METHODS

Patients

FROM NOVEMBER 2000 to October 2005, curative resection of HCC was performed at Osaka City University Hospital in 24 patients seropositive for HB surface antigen (HBsAg) who were negative for antihepatitis C virus antibody and had high serum concentrations of HBV DNA. The patients had not received any lamivudine therapy before the operation. Curative resection was defined as a complete resection of all macroscopically evident tumors. Absence of tumor cells along the parenchymal transection line was confirmed histologically. No remaining tumor was detected in the remnant liver by computed tomography (dynamic study) 3–4 weeks after surgery. The serum concentration of HBV DNA was determined with a transcription-mediated amplification and hybridization protection assay (TMA-HPA) kit, given that serum HBV DNA concentration measured by this method has been shown to be closely related to its hepatitis activity and useful for predicting risk of recurrence after resection of HBV-related HCC.[14,15] In the 24 patients, the serum concentration of HBV DNA was at least 3.7 logarithms of the genome equivalent (LGE) per milliliter, which was considered to represent a high concentration. The patients were told about the previously reported finding that high pre- and postoperative viral concentrations were strong risk factors for HCC recurrence after the operation. Antiviral drug therapeutic effects and side-effects, including the appearance of YMDD mutant viruses, were also explained to the 24 patients. Lamivudine therapy was started in the 14 who then agreed to this therapy and gave their informed consent (lamivudine group), while the control group consisted of the other 10 patients who declined treatment with the drug because of the possibility of adverse events or the necessity of long-term administration of the drug. The 24 patients were followed up until the detection of HCC recurrence or for at least for 6 months in patients without recurrence. The median follow up from operation until the detection of HCC recurrence or the study endpoint (30 April 2006) in this study was 759 days (34–2053). The median follow up for each group was 1117 days (187–2037) for patients receiving lamivudine and 224 days (34–2053) for the controls.

Examination of viral status and active hepatitis

HBsAg and HB envelope antigen (HBeAg) were assayed using an enzyme immunoassay (International Reagents, Kobe, Japan). Anti-hepatitis C virus antibody was assayed using an enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). We used a TMA-HPA kit (Chugai Diagnostics, Tokyo, Japan) to measure serum concentration of HBV DNA. TMA-HPA can quantify 3.7–9.7 LGE/mL (5×10^3–5×10^8 copies/mL). Serum alanine aminotransferase (ALT) activity usually increased immediately following liver resection, then decreased gradually to preoperative activity within 3 weeks. A serum ALT activity at any clinical follow-up assessment (at least every 3 months) of 45 IU/L or less was considered a sustained low ALT activity. The serum concentration of HBV DNA was measured at least twice a year. A serum HBV DNA concentration at any follow up of less than 3.7 LGE/mL was considered a sustained low concentration in the absence of YMDD mutant viruses. YMDD mutant viruses were detected by a reverse hybridization line probe assay (INNO-LiPA HBV DR, Innogenetics, Ghent, Belgium) when serum HBV DNA returned to concentrations above the reference range (≥3.7 LGE/mL) after a decrease to the reference range (<3.7 LGE/mL) with lamivudine therapy.[16,17]

Antiviral treatment

Fourteen patients received lamivudine therapy (100 mg/day), beginning 2 weeks to 2 months after surgery. The period of lamivudine administration was 6 months to 65 months (mean, 32 months). When active hepatitis with a high viral concentration reemerged following proliferation of YMDD mutant viruses, adefovir dipivoxil was administered if patients gave their informed consent. Interferon therapy was carried out in one patient after administration of lamivudine for 6 months, before any appearance of YMDD mutant viruses, to avoid the emergence of YMDD mutant virus.

Pathologic examination

Histologic grading of tumors with respect to differentiation was carried out using the classification of Edmondson and Steiner[18] with certain modifications.[19] When clusters of cancer cells were present in the portal vein, we defined the case as positive for portal invasion. Cancer cells in intracapsular blood vessels, considered branches of the portal vein, also indicated portal vein invasion. When the tumor-free surgical margin was less than 5 mm according to pathologic examination, the margin was defined as positive. Noncancerous hepatic tissues also were examined pathologically. The histologic activity index (HAI) score[20] was used to evaluate the severity of active hepatitis and the degree of fibro-

sis. An HAI score of 0 (based on components 1–3) indicated no activity (grade 0); a score of 1–3, minimal activity (grade 1); a score of 4–8, mild activity (grade 2); a score of 9–12, moderate activity (grade 3); and a score of greater than 12, severe activity (grade 4). The histologic fibrosis score (stage) was determined from component 4 in the HAI scoring. A score of 1 indicated portal fibrous expansion; 2, portal-to-portal septa without architectural distortion; 3, portocentral septa with architectural distortion; and 4, cirrhosis.

Detection of tumor recurrence

Serum α-fetoprotein concentrations were measured every three months. Ultrasonography, computed tomography, magnetic resonance imaging, chest radiography, or a combination of these was performed every three months. When tumor recurrence was suspected on the basis of a tumor marker, radiologic studies, or both, angiography or biopsy was performed to obtain a definitive diagnosis.

Statistics

Student's *t*-test was used to analyze differences in age and tumor size. The Mann–Whitney *U*-test was used to analyze the differences in results of laboratory tests. Fisher's exact test was used to compare categorical data between groups. Tumor-free survival rates were calculated by the Kaplan–Meier method, and the significance of differences in rates between the groups was assessed by the log-rank test. For multivariate analysis, the Cox proportional hazards model with a stepwise method was used. Variables significant at a *P*-value less than 0.1 on univariate analysis were subjected to multivariate analysis. A *P*-value less than 0.05 was considered indicative of significance.

RESULTS

CLINICOPATHOLOGIC FINDINGS IN the lamivudine and control groups are described in Table 1. Age, gender distribution, the proportion of patients with

Table 1 Clinicopathologic findings of patients with and without lamivudine therapy

Findings	Lamivudine therapy		*P*-value
	Yes, $n = 14$	No, $n = 10$	
Age, years, mean ± SD	55 ± 8	55 ± 5	0.993
Gender, men : women	10:4	7:3	>0.999
HBeAg positivity§	11	5	0.204
HBV DNA concentration (LGE/ml)	6.0 ± 1.2	6.0 ± 1.2	0.975
Total bilirubin, mg/dL	0.8 (0.4–1.3)	0.8 (0.4–1.3)	0.656
Albumin, g/dL	3.8 (3.3–4.3)	3.7 (2.6–4.2)	0.426
AST‡, IU/L	44 (35–109)	40 (25–128)	0.578
ALT†, IU/L	53 (25–141)	56 (30–125)	0.558
Child–Pugh classification(A : B)	11:3	8:2	>0.999
α-Fetoprotein, >20 ng/mL	11	7	0.665
Tumor size, cm	2.4 ± 0.7	2.8 ± 1.4	0.329
Multiple tumors	5	5	0.679
Differentiation of main tumor			
Moderately	7	3	0.421
Poorly	7	7	
Portal invasion	4	4	0.673
TMN stage (1:2:3)	2:7:5	3:2:5	0.305
Severity of active hepatitis			
Grade 1, minimal	5	3	>0.999
Grade 2, mild	9	7	
Degree of fibrosis			
Stages 1–3, mild to moderate	8	6	>0.999
Stage 4, cirrhosis	6	4	
Anatomic resection	8	6	>0.999

†ALT, alanine aminotransferase; ‡AST, aspartate aminotransferase; §HBeAg, hepatitis B envelope antigen.
Results of laboratory tests are expressed as medians, with ranges in parentheses.

Table 2 Changes in number of patients with low serum concentration of HBV DNA after surgery

	No. of patients with low serum concentration of HBV DNA (<3.7 LGE/ml)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	10/10	1/8	1/4	1/4	1/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Lamivudine	14/14	11/14	8/11	8/11	6/11	4/8	4/8	4/7	4/6	4/5	3/4	1/1

$p = 0.0028$.

HBeAg positivity, viral load, the results of laboratory tests, and Child-Pugh classification[21] did not differ between groups. Type of resection, tumor size, tumor number, differentiation of main tumor, prevalence of portal invasion, cancer stage according to UICC classification,[22] severity of active hepatitis as well as degree of hepatic fibrosis in non-cancerous hepatic tissue showed no difference between groups. No patients had portal invasion detected by preoperative medical imagings. The changes in the number of patients with low serum concentration of HBV DNA and low ALT activity after the operation until the detection of HCC recurrence or the study endpoint in both groups are shown in Tables 2 and 3, respectively. In one patient in the lamivudine group, serum HBeAg changed from positive to negative after the operation.

The tumor-free survival rate was significantly higher in the lamivudine group than in the control group (Fig. 1, $P = 0.0086$). In one patient in the lamivudine group and four patients in the control group, multiple recurrent tumors were detected in the remnant liver. In one patient in the lamivudine group and in one patient in the control group, a single recurrent tumor was detected. By univariate analysis using preoperative factors, multiple tumors was also a risk factor for short tumor-free survival ($P = 0.0036$). By multivariate analysis, lack of lamivudine therapy and multiple tumors were independent risk factors for short tumor-free survival (risk ratio, 18.306; 95% confidence interval, 1.707–196.327; $P = 0.0163$, 16.949; 1.686–166.667; $P = 0.0162$, respectively). Thus, the lack of lamivudine therapy was an independent risk factor for recurrence.

A sustained low serum concentration of HBV DNA was observed in no patient in the control group and in eight patients in the lamivudine group. Although HCC did not recur in the eight patients with sustained low serum concentrations of HBV DNA, HCC recurred in seven of 16 patients with high serum concentrations of HBV DNA (Fig. 2). The proportion of patients with multiple tumors was significantly higher in the seven patients than in nine patients without recurrence. In the lamivudine group, HCC recurred in two patients, who had sustained high serum concentrations of HBV DNA. Sustained normal ALT activity was observed in five patients in the control group and in 10 patients in the lamivudine group. In one patient in the control group, ulsodeoxycholic acid was administered because serum ALT activity was more than 90 IU/L (twice the reference range). In four other patients, no treatment was performed because ALT activity was less than 90 IU/L. Although HCC recurred in two of the 15 patients with sustained normal ALT activity, HCC recurred in five of nine patients with high serum ALT activity (Fig. 3, $P = 0.0346$).

Although three patients in the control group died of HCC recurrence, no patients died in the lamivudine group.

In four patients, YMDD mutant viruses were detected at 10, 11, 21 and 43 months after beginning administration of lamivudine and ALT activity increased in three of the four patients. In two of the three patients with sustained high ALT activity, adefovir dipirvoxil was administered. In one patient, YMDD mutant virus was detected when the HCC recurrence was detected. In the three other patients with YMDD mutant viruses, HCC did not recur.

Table 3 Change in number of patients with low alanine aminotransferase activity after surgery

	No. of patients with low alanine aminotransferase activity (≤45 IU/l)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	2/10	5/8	2/4	2/4	2/2	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Lamivudine	6/14	10/14	7/11	7/11	6/11	5/8	5/8	5/7	6/6	5/5	2/4	1/1

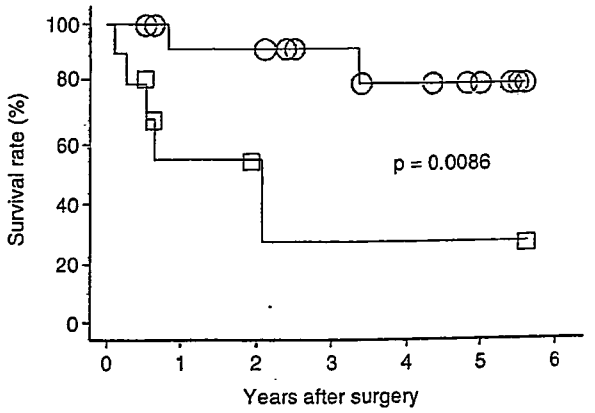

Figure 1 Tumor-free survival rates in lamivudine and control groups. (○), 14 patients with postoperative lamivudine therapy; (□), 10 patients without postoperative lamivudine therapy.

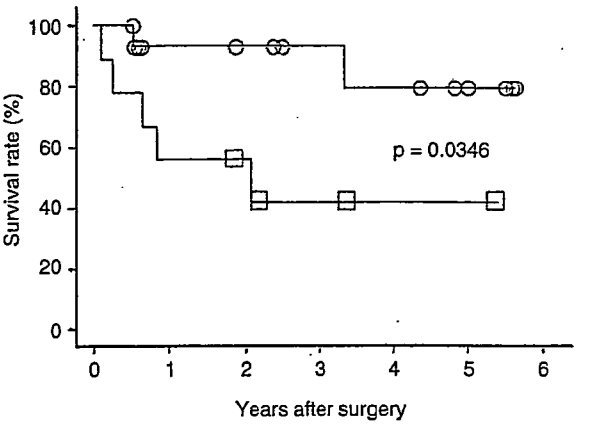

Figure 3 Tumor-free survival rates in patients with and without sustained low serum alanine aminotransferase activity. (○), 15 patients with sustained low serum alanine aminotransferase activity; (□), nine patients with high serum alanine aminotransferase activity.

DISCUSSION

THIS STUDY SHOWED that lamivudine therapy improved the tumor-free survival rate after liver resection for HCC in patients with a high serum concentration of HBV DNA.

This study did not have a randomized prospective design. A well-designed prospective study is difficult to conduct because lamivudine therapy already has been developed as a treatment for chronic hepatitis B and is presently used in clinical practice.[3–8] Despite its status as an established treatment for this form of hepatitis, whether lamivudine therapy is effective for the suppression of tumor recurrence after HBV-related HCC has not been determined. We have reported high preoperative viral concentration and sustained high viral concentration after liver resection to be risk factors for HCC recurrence,[1,2] defining a high-risk patient group. Of 24 patients presently studied, 14 chose lamivudine therapy after explanation of these previous results of liver resection for HBV-related HCC, as well as likely advantages and disadvantages of lamivudine in such a high-risk group. As shown in Table 1, no difference in background characteristics was evident between the lamivudine and control groups. The tumor-free survival rate was significantly higher in the lamivudine group than in the control group, with the absence of lamivudine therapy being an independent risk factor for short tumor-free survival. Thus, this study showed that lamivudine therapy suppressed tumor recurrence after curative resection in these HBV-positive patients.

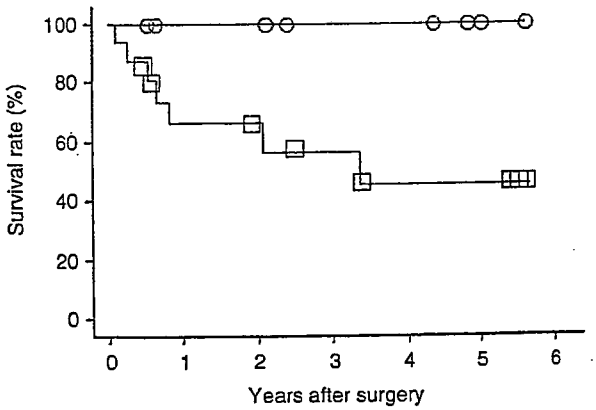

Figure 2 Tumor-free survival rates in patients with and without sustained low serum concentrations of HBV DNA. (○), eight patients with sustained low serum concentration of HBV DNA; (□), 16 patients with high serum concentrations of HBV DNA.

Recurrences after surgery include both intrahepatic metastases from the primary HCC and newly developed HCC (multicentric carcinogenesis). Although the mechanism by which lamivudine therapy suppressed tumor recurrence after surgery was not determined in this investigation, one likely factor underlying the low recurrence rate in the lamivudine group would be a decrease in multicentric carcinogenesis after surgery. Regeneration of hepatocytes amid a background of necrosis during active hepatitis may directly induce DNA mutation, as well as chromosomal rearrangement resulting in genetic instability. Sustained high serum concentrations of HBV and

persistent or intermittent elevation of ALT activity have been linked closely to carcinogenesis in patients with hepatitis B.[23–25] Two recent studies demonstrated that lamivudine therapy reduced the development of HCC in patients with chronic hepatitis B.[12,13] In the present study, HCC did not recur in patients with a sustained low serum concentration of HBV DNA. HCC recurred in only two of 15 patients with sustained normal ALT activity, although HCC recurred in five of nine patients with high serum ALT activity. Thus, sustained serum concentration of HBV DNA and the remission of active hepatitis brought about by lamivudine therapy may decrease multicentric carcinogenesis after surgery.

In our previous study, the percentage of intrahepatic metastases from similarly differentiated main tumors tended to be higher in patients with a high viral concentration than in those with a low viral concentration.[1] HCC thus may behave more aggressively in patients with a high viral concentration, even when histologic differentiation of the tumor is the same; a decrease in viral concentration with lamivudine therapy thus may affect important biologic characteristics of HCC, although lamivudine itself does not have anticancer effects. For example, active hepatitis induces upregulation of adhesion molecules on cells lining hepatic sinusoids, which may enhance the likelihood of metastasis.[26] Remission of active hepatitis in response to lamivudine therapy thus may decrease metastatic potential.

Long-term lamivudine administration is associated with an increasing rate of emergence of drug-resistant viral strains.[9–11] YMDD mutant viruses appeared in four patients in this series. YMDD mutant viruses often cause flare-ups of ALT activity and hepatic decompensation in patients with cirrhosis. In fact, ALT activity increased above the reference range in three of the four patients with YMDD mutant virus. Adefovir dipivoxil was administered in two of the three patients with high ALT activity. In addition to hepatitis exacerbations, viral breakthrough itself may increase the likelihood of the development of HCC,[27] a particular risk in patients with cirrhosis and YMDD mutations.[28] Although HCC did not recur after the appearance of YMDD mutant virus in this series, early identification of YMDD mutant viruses is important. When such mutant viruses are identified, close follow up is necessary to prevent hepatic failure and recurrence of HCC. Several investigators have reported that interferon therapy reduced the incidence of HCC,[29–32] although some others found no decrease.[33,34] Recently, Sun *et al.* reported that interferon therapy improved the overall survival of patients with HBV-related HCC after curative resection, probably by postponing recurrence.[35] Lamivudine appears to be superior to interferon in delaying HCC development.[36] In our series, interferon was administered after lamivudine therapy for 6 months in one patient, who then showed a decreased serum concentration of HBV DNA and ALT activity without appearance of YMDD mutant viruses. A combination of lamivudine and interferon therefore may represent a useful alternative treatment regimen.

This study fell somewhat short of conclusiveness because the number of patients was small. The effects of HBV genotypes on recurrence after surgery also are unclear. The effects of YMDD mutant virus and adefovir dipivoxil on HCC recurrence also should be evaluated. A larger investigation is necessary to clarify the effects of and indications for lamivudine.

In conclusion, lamivudine therapy improved the tumor-free survival rate after curative resection of HBV-related HCC in patients with a high serum concentration of HBV DNA, although careful follow up was necessary for timely detection of YMDD mutant viruses to minimize their adverse effects.

REFERENCES

1 Kubo S, Hirohashi K, Tanaka H *et al.* Effect of viral status on recurrence after liver resection for patients with hepatitis B virus-related hepatocellular carcinoma. *Cancer* 2000; 88: 1016–24.
2 Kubo S, Hirohashi K, Tanaka H *et al.* Virologic and biochemical changes and prognosis after liver resection for hepatitis B virus-related hepatocellular carcinoma. *Dig Surg* 2001; 18: 26–33.
3 Dienstag JL, Perrillo RP, Schiff ER *et al.* A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995; 333: 1657–61.
4 Nevens F, Main J, Honkoop P *et al.* Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. *Gastroenterology* 1997; 113: 1258–63.
5 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998; 339: 61–8.
6 Leung NW, Lai CL, Chang TT *et al.* Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001; 33: 1527–32.
7 Dienstag JL, Goldin RD, Heathcote EJ *et al.* Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; 124: 105–17.
8 Ohkoshi S, Ogata N, Ichida T. The long-term clinical outcome of 1-year treatment of chronic hepatitis B with lamivudine – 5 years observation. *Hepatol Res* 2003; 27: 13–17.

9 Chayama K, Suzuki Y, Kobayashi M *et al.* Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. *Hepatology* 1998; 27: 1711–16.
10 Lok AS, Hussain M, Cursano C *et al.* Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; 32: 1145–53.
11 Fujioka S, Shimomura H. Two cases of chronic hepatitis B with emergence of lamivudine-resistant virus during long-term therapy. *Hepatol Res* 1999; 12: 97–104.
12 Liaw YF, Sung JJY, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced disease. *N Engl J Med* 2004; 351: 1521–31.
13 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173–84.
14 Kubo S, Hirohashi K, Tanaka H *et al.* Usefulness of viral concentration measurement by transcription-mediated amplification and hybridization protection as a prognostic factor for recurrence after resection of hepatitis B virus-related hepatocellular carcinoma. *Hepatol Res* 2003; 25: 71–7.
15 Sakugawa H, Nakasone H, Nakayoshi T *et al.* Correlation between serum transaminase activity and virus load among patients with chronic liver disease type B. *Hepatol Res* 2001; 21: 159–68.
16 Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. *Hepatology* 2001; 34: 785–91.
17 Lok AS, Zoulim F, Locarnini S *et al.* Monitoring drug resistance in chronic hepatitis B virus (HBV) -infected patients during lamivudine therapy: evaluation of performance of INNO-LiPA HBV DR assay. *J Clin Microbiol* 2002; 40: 3729–34.
18 Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48 900 necropsies. *Cancer* 1954; 7: 462–503.
19 Liver Cancer Study Group of Japan. *Classification of Primary Liver Cancer*, 1st English edn. Tokyo: Kanehara, 1997.
20 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; 19: 1513–20.
21 Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; 60: 646–9.
22 UICC. Liver. In: Spiessl B, Beahrs OH, Hermanek P *et al.*, eds. *TMN Atlas: illustrated guide to the YNM/pTNM classification of malignant tumours*, 3rd edn. Berlin: Springer-Verlag, 1992; 104–111.
23 Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology* 1988; 8: 493–6.
24 Lok AS, Lai CL. Acute exacerbations in Chinese patients with chronic hepatitis B virus (HBV) infection: incidence, predisposing factors and etiology. *J Hepatol* 1990; 10: 29–34.
25 Tang B, Kruger WD, Chen G *et al.* Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers. *J Med Virol* 2004; 72: 35–40.
26 Volpes R, van den Oord JJ, Desmet VJ. Vascular adhesion molecules in acute and chronic liver inflammation. *Hepatology* 1992; 15: 269–75.
27 Andreone P, Gramenzi A, Cursaro C *et al.* High risk of hepatocellular carcinoma in anti-HBe positive liver cirrhosis patients developing lamivudine resistance. *J Viral Hepat* 2004; 11: 439–42.
28 Di marco V, Di Stefano R, Ferraro D *et al.* HBV-DNA suppression and disease course in HBV cirrhosis patients on long-term lamivudine therapy. *Antivir Ther* 2005; 10: 431–9.
29 Ikeda K, Saitoh S, Suzuki Y *et al.* Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. *Cancer* 1998; 82: 827–35.
30 Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999; 29: 971–5.
31 Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis. *J Hepatol* 2001; 34: 306–13.
32 van Zonneveld M, Honkoop P, Hansen BE *et al.* Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. *Hepatology* 2004; 39: 804–10.
33 Yuen MF, Hui CK, Cheng CC *et al.* Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: the effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology* 2001; 34: 139–45.
34 Lampertico P, Del Ninno E, Vigano M *et al.* Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. *Hepatology* 2003; 37: 756–63.
35 Sun HC, Tang ZY, Wang L *et al.* Postoperative interferon α treatment postponed recurrence and improved overall survival in patients after curative resection of HBV-related hepatocellular carcinoma: a randomized clinical trial. *J Cancer Res Clin Oncol* 2006; 132: 458–65.
36 Colombo M, Donato MF. Prevention of hepatocellular carcinoma. *Semin Liver Dis* 2005; 25: 155–61.

Original Paper

Risk Factors for Massive Blood Loss during Liver Resection for Hepatocellular Carcinoma in Patients with Cirrhosis

Shoji Kubo MD[1], Shigekazu Takemura MD[1], Satoshi Yamamoto MD[1], Seikan Hai MD[1] Tsuyoshi Ichikawa MD[1], Shintaro Kodai MD[1], Hiroji Shinkawa MD[1], Taichi Shuto MD[2] Kazuhiro Hirohashi MD[2], Hiromu Tanaka MD[1]

[1]Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine
[2]Department of General Practice, Osaka City University Hospital, Osaka, Japan
Corresponding Author: Shoji Kubo, MD, Department of Hepato-Biliary-Pancreatic 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: m7696493@msic.med.osaka-cu.ac.jp

KEY WORDS:
Liver resection; Hepatocellular carcinoma; Liver cirrhosis; Portal invasion; Hepatic vein involvement; Autologous blood storage

ABBREVIATIONS:
Hepatocellular Carcinoma (HCC); Odds Ratio (OR); Confidence Interval (CI): Central Venous Pressure (CVP); Inferior Vena Cava (IVC)

ABSTRACT

Background/Aims: Liver resection for hepatocellular carcinoma in patients with cirrhosis carries risk of major hemorrhage and sometimes requires blood transfusion. We investigated risk factors for massive blood loss during liver resection and indications for storing blood for autologous intraoperative transfusion.

Methodology: We analyzed clinical records of 100 patients with cirrhosis who underwent liver resection for hepatocellular carcinoma. Autologous blood was stored preoperatively for 19 patients.

Results: Intraoperative blood loss ranged from 5 to 3000mL (mean, 640). Liver resection was performed without transfusion in 67 patients and with autologous blood storage in 17 patients not receiving homologous blood. In the other 16 patients, homologous blood was transfused. Univariate analysis identified youth, large tumors (>4cm), major hepatectomy, portal tumor involvement, hepatic vein involvement, and prolonged operation time as risk factors for massive blood loss; multivariate analysis identified portal involvement and hepatic vein involvement as independent risk factors. Blood loss exceeded 1000mL in the 4 transfused group B patients and 3 of the 4 patients had hepatic vein involvement.

Conclusions: Portal involvement and hepatic vein involvement were risk factors for massive blood loss during liver resection for hepatocellular carcinoma in patients with cirrhosis. Autologous blood storage is indicated in patients with such risk factors.

INTRODUCTION

Despite recent advances in instrumentation and techniques including hepatic inflow occlusion and total vascular exclusion, liver resection is particularly associated with hemorrhage, sometimes requiring blood transfusion. Most hepatocellular carcinomas (HCC) are associated with chronic liver disease including cirrhosis, inducing a coagulopathy. As a result, reported transfusion rate and mean blood loss during liver resection for HCC exceed 30% and 1000mL respectively (1-4). Massive blood loss during liver resection has been reported to be a risk factor for postoperative hepatic failure (1,2,5,6), while homologous blood transfusion carries risks including viral hepatitis and graft-*versus*-host disease. Poor outcomes in patients receiving homologous blood transfusion recently have been reported (7-9), while some investigators have noted efficacy of autologous blood transfusion in liver resection (10-14). As few reports have analyzed risk factors for massive blood loss during liver resection for HCC in patients with cirrhosis, we carried out such a study including the issue of indications for preoperative autologous blood storage.

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METHODOLOGY

From early 1999 to August 2005, we performed liver resection for HCC in 100 patients with cirrhosis. Data from these 100 consecutive patients were analyzed retrospectively. Cirrhosis was diagnosed pathologically using the resection specimens. Subjects consisted of 70 men and 30 women, with a mean age of 65 years (range, 45 to 81 years). Sera from 65 patients were positive for anti-hepatitis C virus antibody alone, 15 patients were seropositive for hepatitis B surface antigen alone, while 1 patient was seropositive for both anti-hepatitis C virus antibody and hepatitis B surface antigen. Sera from 19 other patients were negative for both viral markers.

Before surgery, patients underwent ultrasonography, computed tomography, and angiography. Intraoperative ultrasonography also was performed. The imaging reliably detected portal involvement (portal vein tumor thrombi at the right and left portal veins and the bifurcation of the veins). When the trunk of the right, middle, and/or left hepatic vein was compressed or when tumor thrombi were present in hepatic veins, the hepatic vein was assumed to be

involved. These cases required exposure and dissection or cutting of the hepatic veins. In patients who underwent resection of at least one Couinaud segment (15), an ultrasonic dissector (CUSA Excel, Valleylab, Boulder, CO) was used for hepatic dissection during total or unilateral intermittent clamping of hepatic vascular inflow. In patients who underwent limited liver resection (resection of less than one segment), hepatic parenchyma was dissected with the ultrasonic dissector after coagulation along the dissection line with a microwave tissue coagulator (Microtaze; Heiwa Electronics Industry, Tokyo, Japan).

Operative blood loss was estimated as the sum of blood volumes absorbed by gauze sponges or removed by suctioning. The need for blood transfusion was determined by the surgeons together with anesthesiologists, guided by intraoperative monitoring of blood loss, vital signs, urinary output, and laboratory test results. A restrictive blood transfusion policy during liver resection in patients with cirrhosis, proposed by Makuuchi *et al.* (16), was used.

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.

Autologous blood was stored when the conditions below were met. Patients with hemoglobin of at least 11.0g/dL or hematocrit of at least 33%, and without acute infection were offered the option of preoperative autologous blood storage. After informed consent, 400mL (one collection) to 1200mL (three collections) of blood was taken, with the multiple collections carried out at weekly intervals.

A histologic grade for tumor differentiation was assigned using the Edmondson-Steiner classification (17) with modification according to the Liver Cancer Study of Japan (18). Noncancerous hepatic tissues also were examined pathologically. A histologic activity index (19) was used to evaluate the degree of fibrosis (cirrhosis).

Student's *t* test was used to analyze differences in age and tumor size. The Mann-Whitney U test was used to analyze differences in laboratory test results. Fisher's exact test or the chi-squared test was used to compare categoric data between groups. Odds ratio (OR) and 95% confidence interval (CI) were calculated using a conditional regression model. For multivariate analysis, the significant variables ($p<0.1$) were modeled using conditional logistic regression. The adjusted OR and 95% CI for each variable were estimated using the logistic regression coefficient.

RESULTS

Intraoperative blood loss ranged from 5 to 3000mL (mean, 640). Liver resection was performed without blood storage or transfusion in 67 patients (group A). In 17 patients, autologous blood was stored and liver resection was performed with only autologous blood transfusion, if needed (group B); 16 other patients received homologous blood (group C); including 2 patients also receiving autologous blood stored preoperatively. Intraoperative blood loss was 437±374mL (range, 5 to 1800) in group A, 695±445mL (150 to 1855) in group B, and 1432±773mL (355 to 3000) in group C. Of the 16 patients in group C, 11 had blood loses exceeding 1000mL; 5 of 67 patients in group A and 4 of 17 patients in group B had such losses. The proportion of patients with massive blood loss (>1000mL) was significantly higher in group C than in group A or B ($p<0.0001$ and $p=0.0149$ respectively). In group C, homologous blood transfusion was given in 2 patients with anemia, 1 patient with chronic renal failure, and 1 patient with ulcerative colitis, even though blood loss was less than 1000mL. In 10 of the 12 remaining patients in group C, blood loss exceeded 1000mL. Thus, homologous blood often was transfused when blood loss exceeded 1000mL.

TABLE 1 Clinicopathologic and Operative Factors in Patients with Massive vs. Lesser Intraoperative Blood Loss

Factors	Intraoperative blood loss (mL) ≤1000 (n=82)	>1000 (n=18)	*p* value
Age	66.3±8.0	61.4±7.3	0.0204
Gender, male:female	60:22	10:8	0.162
Hemoglobin, g/dL	13.4 (11.1, 15.5)	13.1 (10.9, 15.0)	0.419
Platelet count, x10⁴/mm³	13.2 (7.8, 19.4)	12.0 (6.9, 17.6)	0.131
Prothrombin time (sec)	11.3 (9.8, 12.7)	11.0 (9.3, 11.9)	0.336
Total bilirubin, g/dL	0.9 (0.5, 1.3)	0.8 (0.5, 1.2)	0.708
Albumin, g/dL	3.7 (3.2, 4.1)	3.8 (3.3, 4.2)	0.143
AST, IU/L	56 (33, 100)	56 (32, 89)	0.545
ALT, IU/L	58 (24, 107)	56 (26, 99)	0.851
$ICGR_{15}$, %	17.7 (9.4, 28.0)	15.0 (6.6, 26.5)	<0.0001
AFP elevation (>20ng/mL)	46	12	0.444
Tumor size, cm	2.8±1.6	3.8±2.1	0.0162
Single tumor number	45	11	0.794
Portal involvement	2	4	0.0091
Hepatic vein involvement	8	11	<0.0001
Major hepatectomy	1	5	0.0006
Operation time, min	235±83	373±158	<0.0001

AST: aspartate aminotransferase; ALT: alanine aminotransferase; $ICGR_{15}$: indocyanine green retention rate at 15 min; AFP: α-fetoprotein. For continuous variables, values are means ± SD, or medians followed in parentheses by 10% and 90% percentiles.

In addition to the above transfusion-defined groups, subjects were divided into a group of 18 patients with massive blood loss (>1000mL) and group of 82 patients without massive blood loss (**Table 1**). In patients with massive blood loss, mean age was significantly lower, and mean tumor size was significantly greater, than in patients with less loss. The proportion of patients with a large tumor (4cm or greater in diameter) was significantly higher among patients with massive blood loss than among patients with less loss ($p<0.0001$). The proportion of patients who underwent major hepatectomy (bisegmentectomy) was significantly higher for patients with massive blood loss than for patients with less loss. Operation time was significantly longer in the patients with massive loss.

Proportions of patients with portal tumor involvement and hepatic vein involvement were significantly higher among patients with massive blood loss. In two

TABLE 2 Odds Ratio for Massive Blood Loss (>1000mL) Evaluated by Univariate Analysis

Factors	Odds ratio 95%	Confidence interval	p value
Age (per 1 year)	0.928	0.870-0.991	0.0248
Tumor size (>4cm)	4.857	1.636-14.424	0.0044
Portal invasion	11.429	1.908-68.451	0.0076
Hepatic vein invasion	14.536	4.396-48.067	<0.0001
Major hepatectomy	31.154	3.364-288.477	0.0248
Operation time (per 1 min)	1.013	1.006-1.020	0.0003

TABLE 3 Adjusted Odds Ratio for Massive Blood Loss (>1000mL) Evaluated by Multivariate Analysis of Preoperative Factors

Factors	Adjusted odds ratio	95% Confidence interval	p value
Age	0.964	0.883-1.053	0.421
Tumor size (>4cm)	1.707	0.386-7.547	0.481
Portal invasion	11.798	1.335-104.252	0.0264
Hepatic vein invasion	12.274	2.885-52.224	0.0007

patients, tumor thrombus at the bifurcation of the right and left portal veins was removed. Of the six patients who underwent major hepatectomy, five were younger than the mean age for all subjects. Six patients who underwent major hepatectomy had at least one of three tumor-related risk factors (large tumor, portal involvement and/or hepatic vein involvement). Proportions of patients with a large tumor (>4cm) or portal involvement were significantly higher in major hepatectomy patients than in minor hepatectomy patients ($p=0.0240$ and $p<0.0001$ respectively). The proportion of patients with hepatic vein involvement tended to be higher in the major hepatectomy group than in the minor hepatectomy group ($p=0.0807$). Thus, major hepatectomy typically was performed in young patients with advanced HCC. Proportions of patients with portal involvement or hepatic vein involvement were significantly higher in cases with a long operation time (≥360 min) than in cases with a shorter operation ($p=0.0023$ and $p=0.0026$ respectively). The proportion of patients with large tumors tended to be higher in cases with a long operation time ($p=0.0697$).

By univariate analysis, the OR for massive blood loss (>1000mL) increased as age decreased (**Table 2**). Large tumor (>4cm), major hepatectomy, portal involvement, hepatic vein involvement, and prolonged operation time were significant risk factors for massive blood loss. We next calculated an adjusted OR using only preoperative variables to predict risk for massive blood loss before surgery. Multivariate analysis identified hepatic vein involvement and portal vein involvement as independent risk factors for massive blood loss (**Table 3**).

Although blood loss exceeded 1000mL in 4 patients in group B, the operation could be performed safely using only autologous blood transfusion without homologous transfusion. Of these 4 patients, 3 had hepatic vein involvement. In the other 13 group B patients, blood loss was less than 1000mL.

DISCUSSION

Youths, large tumors (>4cm), portal involvement, hepatic vein involvement, major hepatectomy, and prolonged operation time were risk factors for massive blood loss by our present univariate analysis, while portal involvement and hepatic vein involvement were independent risk factors for massive blood loss by multivariate analysis including only preoperative factors. Several previous studies identified factors affecting blood loss during liver resection as tumor size, number of tumors, tumor stage, macroscopic vascular invasion, major hepatectomy, central venous pressure (CVP), and operation time (3,20-23).

Although young patients had a significantly greater risk by univariate analysis, most patients who underwent major hepatectomy were young patients with portal involvement and/or hepatic vein involvement. Thus, amount of blood loss was affected by type of operation and advanced HCC stage rather than age itself. Proportions of patients with large tumors, portal involvement, or hepatic vein involvement were higher in major hepatectomy patients than in minor hepatectomy patients, and higher in association with long than short operations. Accordingly, prolonged major hepatectomy typically was performed for large, advanced HCC with portal involvement and/or hepatic vein involvement, resulting in massive blood loss.

By multivariate analysis using preoperative factors, portal involvement and hepatic vein involvement were independent risk factors for massive blood loss. In two patients, portal thrombectomy, a procedure with serious risk of hemorrhage, was required by portal tumor thrombus situated at the bifurcation of the right and left portal veins. Massive bleeding usually occurs from the major hepatic veins (right, middle, and left hepatic veins), inferior vena cava (IVC), and direct tributaries of the major hepatic veins or IVC. In some studies, maintenance of a low CVP (below 5 cm H2O) was effective in reducing blood loss during hepatectomy (20,23-26), although in the experiences of Hasegawa *et al.* (27) decreasing CVP by hypoventilation did not reduce blood loss during liver resection. Extrahepatic control of the hepatic veins and meticulous ligation of small tributaries entering the hepatic veins and IVC is important for preventing bleeding during parenchymal dissection.

Kajikawa *et al.* (12) performed transfusion when blood loss exceeded 2000mL in patients with cirrhosis underwent liver resection, while Wu *et al.* (3) found that no blood transfusion was needed in some patients even when estimated blood loss exceeded 2000mL. Miyagawa *et al.* (28) administered transfusions in liver resection when estimated blood loss exceeded 1500mL. In our present study, mean blood loss in group C was 1432±773mL, with blood loss exceeding 1000mL in 11 of 16 patients; the largest blood loss not requiring homologous blood transfusion was 1800mL. Thus, need for transfusion was likely when blood loss

exceeded 1000mL. In group B, blood loss exceeded 1000mL in 4 of 17 patients. Assuming that blood transfusion would be necessary only when blood loss exceeded 1000mL, autologous blood transfusion was not necessary in the other 13 patients. In addition, autologous blood transfusion might not have been necessary in all of the 4 patients who received it, since the volume lost ranged from 1045 to 1855mL. Preoperative storage of autologous blood therefore is not necessary for most patients, although autologous blood should be useful for patients with HCC who have risk factors for massive blood loss. In this study, portal involvement and hepatic vein involvement were independent risk factors for such loss. In patients with major venous involvement, autologous blood is useful in avoiding homologous blood transfusion.

In conclusion, patients with cirrhosis who have portal and/or hepatic vein tumor involvement are at a risk for massive blood loss during liver resection for HCC. When specific contraindications are not present, autologous blood storage is indicated in such patients.

REFERENCES

1 **Shimada M, Matsumata T, Akazawa K, Kamakura T, Itasaka H, Sugimachi K, et al:** Estimation of risk of major complications after hepatic resection. Am J Surg 1994; 167:399-403.
2 **Taniguchi H, Takahashi T:** Analysis of 210 elective hepatic resections. Hepatogastroenterology 1997; 44:1624-1631.
3 **Wu CC, Kang SM, Ho WM, Tang JS, Yeh DC, Liu TJ, et al:** Prediction and limitation of hepatic tumor resection without blood transfusion in cirrhotic patients. Arch Surg 1998; 133:1007-1010.
4 **Poon RTP, Fan ST, Lo CM, Ng IOL, Liu CL, Lam CM, et al:** Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. Ann Surg 2001; 234:63-70.
5 **Gozzetti G, Mazziotti A, Grazi GL, Jovine E, Gallucci A, Gruttadauria S, et al:** Liver resection without blood transfusion. Br J Surg 1995; 82:1105-1110.
6 **Fan ST, Lai ECS, Lo CM, Chu KM, Liu CL, Wong J:** Hepatectomy with an ultrasonic dissector for hepatocellular carcinoma. Br J Surg 1996; 83:117-120.
7 **Asahara T, Katayama K, Itamoto T, Yano M, Hino M, Okamoto Y, et al:** Perioperative blood transfusion as a prognostic indicator in patients with hepatocellular carcinoma. World J Surg 1999; 23:676-680.
8 **Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, et al:** Perioperative blood transfusion promotes recurrence of hepatocellular carcinoma after hepatectomy. Surgery 1994; 115:303-309.
9 **Kwon AH, Matsui Y, Kamiyama Y:** Perioperative blood transfusion in hepatocellular carcinomas: influence of immunologic profile and recurrence free survival. Cancer 2001; 92:771-778.
10 **Fujimoto J, Okamoto E, Yamanaka N, Oriyama T, Furukawa K, Kawamura E, et al:** Efficacy of autotransfusion in hepatectomy for hepatocellular carcinoma. Arch Surg 1993; 128:1065-1069.
11 **Kajikawa M, Nonami T, Kurokawa T, Hashimoto S, Harada A, Nakao A, et al:** Autologous blood transfusion for hepatectomy in patients with cirrhosis and hepatocellular carcinoma: use of recombinant human erythropoietin. Surgery 1994; 115:727-734.
12 **Chan ACW, Blumgart LH, Wuest DL, Melendez JA, Fong Y:** Use of preoperative autologous blood donation in liver resections for colorectal metastases. Am J Surg 1998; 175:461-465.
13 **Shinozuka N, Koyama I, Arai T, Numajiri Y, Watanabe T, Nagashima N, et al:** Autologous blood transfusion in patients with hepatocellular carcinoma undergoing hepatectomy. Am J Surg 2000; 179:42-45.
14 **Obayashi T, Taniguchi H, Mugitani T, Koh T, Kitagawa K, Kunishima S, et al:** Safety and utility of autologous blood transfusion for resection of metastatic liver tumor. Hepatogastroenterology 2001; 48:812-817.
15 **Couinaud C:** Lobes et segments hepatiques. Notes sur larchitecture anatomique du foie. Press Med 1954; 62:709-711.
16 **Makuuchi M, Takayama T, Gunvin P, Kosuge T, Yamazaki S, Hasegawa H:** Restrictive *versus* liberal blood transfusion policy for hepatectomies in cirrhotic patients. World J Surg 1989; 13:644-648.
17 **Edmonson HA, Steiner PE:** Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer 1954; 7:462-503.
18 **Liver Cancer Study Group of Japan:** Classification of primary liver Cancer, 1st English ed. Tokyo, Kanehara & Co., 1997.
19 **Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ:** Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19:1513-1520.
20 **Cunningham JD, Fong Y, Shriver C, Melendez J, Marx WL, Blumgart LH:** One hundred consecutive hepatic resections: blood loss, transfusion, and operative technique. Arch Surg 1994; 129:1050-1056.
21 **Mariette D, Smadja C, Naveau S, Borgonovo G, Vons C, Franco D:** Preoperative predictors of blood transfusion in liver resection for tumor. Am J Surg 1997; 173:275-279.
22 **Belghiti J, Hiramatsu K, Benoist S, Massault PP, Sauvanet A:** Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. J Am Coll Surg 2000; 191:38-46.
23 **Itamoto T, Katayama K, Nakahara H, Tashiro H, Asahara T:** Autologous blood storage before hepatectomy for hepatocellular carcinoma with underlying liver disease. Br J Surg 2003; 90:23-28.
24 **Rees M, Plant G, Wells J, Bygrave S:** One hundred and fifty hepatic resections: evolution of technique towards bloodless surgery. Br J Surg 1996; 83:1526-1529.
25 **Johnson M, Mannar R, Wu AVO:** Correlation between blood loss and inferior vena caval pressure during liver resection. Br J Surg 1998; 85:188-190.
26 **Jones RM, Moulton CE, Hardy KJ:** Central venous pressure and its effect on blood loss during liver resection. Br J Surg 1998; 85:1058-1060.
27 **Hasegawa K, Takayama T, Orii R, Sano K, Sugawara Y, Imamura H, et al:** Effect of hypoventilation on bleeding during hepatic resection: a randomized controlled trial. Arch Surg 2002; 137:311-315.
28 **Miyagawa S, Makuuchi M, Kawasaki S, Kakazu T:** Criteria for safe hepatic resection. Am J Surg 1995; 169:589-594.

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SURGERY TODAY

Surgical Treatment for Hepatocellular Carcinoma Detected After Successful Interferon Therapy

SHOJI KUBO[1], HIROMU TANAKA[1], SHIGEKAZU TAKEMURA[1], SATOSHI YAMAMOTO[1], SEIKAN HAI[1], TSUYOSHI ICHIKAWA[1], SHINTARO KODAI[1], HIROJI SHINKAWA[1], TAICHI SHUTO[2], and KAZUHIRO HIROHASHI[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 General Practice, Osaka City University Hospital, Osaka, Japan

Abstract

Purpose. Interferon therapy suppresses the development of hepatocellular carcinoma (HCC) and tumor recurrence after a resection of HCC in patients with chronic hepatitis C. However, the value of a liver resection and which method is best for the treatment of HCC detected after successful interferon therapy remains to be clarified. The risk factors for tumor recurrence after a liver resection for HCC detected after successful interferon therapy were investigated to determine the appropriate operative method for such HCC.

Methods. Risk factors including the clinicopathologic findings and the operative methods for tumor recurrence were evaluated by univariate and multivariate analyses in 24 patients who underwent liver resection for HCC detected after successful interferon therapy (sustained viral response or biochemical response).

Results. According to a univariate analysis, large tumor (>2 cm, $P = 0.0326$), multiple tumors ($P = 0.0372$), nonanatomic resection ($P = 0.0103$), and positive surgical margin (<5 mm of a free surgical margin, $P = 0.0245$) were possible risk factors for short tumor-free survival time after surgery. A multivariate analysis showed that large tumor ($P = 0.0407$), nonanatomic resection ($P = 0.0215$), and positive surgical margin ($P = 0.0253$) were independent risk factors for a short tumor-free survival time after surgery.

Conclusion. An anatomic resection with an appropriate surgical margin (≥5 mm of a free surgical margin) is recommended for patients with HCC detected after successful interferon therapy.

Key words Hepatocellular carcinoma · Interferon therapy · Liver resection · Anatomic resection · Chronic hepatitis C

Introduction

Hepatitis C virus (HCV) is one of the most common causes of hepatocellular carcinoma (HCC). The results of treatment for HCV-related HCC are still unsatisfactory because of a high rate of HCC recurrence and the progression of underlying chronic hepatitis and cirrhosis.[1–3] Recently, interferon (IFN) therapy has been shown to improve the liver function and histology, while suppressing the development of HCC in patients with chronic hepatitis C.[4–11] Previous studies have indicated that HCC is less likely to develop in patients in whom IFN effectively normalized the serum alanine aminotransferase (ALT) activity, even when HCV RNA did not disappear.[9,10] In addition, active hepatitis is a risk factor for tumor recurrence after a liver resection for HCV-related HCC[1,2] and postoperative IFN therapy suppresses recurrences while prolonging the survival time after treatment for HCC.[12–15]

Although HCCs can even develop in patients successfully treated with IFN,[16] we previously reported the tumor-free survival rate to be significantly higher in patients in whom IFN therapy was effective than in patients in whom IFN therapy was not effective and in patients who did not undergo IFN therapy.[17,18] Therefore, a liver resection offers hope for the low incidence of postoperative recurrences in HCC patients when previous IFN therapy has controlled their active hepatitis associated with HCV. However, the value of a liver resection for HCC detected after successful IFN therapy is still unclear. In this study, the risk factors for tumor recurrence after a liver resection for HCC detected after successful IFN therapy were investigated to determine the appropriate operative method for such HCC.

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Patients and Methods

Patients

The subjects in this study consisted of 24 patients who underwent a curative liver resection for HCC detected after successful IFN therapy. HCC was detected from 3 months to 13 years (mean, 4 years 5 months) after the end of IFN therapy. Curative surgery was defined as a complete removal of all macroscopic tumor masses and no histologic evidence of any tumor cells along the parenchymal transection line. Before the detection of HCC, IFN-α was administered in 10 patients, IFN-α2a in 1 patient, IFN-α2b in 11 patients, and IFN-β in 2 patients. The response to IFN therapy was classified based on the changes in the HCV RNA levels and the serum ALT activity during and immediately after IFN administration, and for at least 1 year after IFN therapy. Nineteen patients obtained a sustained viral response, which 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 a transient disappearance of serum HCV RNA, was demonstrated in 5 patients. A hemihepatectomy, bi-segmentectomy, and segmentectomy, are all assumed to be anatomic resections. The median follow-up from the operation until the detection of HCC recurrence or study endpoint (September 30, 2006) was 678 days (range, 125–4580).

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.

Detection of recurrences

The serum concentration of α-fetoprotein and protein induced by the absence of both vitamin K and antagonist II were measured 1 month after surgery and then every 3 months thereafter. Ultrasonography, computed tomography, chest radiography, or some combination of these tests were done 1 month after surgery and then every 3 months thereafter. When a recurrence of the HCC was strongly suspected based on the findings of tumor markers or imaging, selective hepatic angiography, ultrasound-guided biopsy, or both was conducted to establish a definitive diagnosis.

Histology

The guidelines of the Liver Cancer Study Group of Japan[19] were used to categorize the histologic findings. The tumor number was determined by macroscopic and microscopic examinations. The histologic grade of differentiation (well, moderate, or poor) of HCC was determined according to a modification of Edmondson and Steiner.[20] The grade (grade of active hepatitis) and stage (degree of hepatic fibrosis) in the noncancerous hepatic tissue specimens were determined based on the score of the histologic activity index,[21] which was determined by four events, i.e., periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis.

Statistics

The survival rates were calculated by the Kaplan–Meier method and then 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 (September 30, 2006) in patients who did not develop a recurrence. Cox's proportional hazard model with a stepwise variable selection was used for a multivariate analysis. A P value of less than 0.05 was considered to be significant. The variables were selected based on the findings of previous studies or our own clinical experience. The variables chosen were age (≥65 or <65), gender, history of alcohol abuse (intake of at least 86 g of ethanol daily for at least 10 years),[22] a history of blood transfusion, anti-hepatitis B core antibody (positive or negative), total bilirubin (≥1 or <1 mg/dl), albumin concentration (≥4.0 or <4.0 g/dl), indocyanine green retention rate at 15 min ($ICGR_{15}$, ≥10 or <10%), a platelet count (≥10×10^4 or <10×10^4/μl), serum α-fetoprotein concentration (>20 or ≤20 ng/ml), the largest diameter of the main tumor (>2 or ≤2 cm), the degree of differentiation of the main tumor (well-, moderately vs. poorly differentiated HCC), the number of tumors (single or multiple including intrahepatic metastasis), microscopic portal invasion, the grading score (0, 1, or 2 to 4), the staging score (0 to 2 or 3, 4), the operative methods (anatomic or nonanatomic resection), and the surgical margin. When the surgical free margin based on a pathologic examination was less than 5 mm, it was defined as a positive surgical margin. The statistical analysis was performed using the StatView program (SAS Institute, Cary, NC, USA).

Results

Of the 24 patients, HCC recurred in 8 patients. In 5 of the 8 patients, multiple recurrent tumors were detected in the remnant liver within 3 years after the operation. In 3 other patients, a solitary recurrent tumor was detected in the remnant liver. In 2 of the 3 patients, the recurrent tumor was treated with microwave coagula-

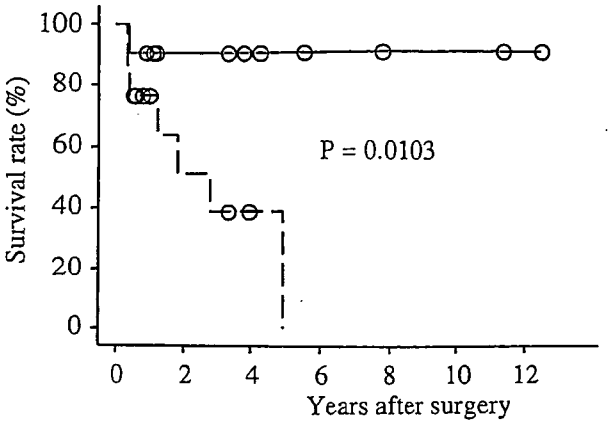

Fig. 1. Tumor-free survival rate after a resection of hepatocellular carcinoma in patients who underwent an anatomic resection or a nonanatomic resection. *Continuous line*, anatomic resection (n = 11); *dotted line*, nonanatomic resection (n = 13)

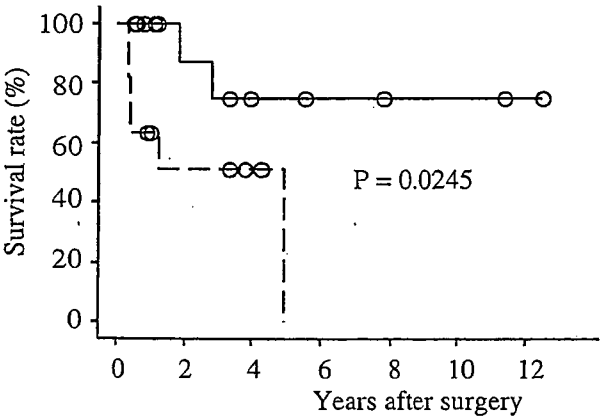

Fig. 2. Tumor-free survival rate after a resection of hepatocellular carcinoma in patients with a positive or negative surgical margin. *Continuous line*, negative surgical margin (n = 13); *dotted line*, positive surgical margin (n = 11)

tion therapy or radiofrequency ablation therapy and the patients are still alive without HCC recurrence (6 years 4 months after the therapy, 1 month after the therapy, respectively). In the other patient, a second liver resection was performed and the patient is alive without HCC recurrence (2 years 6 months after the second operation). Based on a univariate analysis, large size tumor (P = 0.0326), multiple tumors (P = 0.0372), nonanatomic resection (P = 0.0103), and positive surgical margin (P = 0.0245) were possible risk factors for a short tumor-free survival time after the operation (Table 1). The tumor-free survival rates in patients who underwent an anatomic resection or nonanatomic resection and those in patients with positive or negative surgical margin are shown in Figs. 1 and 2. A multivariate analysis showed a large tumor (risk ratio, 0.015; 95% confidence interval, 0.000257–0.836; P = 0.0407), a nonanatomic resection (0.0396, 0.00252–0.622, P = 0.0215), and a positive surgical margin (0.067, 0.006–0.716, P = 0.0253) to all be independent risk factors for a short tumor-free survival time after the operation (Table 2). In the 8 patients with HCC recurrence, 7 patients had a larger tumor (>2 cm) and 5 patients had multiple tumors. Seven patients underwent a nonanatomic resection while another patient with portal invasion in the left portal vein underwent an extended left lobectomy. The surgical margin was positive in 6 of the 8 patients.

Discussion

Our previous study showed that an advanced age, HCV viremia, elevated aspartate aminotransferase (AST) activity, elevated ALT activity, large tumor size, multiple tumors, moderately or poorly differentiated HCC, portal invasion, and high-grade score (active hepatitis) were possible risk factors for HCC recurrence after a liver resection for HCV-related HCC.[3] Some recurrences are thought to result from intrahepatic metastases originating from the primary cancer and some from multicentric (multifocal) carcinogenesis after surgery.[23–25] Studies of the risk factors for multicentric occurrence of HCC or recurrence after resection of HCC suggest that chronic active hepatitis and hepatic fibrosis are important factors in multicentric carcinogenesis after surgery.[2,3,26,27] It is thus important to consider the potential for hepatic carcinogenesis that is related to active hepatitis and hepatic fibrosis to determine the appropriate treatment for HCC patients with chronic hepatitis C.[28] On the other hand, many studies have shown that IFN therapy improves active hepatitis and hepatic fibrosis, thus resulting in a decreased incidence of HCC development.[4–10] Several studies have also confirmed that IFN therapy suppresses the incidence of HCC recurrence after treatment for HCV-related HCC.[12,13,15] Although HCC develops even in patients who were treated successfully with IFN, the prognosis after treatment for such HCC is better than in patients with active hepatitis caused by HCV.[16,17,29] These findings indicate that successful IFN treatment for HCV suppresses the potential for carcinogenesis while decreasing the development of multicentric carcinogenesis after surgery for primary HCC.

In this study, 8 patients had HCC recurrence after the operation. Of the 8 patients, 7 had a large-sized tumor (>2 cm) and 5 had multiple tumors. Seven patients underwent a nonanatomic resection and the surgical margin was positive in 6 patients. A univariate analysis showed the risk factors for recurrence include tumor

Table 1. Tumor-free survival rate after a resection of hepatocellular carcinoma

Variable (*n*)	Survival rate (%)			*P* value
	4 years	8 years	12 years	
Age (years)				
≥65 (10)	67	67	67	0.145
<65 (14)	70	0	0	
Gender				
Male (18)	72	54	54	0.209
Female (6)	33	—	—	
Alcohol abuse				
Yes (4)	51	51	—	0.771
No (20)	68	52	52	
Blood transfusion				
Yes (6)	67	—	—	0.925
No (18)	58	47	47	
Anti-hepatitis B core antibody				
Positive (13)	73	73	73	0.300
Negative (11)	54	—	—	
Total bilirubin (mg/dl)				
≥1.0 (11)	76	—	—	0.255
<1.0 (13)	55	37	37	
Albumin (g/l)				
≥4.0 (16)	81	55	—	0.0884
<4.0 (8)	38	38	38	
$ICGR_{15}$ (%)				
≥10 (17)	62	62	62	0.889
<10 (7)	67	—	—	
Platelet count ($\times 10^4/mm^3$)				
≥10.0 (21)	73	58	58	0.358
<10.0 (3)	—	—	—	
α-Fetoprotein (ng/ml)				
>20.0 (12)	67	—	—	0.958
≤20.0 (12)	63	63	63	
Tumor size (cm)				
≥2.0 (15)	37	37	—	0.0326
<2.0 (9)	100	67	67	
Differentiation of tumor				
Well or moderately (11)	77	63	63	0.0918
Poorly (13)	52	—	—	
Tumor number				
Single (14)	73	73	73	0.0372
Multiple (10)	47	—	—	
Microscopic portal invasion				
Yes (11)	73	—	—	0.922
No (13)	63	42	42	
Grading score				
0, 1 (11)	82	—	—	0.428
2–4 (13)	57	43	43	
Staging score				
0–2 (11)	76	—	—	0.581
3, 4 (13)	56	56	56	
Operative method				
Anatomic (11)	91	91	91	0.0103
Nonanatomic (13)	38	0	0	
Surgical margin				
Positive (11)	51	0	0	0.0245
Negative (13)	75	75	75	

$ICGR_{15}$, indocyanine green retention rate at 15 min

Table 2. Independent risk factors for short tumor-free survival time after liver resection for hepatocellular carcinoma (multivariate analysis)

Variable	Risk ratio	95% confidence interval	*P*
Large tumor (>2 cm)	0.015	0.000257–0.836	0.0407
Nonanatomic resection	0.0396	0.00252–0.622	0.0215
Positive surgical margin	0.067	0.006–0.716	0.067

factors such as large HCC and multiple tumors as well as operative factors such as a nonanatomic resection and a positive surgical margin. A multivariate analysis showed a large tumor, a nonanatomic resection, and a positive surgical margin to be independent risk factor for short tumor-free survival time after the operation. The hepatitis activity was not a risk factor in patients who were treated successfully with IFN therapy. In this study, multiple recurrent tumors were detected in the remnant liver within 3 years after the operation in 5 of 8 patients with an HCC recurrence; the tumors were thought to be intrahepatic metastases originating from the primary tumor in at least 5 of the 8 patients.[23,25] These findings indicate that HCC recurrences after surgery are mainly due to intrahepatic metastases that had not been resected in such patients; successful IFN therapy might thus have decreased the incidence of multicentric carcinogenesis after the resection of the primary HCC. Anatomic resections along the portal system can remove occult intrahepatic metastases, which spread mainly through the portal vein. On the other hand, a nonanatomic resection and ablation therapy including microwave coagulation therapy and radiofrequency ablation therapy are thus considered to be disadvantageous from the stand point of eradiating such intrahepatic metastases.[30] A liver resection with an appropriate surgical margin also can remove small metastatic lesions surrounding the main tumor. Therefore, an anatomic resection with a sufficient surgical margin is recommended in HCC patients who were treated successfully by IFN therapy. Recently, the value of an anatomic resection for HCC has been reported.[30] The value of an anatomic resection should be emphasized especially in patients with HCC detected after successful IFN therapy.

On the other hand, two patients who underwent microwave coagulation therapy or a second liver resection for a solitary recurrent tumor have survived without any HCC recurrence for a long time after treatment for their first recurrence. In such patients, successful IFN therapy possibly suppressed the risk of carcinogenesis, thus inducing a prolonged tumor-free survival time after treatment for their first recurrence. Similar findings have been reported by Shiratori et al.[15]

In conclusion, an anatomic resection with an appropriate surgical margin is recommended for patients with HCC detected after successful IFN therapy. However, the number of patients in this study was too small to yield any definitive conclusions.

References

1. Shuto T, Hirohashi K, Kubo S, Tanaka H, Tsukamoto T, Yamamoto T, et al. Changes and results of surgical strategies for hepatocellular carcinoma: results of a 15-year study on 452 consecutive patients. Surg Today 1998;28:1124–9.
2. Kubo S, Nishiguchi S, Shuto T, Tanaka H, Tsukamoto T, Hirohashi K, et al. Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. Jpn J Cancer Res 1999;90:162–70.
3. Kubo S, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, Ikebe T, et al. Risk factors for recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. World J Surg 2000;24:1559–65.
4. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima 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.
5. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 1998;27:1394–402.
6. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, 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.
7. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. Lancet 1998;351:1535–9.
8. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata 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. Nishiguchi S, Shiomi S, Nakatani S, Takeda T, Fukuda K, Tamori A, et al. Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. Lancet 2001;357:196–7.
10. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, 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.
11. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, 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. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary

tumor—a prospective randomized study of hepatitis C virus-related liver cancer. Hepatology 2000;32:228–32.
13. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, et al. Effects of long-term postoperative interferon-α therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma: A randomized, controlled trial. Ann Intern Med 2001;134:963–7.
14. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. Br J Surg 2002;89:418–22.
15. Shiratori Y, Shiina S, Teratani T, Imamura M, Obi S, Sato S, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med 2003;138:299–306.
16. Kubo S, Nishiguchi S, Tamori A, Hirohashi K, Tanaka H, Tsukamoto T, et al. Resected cases of hepatocellular carcinoma detected after interferon therapy for chronic hepatitis C. Hepatogastroenterology 2000;47:1100–2.
17. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, et al. Influence of previous interferon therapy on recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. Jpn J Cancer Res 2001;92:59–66.
18. Uenishi T, Kubo S, Hirohashi K, Tanaka H, Shuto T, Yamamoto T, et al. Relationship between response to previous interferon therapy and postoperative recurrence of hepatitis C virus-related hepatocellular carcinoma. Hepatol Res 2002;24:404–12.
19. Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 4th ed. (in Japanese). Tokyo: Kanehara; 2000.
20. Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48900 necropsies. Cancer 1954;7: 462–503.
21. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.
22. Liver Cancer Study Group of Japan. Primary liver cancer in Japan: Clinicopathological features and results of surgical treatment. Ann Surg 1990;211:277–87
23. Kumada T, Nakano S. Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 1997;25:87–92.
24. Kubo S, Kinoshita H, Hirohashi K, Tanaka H, Shuto T, Okuda T, et al. Patterns of and risk factors for recurrence after liver resection for well-differentiated hepatocellular carcinoma: a special reference to multicentric carcinogenesis after operation. Hepatogastroenterology 1999;46:3212–5.
25. Sakon M, Umeshita K, Nagano H, Eguchi H, Kishimoto S, Miyamoto A, et al. Clinical significance of hepatic resection in hepatocellular carcinoma: analysis by disease-free survival curves. Arch Surg 2000;135:1456–9.
26. Kubo S, Yamamoto T, Ikebe T, Shuto T, Hirohashi K, Tanaka H, et al. Relationship between multicentric occurrence of hepatocellular carcinoma and histology of noncancerous hepatic tissue in patients with chronic hepatitis C. Jpn J Cancer Res 1999;90: 1076–80.
27. Tarao K, Takemiya S, Tamai S, SugimasaY, Ohkawa S, Akaike M, et al. Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. Cancer 1997;79:688–94.
28. Kubo S, Tsukamoto T, Hirohashi K, Tanaka H, Shuto T, Takemura S, et al. Appropriate surgical management of small hepatocellular carcinomas in patients infected with hepatitis C virus. World J Surg 2003;27:437–42.
29. Ikeda K, Kobayashi M, Saitoh S, Someya T, Hosaka T, Akuta N, et al. Recurrence rate and prognosis of patients with hepatocellular carcinoma that developed after elimination of hepatitis C virus RNA by interferon therapy. Oncology 2003;65:204–10.
30. Hasegawa K, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, et al. Prognostic impact of anatomic resection for hepatocellular carcinoma. Ann Surg 2005;242:252–9.

Original Paper

A Histopathological Study on Combined Hepatocellular and Cholangiocarcinoma: Cholangiocarcinoma Component is Originated from Hepatocellular Carcinoma

Tomoko Wakasa[1], Kenichi Wakasa[1], Taichi Shutou[2], Seikan Hai[2], Shoji Kubo[2]
Kazuhiro Hirohashi[2], Koji Umeshita[3], Morito Monden[3]
[1]The Department of Diagnostic Pathology and [2]Department of Hepatobiliary and Pancreatic Surgery
Graduate School of Medicine, Osaka City University, Osaka, Japan
and [3]Department of Surgery and Clinical Oncology, Graduate School of Medicine, Osaka University
Suita, Japan
Corresponding Author: Tomoko Wakasa, MD, The Department of Diagnostic Pathology
Graduate School of Medicine, Osaka City University Graduate School of Medicine, 1-5-7, Asahi-machi
Abeno-ku, Osaka City, Osaka 545-8586, Japan
Tel: +81 6 6645 2225, Fax: +81 6 6646 3798, E-mail: haba@med.osaka-cu.ac.jp

KEY WORDS:
Combined hepatocellular and cholangiocarcinoma; Hepatic neoplasm; Ki-67 labeling index; Metaplasia

ABBREVIATIONS:
Alpha-Fetoprotein (AFP); Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II); Carcinoembryonic Antigen (CEA); Carbohydrate Antigen (CA19-9); Hepatitis B virus surface Antigen (HBsAg); Hepatitis C Virus Antibody (HCVAb)

ABSTRACT

Background/Aims: Combined hepatocellular and cholangiocarcinoma of the liver is relatively infrequent, and its pathogenesis remains obscure. The aim of this study is to investigate its clinical and pathological features and proliferative activity.

Methodology: In this study, we investigated the histopathological features, Ki-67 labeling index, and p53 immunohistochemistry of 18 surgically resected cases of combined hepatocellular and cholangiocarcinoma among 1102 consecutive cases of surgically resected primary liver cancers. All tumors were compatible with the WHO definition of this tumor.
Microscopically, we classified the cases into the following three categories according to the arrangement of the hepatocellular carcinoma and cholangiocarcinoma components; 1) Type I in which hepatocellular carcinoma and cholangiocarcinoma formed nodules that could easily be distinguished from each other, 2) Type II in which the both components were finely mixed, so that the two components were almost indistinguishable, and 3) Type III in which the tumors had lobular structures with hepatocellular carcinomas existing centrally and cholangiocarcinomas existing peripherally.

Results: Microscopically, the tumors were classified into type I 7 tumors, type II 5 tumors, and type III 6 tumors. In one case of type I, well differentiated hepatocellular carcinoma demonstrated cholangiocarcinoma in "nodules-in-nodules" fashion. The average of Ki-67 labeling index of hepatocellular carcinoma component of combined hepatocellular and cholangiocarcinoma was 4.4±3.4% and the index of cholangiocarcinoma component was 11.0±8.5%, which is significantly higher than that of the hepatocellular carcinoma component. On p53 immunohistochemistry, 5 of 18 cases (29.4%) were positive. In one case, the cholangiocarcinoma component was positive for p53, but the hepatocellular carcinoma component was negative. In the other 4 cases, both the hepatocellular carcinoma and cholangiocarcinoma components were positive.

Conclusions: Microscopically, type III seems to be a feature of metaplasia or proliferation of bipotential progenitor cells. Metaplasia of hepatocellular carcinoma to intrahepatic cholangiocarcinoma is assumed to be one of the pathogenic pathways of combined hepatocellular and cholangiocarcinoma.

INTRODUCTION

Primary liver cancers can be classified either into hepatocellular carcinoma, originating from the hepatocytes, or intrahepatic cholangiocarcinoma, originating from the intrahepatic bile duct epithelium. However, there are occasional cases that present both hepatocellular carcinoma and intrahepatic cholangiocarcinoma in the same liver. Such tumors are designated as combined hepatocellular and cholangiocarcinoma. But histological criteria for the combined hepatocellular and cholangiocarcinoma have not been universally agreed upon. Allen *et al.* (1) subclassified combined hepatocellular and cholangiocarcinoma into 1: collision type, 2: combined type, and 3: mixed type. However, from the clinicopathological features, it would be better to exclude the collision type from combined hepatocellular and cholangiocarcinoma. In the World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of the Digestive System, combined hepatocellular and cholangiocarcinoma is diagnosed when lesions containing unequivocal elements of both hepatocellular and cholangiocar-

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cinoma that are intimately admixed (2).

In 1985, Goodman *et al.* (3) classified the combined hepatocellular and cholangiocarcinoma into the following three categories: 1) Hepatocellular carcinoma and intrahepatic cholangiocarcinoma could be clearly distinguished. 2) Cholangiocarcinoma lesions with tubular pattern were contiguous to the hepatocellular carcinoma lesions with a trabecular or solid pattern and which shares hepatocellular carcinoma and cholangiocarcinoma transitional features. 3) Hepatocellular carcinoma and cholangiocarcinoma are almost indistinguishable, the tumor could be interpreted as either intrahepatic cholangiocarcinoma or poorly differentiated hepatocellular carcinoma, and these were therefore considered as an intermediate type between hepatocellular carcinoma and intrahepatic cholangiocarcinoma cases.

Combined hepatocellular and cholangiocarcinoma is relatively infrequent, and its clinicopathological features are still obscure. In this study, we investigated the clinicopathological features of 18 surgically resected combined hepatocellular and cholangiocarcinoma classified into Allen type 2 and 3. Also, we evaluated the proliferative activity using the immunostaining of Ki-67 between the hepatocellular carcinoma component and cholangiocarcinoma component.

METHODOLOGY

Among 1102 consecutive cases of primary liver cancers surgically resected at Osaka City University Hospital and Osaka University Hospital, between January 1986 and December 2000, 18 cases were combined hepatocellular and cholangiocarcinoma, 1036 cases were hepatocellular carcinoma, 45 cases were intrahepatic cholangiocarcinoma. The 18 cases of combined hepatocellular and cholangiocarcinoma were examined in this study. The resected liver specimens were fixed in 10% neutralized buffered formalin immediately after hepatectomy, cut into slices, embedded in paraffin, prepared into 4-μm sections and were routinely stained with hematoxylin-eosin, diastase digested periodic acid-Schiff (PAS), and alcian blue. In addition, all 18 cases were immunohistochemically examined for cytokeratin 7 (OV-TL 12/30, DAKO, Glostrup, Denmark), cytokeratin 19 (RCK108, DAKO, Glostrup, Denmark), alpha fetoprotein (rabbit polyclonal antibody, DAKO, Glostrup, Denmark), fibrinogen (rabbit polyclonal antibody, DAKO, Glostrup, Denmark), p53 (DO-7, DAKO, Glostrup, Denmark), and Ki-67 antigen (rabbit polyclonal antibody, DAKO, Glostrup, Denmark). Methods of immunohistochemistry, other than Ki-67, were the labeled streptoavidin-biotin method. The method of immunohistochemistry of Ki-67 was the enhanced polymer one-step staining method.

We identified the hepatocellular carcinoma component based on the following criteria. 1) The trabecular structure surrounded by sinusoid-like vessels. 2) Eosinophilic granularity of the cytoplasm. 3) Bile production. In some cases the immunohistochemical staining of alpha-fetoprotein or fibrinogen was required to help in recognizing the hepatocellular carcinoma component. On the other hand, the cholangiocarcinoma component was defined as follows: 1) Tumor cells were cuboidal or columnar, and had rather basophilic cytoplasm. 2) The nuclei were rather oval or spindle shaped 3) Tumor cells were positive in either alcian blue stain or diastase digested PAS stain **(Figure 1)**.

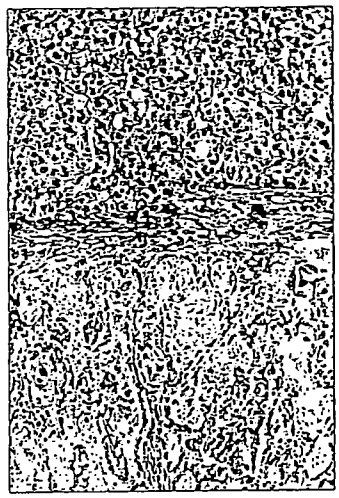

FIGURE 1
The microscopic finding of a combined hepatocellular and cholangiocarcinoma. The upper side of the figure shows the hepatocellular carcinoma component of the combined hepatocellular and cholangiocarcinoma. It shows the trabecular structure surrounded by sinusoid-like vessels, and the eosinophilic granularity of the cytoplasm. The lower side of the figure shows the cholangiocarcinoma component. Tumor cells are cuboidal or columnar, and have rather basophilic cytoplasm (HE).

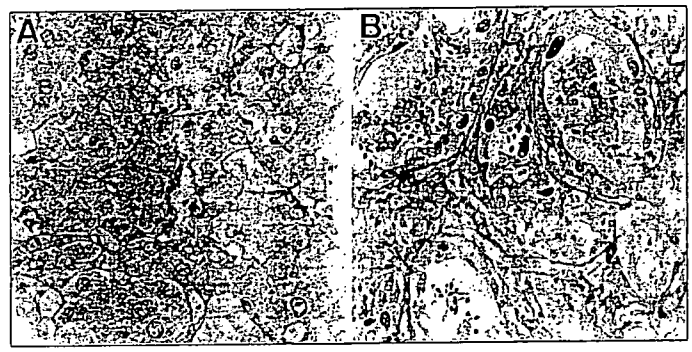

FIGURE 2 Immunohistochemistry for Ki-67. **(A)** The area of a hepatocellular carcinoma component. The Ki-67 labeling index was 6.7% in this area. **(B)** The area of a cholangiocarcinoma component. The Ki-67 labeling index was 13.5% in this area.

For the assessment of the Ki-67 labeling index in each case, the sections were counted at high power magnifications; 1000 or more nuclei were counted in the hepatocellular carcinoma components and cholangiocarcinoma components, and the number of cells showing positive nuclear staining, regardless of staining intensity, was recorded **(Figure 2)**. Two independent observers without prior knowledge evaluated the Ki-67 labeling index. Necrotic areas were not included in the counting. For comparison with the combined hepatocellular and cholangiocarcinoma, 11 cases of moderately differentiated hepatocellular carcinoma and 8 cases of intrahepatic cholangiocarcinoma were also stained with Ki-67 and counted for the number of cells showing positive nuclear staining in the same way.

The clinical backgrounds regarding the average age, male: female ratio, hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb) positivity, alpha-fetoprotein level, and the presence of chronic liver diseases were analyzed. Disease-free survival was mea-

sured from the date of hepatic resection to the date when recurrent disease was diagnosed or absence of detectable tumor, to the date of death or the date of last follow-up.

The Student's *t*-test was used for the statistical analysis of categorical data. A level of $P<0.05$ was considered statistically significant.

RESULTS

Clinical Features and Prognosis

Of 1102 patients with primary liver cancers, 1036 (94.0%) patients had hepatocellular carcinoma, 45 (4.0%) had intrahepatic cholangiocarcinoma, 3 (0.3%) had both hepatocellular carcinoma and intrahepatic cholangiocarcinoma independently, and 18 (1.6%) had combined hepatocellular and cholangiocarcinoma. The average patient age was 61.0 years for hepatocellular carcinoma, 60.8 years for intrahepatic cholangiocarcinoma, 62.3 years for collision type hepatocellular carcinoma and intrahepatic cholangiocarcinoma, and 58.2 years for combined hepatocellular and cholangiocarcinoma.

TABLE 1 Clinicopathological Findings of Combined Hepatocellular and Cholangiocarcinoma

Case	Age	Sex	AFP (ng/mL)	PIVKAII (AU/mL)	CEA (ng/mL)	CA19-9 (U/mL)	HBsAg	HCV
1	56	M	8.5	1.18	5.1	356	+	+
2	61	F	526.5	0.07	2.4	23	-	+
3	66	F	49.1	NA	11.8	NA	-	+
4	30	M	1300	0.09	NA	NA	+	-
5	61	M	13.6	0.06>	NA	NA	-	+
6	58	M	3.9	0.06>	NA	NA	-	-
7	41	M	5	NA	NA	NA	-	-
8	56	M	5	NA	20	NA	-	-
9	57	M	5	4.037	4.3	26	-	-
10	47	F	5	0.0625	1.4	504	+	-
11	65	M	6	NA	15.6	NA	-	NA
12	64	M	6	NA	NA	NA	-	NA
13	61	M	117	359	27	50	-	-
14	52	M	5	69	13	NA	+	-
15	74	M	3297	9408	3	37	-	-
16	58	M	6	71	5	NA	-	+
17	72	F	1190	1321	NA	NA	-	+
18	69	M	75	25	4.5	NA	-	+

TABLE 2 Histopathology and Ki-67 Labeling Index of Combined Hepatocellular and Cholangiocarcinoma

Case	Non-tumor liver	Predominant part of tumor	Type of arrangement	Diameter (cm)	Ki-67 Labeling Index HCC (%)	Ki-67 Labeling Index CC (%)
1	CH	HCC	3	9	2.79	7.2
2	CH	HCC	3	5.7	3.7	9.77
3	Cirrhosis	CC	2	3	12.66	3.79
4	NL	HCC	2	3.8	1.61	1.58
5	CH	CC	3	5	2.38	9.95
6	NL	HCC	1	17	7.66	34.07
7	NL	UNDIF	2	6.5	NA	NA
8	Cirrhosis	CC	3	2.4	2.1	7.23
9	CH	HCC	2	5	0.85	12.15
10	CH	CC	1	5.6	NA	NA
11	NL	CC	1	12.5	2.98	5.99
12	Cirrhosis	HCC	1	6.8	1.07	2.71
13	NL	UNDIF	2	9	5.61	5.61
14	CH	CC	3	6.9	2.77	4.6
15	CH	HCC	1	6.8	NA	NA
16	CH	HCC	3	4.3	NA	NA
17	Cirrhosis	HCC	1	3.7	8.9	15.71
18	Cirrhosis	CC	1	3.1	6.72	13.5

NA: not available; CH: chronic hepatitis; HCC: part of hepatocellular carcinoma; CC: part of cholangiocarcinoma; UNDIF: part of undifferentiated carcinoma; NL: normal liver.

Clinicopathological findings of the 18 cases are summarized in **Table 1**. The male to female ratio was 5.2:1 for hepatocellular carcinoma, 2.5:1 for intrahepatic cholangiocarcinoma, all three were male for collision type hepatocellular carcinoma and intrahepatic cholangiocarcinoma, and 3.5:1 for combined hepatocellular and cholangiocarcinoma.

Among 18 cases of combined hepatocellular and cholangiocarcinoma, the serum alpha-fetoprotein (AFP) levels were found to be abnormally high (>20ng/mL) in 7 (38.9%). The serum protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels were examined in 13 patients, and 8 (61.5%) were abnormal (0.1>AU/mL). The serum carcinoembryonic antigen (CEA) levels were examined in 12 patients and 11 (91.7%) were abnormal (>2.4ng/mL). The serum carbohydrate antigen (CA19-9) levels were examined in 6 patients, and 4 (67%) were abnormally high (normal ranges <37 U/mL).

Hepatitis B virus surface antigen (HBsAg) was positive in 4 (22.2%) out of 18 cases. Hepatitis C virus antibody (HCVAb) was positive in 7 (43.8 %) out of 16 cases examined. Both HBsAg and HCVAb were positive in 1 (6.3%) and both were negative in 6 (37.5%) out of 16 cases examined. Hepatitis B virus antigen was positive in 174 (18.5%) cases out of 940 cases of hepatocellular carcinoma and 3 (6.5%) cases out of 46 cases in intrahepatic cholangiocarcinoma. HCVAb was positive in 551 (68.3%) cases out of 807 cases in hepatocellular carcinoma and 8 (19.5%) cases out of 41 cases in intrahepatic cholangiocarcinoma.

Fourteen cases of combined hepatocellular and cholangiocarcinoma were followed after hepatic resections. The 1- and 3-year survival rates after operation were 73.3% and 33.3%, respectively.

At the time of hepatectomy, regional lymphadenectomy was done in 4 cases and metastatic tumors in the lymph node were found in 2 cases, in which the metastatic tumors were intrahepatic cholangiocarcinoma. Recurrence was found in 9 cases, in which 6 cases revealed recurrent tumors in the liver, but we could not perform a pathological examination in all 6 cases. The laboratory data suggested that the recurrent tumors were intrahepatic cholangiocarcinoma in 2 cases, hepatocellular carcinoma in 2 cases, and unknown in the remaining 2 cases. Distant metastasis was found in 4 cases (Lung; 2 cases, paraaortic lymph node; 1 case, bone; 2 case, skin; 1 case).

Gross Findings

Ten cases were the hepatocellular carcinoma predominant type, which resembled hepatocellular carcino-

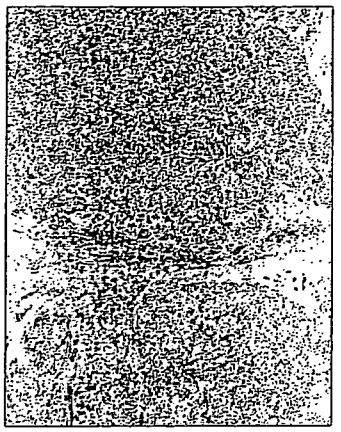

FIGURE 3A
Low power view of type III. The tumor exhibits lobular structures mimicking hepatic lobular structure (HE).

FIGURE 3B
High power view of type III. In the central area of the lobular structures hepatocellular carcinoma components exist and in the periphery cholangiocarcinoma components exist (HE).

ma with a greenish or yellowish color, and were associated with varying degrees of hemorrhage or necrosis and the tumors had demarcated fibrous capsules. The remaining 8 cases were the cholangiocarcinoma predominant type, which resembled intrahepatic cholangiocarcinoma and was gray in color with irregular margins. The largest diameter of the tumors ranged from 2.4 to 17cm (average: 6.5±3.6cm).

Histopathological Findings

Histopathological findings of the 18 combined hepatocellular carcinoma are summarized in **Table 2**. Among the 18 combined hepatocellular and cholangiocarcinomas, 5 cases (27.8%) were associated with liver cirrhosis, and 8 cases (44.4%) were associated with chronic hepatitis. In the remaining 5 cases (27.8%), there was no significant change in non-tumorous liver, in which 1 case was healthy carrier of HBV.

The histological type of the hepatocellular carcinoma component was trabecular type in 15 cases, scirrhous type in one case, compact type in one case, and pseudoglandular type in one case. Differentiation of the hepatocellular components was well-differentiated type in 1 case, moderately differentiated type in 6 cases, and poorly differentiated type in 11 cases.

The cholangiocarcinoma components were well to moderately differentiated tubular adenocarcinoma. Nine (50%) cases were predominantly hepatocellular carcinoma, and 7 (39%) were predominantly cholangiocarcinoma. The remaining 2 (11%) cases were predominantly undifferentiated type. In all cases the areas showing a glandular pattern were depicted as positive in either alcian blue stain or diastase digested PAS stain.

Microscopically, we classified the tumors into the following three categories according to the arrangement of the hepatocellular carcinoma and cholangiocarcinoma components. Type I was the tumor in which hepatocellular carcinoma and cholangiocarcinoma formed nodules that could easily be distinguished from each other. Seven cases were classified into type I. Type II was the tumor in which the both components were finely mixed, so that the two components were almost indistinguishable. Five cases were classified into type II. Type III was the tumor in which the tumors had lobular structures with hepatocellular carcinoma existing centrally and cholangiocarcinomas existing peripherally (**Figure 3A, 3B**). Six cases were classified into type III.

In one case of type I, two nodules were presented and surgically resected. In this case, one nodule was hepatocellular carcinoma and the other was combined hepato-

FIGURE 4A
The macroscopic finding of a combined hepatocellular and cholangiocarcinoma in "nodules-in-nodules" fashion. A well-differentiated hepatocellular carcinoma (arrows) 3.1cm in the maximal diameter contains a cholangiocarcinoma (arrow heads) 1.8cm in the maximal diameter.

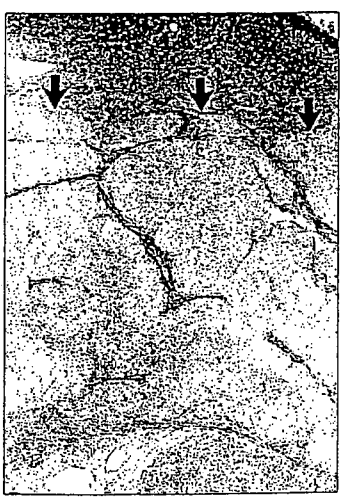

FIGURE 4B
The microscopic finding of Figure 3B. In the lower area a cholangiocarcinoma component exists in a well-differentiated hepatocellular carcinoma component (arrows) in "nodules-in-nodules" fashion. The hepatocellular carcinoma component shows clear cell change (HE).