and Gastroenterological Surgery
Osaka City University Graduate School of Medicine, Osaka, Japan
Corresponding Author: Shogo Tanaka, MD
Department of Hepato-Biliary-Pancreatic and Gastroenterological Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
Tel: +81 6 6645 3841, Fax: +81 6 6646 6057, E-mail: m8827074@msic.med.osaka-cu.ac.jp

ABSTRACT

Background/Aims: Bowel injury associated liver surgery is rare but can be fatal if not adequately treated. The contribution of underlying liver disease and previous hepatectomy to bowel injury in hepatectomy for hepatocellular carcinoma is unknown.

Methodology: Clinical records of 531 patients who underwent hepatic resection without combined resection of the biliary tract or intestine for hepatocellular carcinoma during 13 years were reviewed. Differences in incidence of bowel injury according to presence or absence of liver cirrhosis, technique of hepatectomy, and history of hepatectomy were investigated. Outcome after treatment also was reviewed.

Results: Bowel injury occurred in 5 patients (0.9%). Previous hepatectomy history was associated with an increased incidence of bowel injury [repeat hepatectomy, 3/91 (3.3%), and first hepatectomy, 2/440 (0.5%), $p=0.038$]. Injury was recognized intraoperatively in two patients and postoperatively in three. In the former two patients, the injured bowel was repaired immediately but a fistula still developed in one patient. One patient with a fistula eventually required temporary fecal diversion and eventually limited colectomy. The other three patients were treated by continuous external drainage, but two of them required debridement or colic sleeve resection.

Conclusions: Previous hepatectomy increases the risk for bowel injury during hepatectomy. Care must be taken to prevent adhesion to the hepatic cut surface. Careful use of electrocautery to prevent burn injury also should be taken.

KEY WORDS: Conservative management; Re-laparotomy; Repeated hepatectomy; Enterocutaneous fistula; Total parenteral nutrition

ABBREVIATIONS: Hepatocellular Carcinoma (HCC); Total Parenteral Nutrition (TPN); Transcatheter Arterial Embolization (TAE); Postoperative Day (POD)

INTRODUCTION

Accidental bowel injury during surgery, when promptly recognized and repaired, usually heals without complication (1,2). Unfortunately, however, some injuries lead to wound infection, abscess formation, or enterocutaneous fistula (3). The incidence of bowel injury has been reported to range from 0.06% to 0.6% in laparoscopic cholecystectomy (2,4-6), 0.0004% to 0.08% in Cesarean section (7,8), and 0.5% to 3% in radical prostatectomy (9). However, little attention has been paid to enteric injuries during hepatic resection for hepatocellular carcinoma (HCC). Some surgeons have reported that the presence of underlying hepatic diseases impairs spontaneous healing in patients with uncontrollable bile leakage after hepatic resection (10,11). However, whether this factor affects the bowel is uncertain. Patients with HCC sometimes require repeat hepatectomy two or more times for tumor recurrence (12). In these cases, dense adhesions between cut surface of the liver and intestine is a major cause of bowel injury. This study was undertaken to identify the incidence and risk factors for bowel injury during and following hepatectomy for HCC.

METHODOLOGY

The clinical records of 531 patients who had undergone hepatic resection for HCC without combined resection of the biliary tract or intestine in our department between January 1990 and December 2002 were reviewed. The cohort consisted of 437 males and 94 females, with a mean age of 63 years (range, 2 to 83 years). Prior to surgery, transcatheter arterial embolization (TAE), percutaneous transhepatic portal vein embolization, or both (13-15) were performed in 100, 9, and 34 patients, respectively. The procedure was an initial hepatectomy in 440 patients, a second hepatectomy in 88, and a third hepatectomy in 3. Dissection of dense adhesion was performed using scissors or electrocautery. For subsegmentectomy or more extensive resection, hepatic parenchyma was commonly dissected by ultrasonic dissector (from 1990 to 1999, the SONOP SUS-202 Dissector, Aloka, Tokyo, Japan, and from 2000 to the present, the CUSA EXcel, Valleylab, Boulder, CO), during total (16) or unilateral clamping of hepatic vascular inflow (17). For limited hepatic resection, microwave tissue coagulator (Microtaze®; Heiwa Electronics Industry Inc., Tokyo, Japan) was used mainly. Hepatic anatomy and the

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type of hepatic resection were classified according to Healey's segments (18). A hepatic resection of less than one segment was defined as a limited resection.

Of 531 patients, 268 patients underwent cholecystectomy, intraoperative cholangiography, and the biliary sealing test with 20mL of indocyanine green solution through a balloon catheter made by ourselves (19). And 7 patients underwent lymph node dissection in the hepatoduodenal ligament.

One or two silicone tubes were placed near the cut surface of the liver, and these were removed once the fluid became serous without bile or pus, usually around the third to seventh postoperative day. Prophylactic antibacterial drugs were routinely given for three to five days after surgery.

We analyzed differences in the incidence of accidental bowel injury according to underlying hepatic diseases, liver cirrhosis, types of hepatic tumor, and methods of hepatic resection, and studied the clinical course in patients with accidental bowel injury. Significance of differences of the incidence was determined by Fisher's exact test. A p value less than 0.05 (two-tailed) was considered significant.

This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institute. Written informed consent was obtained from each patient or their parents.

TABLE 1 Incidence of Bowel Injury Associated with Hepatectomy for Hepatocellular Carcinoma

Variables	Number of Patients	Number of patients with bowel injury (%)	p value
Age			0.21
≥ 63	277	4 (1.4%)	
<63	254	1 (0.4%)	
Gender			0.59
Male	437	5 (1.1%)	
Female	94	0 (0%)	
Previous hepatectomy			**0.0375**
+	91	3 (3.3%)	
-	440	2 (0.5%)	
Preoperative embolization			
TAE			0.23
+	98	2 (2%)	
-	433	3 (0.7%)	
PTPE			>0.99
+	9	0 (0%)	
-	522	3 (0.6%)	
TAE and PTPE			>0.99
+	34	0 (0%)	
-	497	5 (1.0%)	
Hepatectomy			0.68
≥1 Couinaud's segment	230	2 (0.9%)	
<1 segment	301	3 (1.0%)	
Cholecystectomy			0.68
+	268	2 (0.7%)	
-	263	3 (1.1%)	
Lymph node dissection			>0.99
+	7	0 (0%)	
-	524	5 (1%)	

TAE: transcatheter arterial embolization; PTPE: percutaneous transhepatic portal vein embolization.

RESULTS

Bowel injury was recognized intraoperatively in two patients, and the repaired intestine leaked in one. In three other patients, the injury was not recognized until after surgery. The overall incidence of accidental bowel injury associated with hepatic resection was 0.9% (5/531). The incidence in patients who had previously undergone hepatectomy was 3.3% (3/91), but prevalence of liver cirrhosis, type of hepatectomy, concomitant cholecystectomy, and lymph node dissection

TABLE 2 Clinical Profiles of Patients with Accidental Bowel Injury Associated with Hepatectomy

Pt. No.	Age/ Sex	Previous Laparotomy	Present hepatectomy	Liver cirrhosis	Detection	Site	Possible causes	Treatments (and outcome)
1	63/M	Anterior-inferior	Limited resection	(+)	POD 7	Colon	Dense adhesion	1) Percutaneous drainage at POD 7 2) Ileostomy at POD 40 (fistula not sealed till POD 86) 3) Closure of the ileostomy at POD 90 (reopen of the colocutaneous fistula) 4) Resection of the fistulous segment of colon at 8 years after hepatectomy (healed)
2	67/M	Anterior segmentectomy	Limited resection (medial segment)	(+)	During operation	Colon	Dense adhesion	1) Repair during operation (healed)
3	62/M	None	Central bisegmentectomy	(+)	POD 17	Jejunum	Uncertain	1) Repair under re-laparotomy at POD 17
4	71/M	None	Posterior segmentectomy	(-)	POD 28–42	Duodenum	Tube for abscess drainage	1) Continuous drainage till POD 59
5	69/M	Anterior segmentectomy total gastrectomy	Limited resection (posterior segment)	(-)	During operation	Colon	Dense adhesion	1) Repair during operation (healed) 2) Continuous drainage till 120 POD (localized infection) 3) Removal of infectious costal bone places at POD 140 (healed)

FIGURE 1 Fistulogram in Case 1 performed 8 years after hepatectomy demonstrates persistent colonic fistula (Arrows).

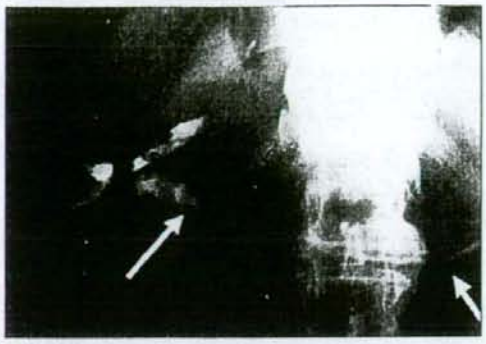

FIGURE 2 Contrast medium injected into a percutaneous abscess drainage catheter in Case 4 creates a duodenogram (Pt No.4). A large arrow indicates the site of injury to the duodenum, and the small arrow indicates greater omentum side of the stomach.

did not affect the incidence of the injury (**Table 1**).

Characteristics, treatment, and outcome in patients with bowel injury are summarized in **Table 2**. Details of three cases (Nos. 1, 4, and 5) are presented as follows;

Case 1: A 63-year-old man with liver cirrhosis, who had undergone an anterior-inferior subsegmentectomy for HCC in 1987, underwent a limited resection of a posterior segment for recurrence in January 1993. Adhesions between the right hepatic flexure of the colon and cut surface of the liver were dense. The patient spiked temperature to 39°C and developed rebound and guarding in the right upper quadrant on postoperative day (POD) 7. As ultrasonogram demonstrated an intraabdominal abscess in the right subphrenic space, percutaneous abscess drainage was performed. Contrast medium injection through the drainage tube demonstrated a fistula tract to the colon at the right hepatic flexure. The fistula failed to close on total parenteral nutrition (TPN) and antibiotics, so an ileostomy was performed on POD 40. Purulent discharge from the fistula persisted until POD 86, and the ileostomy was closed on POD 90. The patient was followed every 3 months, with intermittent recurrence of infection (**Figure 1**). Resection of fistulous segment with end-to-end anastomosis was performed 8 years after the last surgery. The patient is well and asymptomatic 2 years after the last surgery.

Case 4: A 71-year-old man underwent right posterior segmentectomy for HCC in September 2002. Preoperative computed tomography demonstrated a duodenal diverticulum of the anterior wall of the second portion. The patient developed bile leakage on POD 1, and an abscess was diagnosed on POD 7. Percuta-

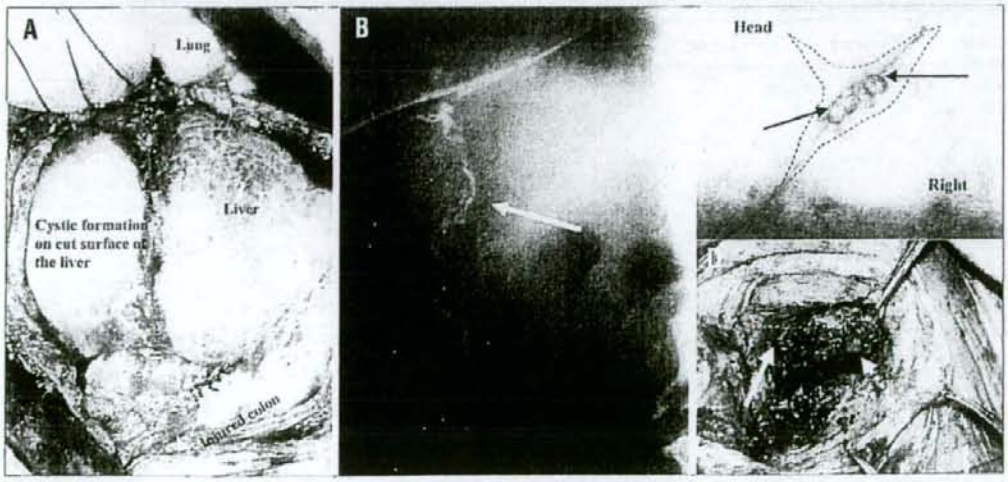

FIGURE 3 (Pt. No. 5) **(A)** Laceration of the anterior wall of the colon during thoracoabdominal hepatectomy (arrow). The injury was repaired by an all-layers suture using 3-0 black silk. **(B)** Contrast medium injection into the percutaneous abscess drainage tube demonstrates a perforation of the colon. Arrow indicates the fistula tract. **(C)** Skin erosion before reoperation. Dotted line indicates skin incision. Arrows indicate the opening of the tract to skin. **(D)** Intraoperative photograph during fistulectomy. Arrow indicates skin erosion and dotted arrow indicates the infected rib pin.

neous transhepatic abscess drainage was performed on POD 28. On POD 42, contrast medium injected into the abscess cavity drained into the duodenum **(Figure 2)**. Oral intake was restricted, and a proton pump inhibitor, and TPN were administered. Thereafter, discharge had gradually decreased and ceased. Diet was restarted without any change in the volume of drainage from the fistula, so the tube was removed 7 days after the additional therapy on POD 59. The duodenocutaneous fistula closed 3 days later. The patient was discharged from the hospital on POD 72, but died of heart failure 10 months after hepatic resection.

Case 5: A 69-year-old man, who had undergone right anterior segmentectomy and total gastrectomy with lymphadenectomy for synchronous HCC and gastric cancer in August 2000, whose postoperative course had been complicated by peritonitis due to failure of the esophagojejunostomy, was admitted in October 2002, for recurrence of HCC in the posterior segment of the liver. To minimize the extent of the intraperitoneal procedure, we chose the thoracoabdominal approach to the hepatic resection. However, adhesions in the peritoneal cavity were too dense to permit identification of the layers of abdominal wall and peritoneum, and a 4-cm full-thickness laceration was made in the transverse colon **(Figure 3A)**. The injured colon wall was thick and edematous, but we repaired it primarily using an all layers closure. To close the thoracic incision and fix the ribs, a biodegradable absorbable pin (Fixsorb Rib, Takiron co., Ltd., Osaka, Japan) was used. The early postoperative course was uneventful, but on POD 7, the patient developed feculent discharge through the surgical incision. An enterocutaneous fistula and fistula-associated abscess that had infiltrated the right thoracic cavity causing an empyema were diagnosed **(Figure 3B)**. A percutaneous intra-abdominal abscess drainage tube and a thoracotomy tube were inserted, oral intake was restricted, TPN and antibiotics were administered, and wound care was performed. A second catheter was placed to facilitate abscess drainage, and oral feeding was restarted after 7 weeks despite a persistent low-grade fever. The thoracotomy tube was removed 8 weeks after its insertion. A fistulogram performed 17 weeks after surgery demonstrated no communication with the colon, and the fistula drainage tube was removed. However, purulent drainage from the fistula tract persisted **(Figure 3C)**, and the rib pin became infected. Partial fistulectomy, including the infected rib pieces and pin, was performed 5 months after the second hepatectomy **(Figure 3D)**. Postoperatively, the patient's general condition continues to be very good, and his weight is normal 14 months after the initial surgery.

DISCUSSION

In this study, incidence of bowel injury associated with hepatic resection was 0.5% (2/440) for first hepatectomy, but was 3.3% (3/91) for subsequent hepatectomy.

Postoperative adhesions have been attributed to intraoperative enteric injury (20). In living donor liver transplantation, difficulty in performing the hepatectomy increases the incidence (6.4% to 20%) of bowel injury, especially in patients who had previous liver-related surgery (21-23). In subsequent hepatectomy for HCC, adhesions between the intestine and cut surface of the liver, as well as between bowel and the parietal peritoneum increase the risk of bowel injury. Moreover, if tumor has recurred near the cut surface, adhesions between the intestine and cut surface of the liver reduces the effectiveness of TAE because of the presence of extrahepatic collateral arteries (24), and TAE increases the risk of gangrene of the gallbladder, and induces adhesions around the hepatoduodenal ligament (25). This indicates that preventable procedure from adhesion after first hepatectomy will be needed to increase the effect of TAE and to reduce the risk of bowel injury for recurrence of HCC; e.g. decollateralization using silicone rubber sheeting.

On the other hand, some bowel injuries are unrecognized intraoperatively. Occult conductive burn injury by electrocautery or pressure necrosis by the drainage tube might have contributed to delayed recognition of bowel injury. Conductive burn injury has been reported primarily in laparoscopic cholecystectomy (2,5,26,27). Unrecognized, this injury can result in full-thickness necrosis of the bowel wall with delayed perforation, and symptoms of peritonitis develop 8 days to 2 to 3 weeks after the actual injury. This emphasizes the need for care in the use of electrocautery and microwave coagulator during hepatic resection.

There are few reports of drainage tube-induced complications, such as penetration into the gastric lumen (28), and lung and bronchial perforation (29,30). We routinely place a Duple drain to drain exudative and transudative ascites and to monitor postoperative bleeding and bile leakage, but we have had no experience of direct bowel injury. However, in Case 4, the percutaneous abscess drainage tube for postoperative bile leakage might have caused duodenal injury. Placement of percutaneous drainage catheter is usually ultrasound-guided, and every effort should be made to define the local anatomy prior to catheter insertion.

When bowel injury is noted at the time of laparotomy, immediate surgical repair is essential (2,20). Ordorica-Flores *et al.* (31) reported that an all layers closure is as safe as a two-layer anastomosis. However, the creation of stoma may be needed if the healing of injured intestine might be impaired. However, in some patients with dense adhesions, even the creation of stoma may be difficult. In such cases, formation of enterocutaneous fistula by drainage tube insertion should be considered (32,33). In Case 5, in a colocutaneous fistula, sepsis might have been prevented during the early postoperative period.

When bowel injury is diagnosed postoperatively, re-laparotomy, to repair the injury or create a stoma,