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Clinicopathologic Features and Outcome After Liver Resection for Hepatocellular Carcinoma in Patients with Concurrent Versus Previous Chronic Hepatitis B

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Abstract

Purpose. We compared the clinicopathologic features affecting outcome after surgery for hepatocellular carcinoma (HCC) between patients with concurrent and previous chronic hepatitis B.

Methods. Group A consisted of 58 patients with concurrent chronic hepatitis B, defined by seropositivity for the hepatitis B surface antigen (HBsAg), and group B consisted of 18 patients whose HCC was detected after disappearance of the HBsAg. We assessed the influence of various characteristics on outcome.

Results. The mean age and percentage of patients suffering from alcohol abuse or diabetes mellitus were significantly greater in group B than in group A, whereas histologic hepatitis activity, hepatic fibrosis, and alanine aminotransferase activity were significantly lower in group B than in group A. The tumor-free survival rates were similar between the two groups, but the risk factors of recurrence differed. In group A, relative youth, high aspartate aminotransferase activity, low platelet count, multiple tumors, large tumor size, portal invasion, cirrhosis, nonanatomic resection, and positive surgical margin were risk factors. In group B, large tumor size and poor differentiation were risk factors.

Conclusion. Hepatitis B status, tumor factors, and the type of operation affected cancer recurrence after surgery for HCC in patients with concurrent chronic HBV, as opposed to only tumor factors in patients with previous chronic hepatitis B.

Key words Hepatocellular carcinoma · Liver resection · Hepatitis B virus · Alcohol abuse · Diabetes mellitus

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Introduction

Hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC). In the natural history of HBV infection, conversion from HB surface antigen (HBsAg) positivity to anti-HB surface antibody (anti-HBs) positivity is associated with the remission of active hepatits.[1] Hepatocellular carcinoma is sometimes detected after recovery from chronic hepatitis B according to the above seroconversion.[2] However, differences in clinicopathologic features and results of surgical treatment between HCC patients with current chronic hepatitis B (those positive for HBsAg) and HCC patients after recovery from chronic hepatitis B (those positive for anti-HB core antibody [anti-HBc] but negative for HBsAg) remain unclear.[3] We investigated these differences to identify the risk factors for postoperative HCC recurrence and formulate therapeutic strategies based on HBV status.

Patients and Methods

Patients

Between April 1990 and the end of 2002, 459 patients underwent liver resection for HCC at our hospital. Sera from 62 patients were positive for HBsAg but negative for antihepatitis C virus antibody (anti-HCV) and curative resection was performed in 58 of these patients (group A). Sera from another 36 patients were positive for anti-HB surface antibody (anti-HBs) or anti-HBc or both, but negative for both HBsAg and anti-HCV. Among these 36 patients, 18 who had been treated previously or followed up for chronic hepatitis B underwent curative resection (group B). Sera from the other 361 patients were positive for anti-HCV, positive for both HBsAg and anti-HCV, or negative for serologic

markers for HBV or HCV (HBsAg, anti-HBs, and anti-HBc). Curative resection was defined as complete removal of all recognizable HCC diagnosed preoperatively or intraoperatively.[4] Patients were examined preoperatively by ultrasonography, computed tomography (CT) with and without intravenous contrast, and angiography. Since May 1993, CT has also been performed when possible during arteriography and during arterioportography. Intraopertive ultrasonography was performed for all patients.

Information about alcohol consumption was obtained during interviews with patients and their families. Alcohol abuse was defined as the intake of 86 g of ethanol daily for at least 10 years, according to the Liver Cancer Study Group of Japan.[5] This study was conducted in accordance with the Helsinki Declaration following the guidelines of the ethics committee at our institution concerning studies on humans.

Viral Markers

Serum samples obtained from patients before surgery were assayed for HBV and HCV. Sera were tested HBsAg, HB envelope antigen (HBeAg), anti-HBs, and anti-HBc using enzyme immunoassays (International Reagents, Kobe, Japan). A titer for anti-HBc representing more than 70% inhibition was scored as positive, and defined as high when inhibition still exceeded 70% after 200-fold dilution of the serum sample. Samples were also tested for anti-HCV using a second- or third-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). Hepatitis B virus DNA was measured in serum by a nested polymerase chain reaction, as reported previously.[6]

Pathologic Examination

Histologic grading of tumors with respect to differentiation was carried out using the classification of Edmondson and Steiner,[7] with certain modifications.[8] Positive portal invasion was defined by clusters of cancer cells in the portal vein. Cancer cells in the 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 were also examined pathologically. The histologic activity index (HAI) score[9] was used to evaluate the severity of active hepatitis and degree of fibrosis. Combined components 1 to 3 of the HAI score that summed to 0 indicated no activity (histologic activity score 0); 1 to 3, minimal activity (histologic activity score 1); 4 to 8, mild activity (histologic activity score 2); 9 to 12, moderate activity (histologic activity score 3); and greater than 12, severe activity (histologic activity score 4). The histologic fibrosis score was determined separately from component 4 of the HAI scoring. A score of 1 indicated portal fibrous expansion; 2, portal-to-portal septa without architectural distortion; 3, portocentral septa wth architectural distortion; and 4, cirrhosis.

Detection of Recurrence

The serum α-fetoprotein concentration was measured every 3 months. Ultrasonography, CT, magnetic resonance imaging (MRI), chest radiography, or a combination of these tests was also performed every 3 months. When tumor recurrence was suspected on the basis of a tumor marker or radiologic studies, angiography or biopsy was done to obtain a definitive diagnosis.

Statistics

Student's *t*-test was used to analyze differences in age. The Mann-Whitney *U*-test was used to analyze the differences in results of laboratory tests and tumor size. The Fisher 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 determined by the log-rank test. For multivariate analysis, the Cox proportional hazards model was used. Variables showing a P value less than 0.1 by univariate analysis were subjected to multivariate analysis. A P value of less than 0.05 was considered significant.

Results

The patients' clinical features, laboratory test results, pathologic findings, and type of operation are shown in Table 1. Sex distribution, the percentage of patients with a history of blood transfusion, and the percentage of patients with symptoms, including abdominal pain, did not differ between the groups. Mean age, the percentage of patients who abused alcohol, and the percentage of patients with diabetes mellitus all were sigificantly higher in group B than in group A. Hepatocellular carcinoma was detected incidentally during follow-up for other diseases or during the course of periodic health examinations in 14 patients from group B. The percentage of relatively young patients aged $<$50 years old was significantly higher in group A (21 of 59 patients) than in group B (1 of 18 patients, $P = 0.0156$). Of the 58 patients in group A, 17 were seropositive for HBeAg. Anti-HBc titers were high in three patients from group B, and HBV DNA was detected in sera from two of them. Although there were no differences between the two groups in the serum

Table 1. Clinicopathologic findings of patients with hepatocellular carcinoma, by hepatitis B virus status

	Hepatitis B surface antigen		
	Positive (n = 58) Group A	Negative (n = 18) Group B	P
Age (mean ± SD)	52 ± 9	61 ± 8	0.0002
Sex (M:F)	44:14	17:1	0.102
Symptoms	10	3	>0.999
History of blood transfusion	5	3	0.385
Alcohol abuse	7	7	0.0169
Esophageal varices	7	1	0.672
Diabetes mellitus	5	6	0.0175
Total bilirubin (mg/dl)	0.9 (0.5, 1.4)	0.8 (0.4, 1.6)	0.269
Albumin (g/dl)	3.7 (3.2, 4.2)	3.6 (3.4, 4.1)	0.573
$ICGR_{15}$ (%)	12.5 (7.5, 23.9)	10.5 (5.0, 22.5)	0.277
AST (IU/l)	47 (26, 112)	41 (25, 76)	0.0512
ALT (IU/l)	63 (24, 125)	35 (18, 71)	0.0219
Platelet count ($\times 10^4/\mu l$)	13.9 (8.1, 24.3)	16.2 (8.4, 29.9)	0.313
α-Fetoprotein (>20 ng/ml)	33	5	0.0571
Tumor size (cm)	5.1 ± 4.4	5.2 ± 5.0	0.975
Tumor number (single)	44	13	0.762
Differentiation of main tumor			
Well	1	1	0.296
Moderate	37	13	
Poor	20	4	
Portal invasion	21	6	>0.999
Histologic activity score = 0, 1	32	15	0.0502
Histologic fibrosis score = 4	28	3	0.0266
Preoperative TAE	16	7	0.389
Preoperative PVE	7	2	>0.999
Anatomic resection	40	10	0.395
Positive surgical margin	23	7	>0.999

Results of laboratory tests are expressed as medians, with 10th and 90th percentiles in parentheses
$ICGR_{15}$, indocyanine green retention rate at 15 min; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TAE, transcatheter arterial embolization; PVE, portal vein embolization

concentrations of total bilirubin and albumin, the indocyanine green retention rate at 15 min, and the platelet count, alanine aminotransferase (ALT) activity was significantly lower in group B than in group A, and aspartate aminotransferase (AST) also tended to be lower in group B than in group A. No significant differences were noted in tumor size, the percentage of patients with multiple tumors, including intrahepatic metastasis, the degree of differentiation of the main tumor, or the percentage of patients with portal invasion. The percentage of patients with a histologic activity score of 0 or 1 tended to be higher in group B than in group A, whereas the percentage of those with a histologic fibrosis score of 4 (cirrhosis) was significantly lower in group B than in group A. There was no significant difference in the percentage of patients who underwent preoperative transcatheter arterial embolization or portal vein embolization (PVE), or in the type of operation or the percentage of patients with a positive surgical margin between the two groups.

In group A, the major postoperative complications were refractory pleural effusion or ascites, or both, in seven patients (three of whom also suffered hepatic failure), intra-abdominal infection in one, and would infection in one (Table 2). In the three patients who suffered postoperative hepatic failure, bi- or trisegmentectomy without preoperative PVE was performed, and more than 60% of the hepatic parenchyma was resected.

Univariate analysis of all subjects identified relative youth (<50 years old, $P = 0.0018$), high AST activity (>40 IU/ml, $P = 0.0303$), low platelet count ($<10 \times 10^4/\mu l$, $P < 0.0001$), a high concentration of α-fetoprotein (>20 ng/ml, $P = 0.0633$), large tumor size (>7 cm in diameter, $P = 0.0092$), multiple tumors ($P = 0.0173$), poorly differentiated HCC ($P = 0.0205$), portal invasion ($P = 0.0391$), cirrhosis ($P = 0.0086$), nonanatomic

Table 2. Postoperative complications of patients after resection of hepatocellular carcinoma, by hepatitis B virus status

	Hepatitis B surface antigen	
	Positive (n = 58) Group A	Negative (n = 18) Group B
Pleural effusion or ascites	7	0
Hepatic failure	3	0
Intra-abdominal infection	1	0
Wound infection	1	0

Table 3. Independent risk factors for recurrence after resection of hepatocellular carcinoma

Variable	Risk ratio	95% CI	P
Age (<50 years)	2.513	1.342–4.694	0.0039
Platelet count (<10 × 10^4/μl)	5.714	2.703–12.05	<0.0001
Tumor size (>7 cm)	2.915	1.395–6.060	0.0044
Poorly differentiated carcinoma	2.268	1.193–4.310	0.0125

CI, confidence interval

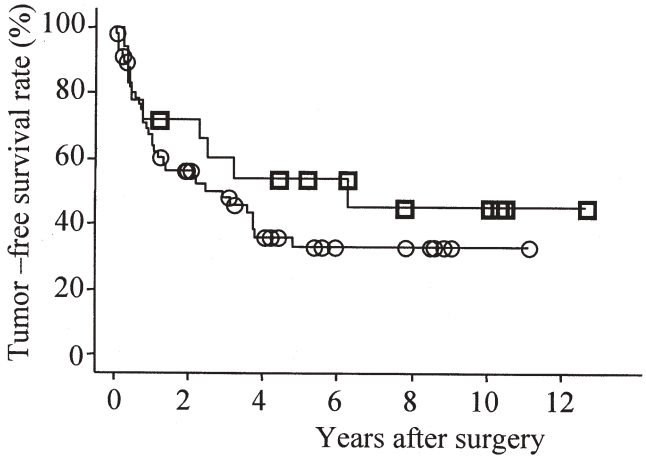

Fig. 1. Tumor-free survival rates after liver resection for hepatocellular carcinoma. *Circles*, 58 patients whose sera were positive for hepatitis B surface antigen but negative for antihepatitis C virus antibody (group A). *Squares*, 18 patients whose sera were positive for antihepatitis B core antibody but negative for hepatitis B surface antigen and anti-hepatitis C virus antibody (group B)

resection (P = 0.0805), and positive surgical margin (P = 0.0125) as possible risk factors for HCC recurrence. The tumor-free survival rate did not differ between groups A and B (Fig. 1, P = 0.292). According to multivariate analysis, relative youth (P = 0.0039), low platelet count (P < 0.0001), large tumor size (P = 0.0044), and poorly differentiated HCC (P = 0.0045) were independent risk factors for HCC recurrence (Table 3).

In group A, relative youth (P = 0.0051), high AST activity (P = 0.0077), low platelet count (P < 0.0001), multiple tumors (P = 0.0001), large tumor size (P = 0.0608), portal invasion (P = 0.0470), cirrhosis (P = 0.0038), nonanatomic resection (P = 0.0006), and positive surgical margin (P = 0.0221) were risk factors for HCC recurrence according to univariate analysis. In group B, large tumor size (P = 0.0366) and poorly differentiated HCC (P = 0.0977) were risk factors for HCC recurrence by univariate analysis. Thus, the risk factors for HCC recurrence differed between the groups, even though the tumor-free survival rates did not. Chronic hepatitis B status, defined by a high AST activity, low platelet count, and cirrhosis; tumor factors such as multiple tumors, large tumor size, poorly differentiated HCC, and portal invasion; and certain characteristics of the operation, such as nonanatomic resection and positive surgical margin, were risk factors for HCC recurrence in group A, whereas only tumor factors such as large tumor size and poorly differentiated HCC were associated with HCC recurrence in group B. In group B, anatomic resection was reserved mainly for large or multiple tumors with a risk of recurrence.

Discussion

Prior or occult HBV infection has been strongly implicated in the development of HCC, even in patients infected with HCV.[10–18] Futhermore, HBV has been detected in the hepatic tissue of some patients after

seroconversion from HBsAg positivity to negativity associated with the remission of active hepatitis, especially those with a high anti-HBc titer.[1,19–24] Other investigators have reported that HBV persists after recovery from acute viral hepatitis.[25,26] Our group B patients were treated or followed up for chronic hepatitis B, and HCC was detected after the disappearance of HBsAg; however, the potential for hepatocarcinogenesis persists even after disappearance of HBsAg in such patients. Sometimes the HBV is still present after the disappearance of HBsAg.[19–22] Indeed, anti-HBc titers were high in three of our patients, and HBV DNA was detected by nested polymerase chain reaction in sera from two of them. Hepatitis B virus genes are also often detected in HCC tissues in patients with anti-HBc.[10–14] The mean age of the patients was significantly higher in group B than in group A, and the ALT and AST activity was lower in group B than in group A. Thus, it is important to follow up patients with previous chronic hepatitis B because HCC can develop even after the disappearance of the HBsAg, although the adequate follow-up time is still unclear.

In group A, active hepatitis and hepatic fibrosis as well as genetic changes induced by HBV are related to hepatocarcinogenesis. Although the percentage of patients with active hepatitis and liver cirrhosis was lower in group B than in group A, 15 patients in group B showed various degrees of histologic hepatitis activity or liver cirrhosis, or both. The group B patients may be at risk of carcinogenesis because of their past chronic hepatitis B, even though their liver histology might improve after the remission of active hepatitis. On the other hand, the percentage of patients suffering from alcohol abuse and diabetes mellitus, each reported to be a risk factor for HCC development in patients without viral hepatitis,[27,28] were significantly higher in group B than in group A. Another possible mechanism is that not only previous or occult HBV infection, but additional factors including alcohol abuse and diabetes mellitus, may contribute to hepatocarcinogenesis. Thus, the role of occult HBV infection in patients with other predisposing factors of chronic liver disease should be clarified.

The incidence of postoperative complications was significantly higher in group A than in group B, especially complications specifically related to liver dysfunction such as pleural effusion, ascites, and hepatic failure. Active hepatitis, cirrhosis, and a high serum concentration of type IV collagen 7s domain have all been reported as risk factors for postoperative hepatic failure.[29,30] Our three patients with postopertive hepatic failure had undergone bi- or trisegmentectomy involving resection of more than 60%, without preoperative PVE. These three patients had active hepatitis and two also had cirrhosis. It is well known that PVE increases the safety[31–33] and improves outcome after major hepatectomy.[34] Preoperative PVE might have been necessary in these three patients, although active hepatitis limits the effectiveness of PVE.[35]

The tumor-free survival rates were not significantly different in our two study groups, in agreement with a previous report.[3] The possible risk factors for HCC recurrence by univariate analysis were relative youth, high AST activity, low platelet count, high α-fetoprotein concentration, multiple tumors, large tumor size, poorly differentiated HCC, portal invasion, liver cirrhosis, nonanatomic resection, and positive surgical margin. By multivariate analysis, relative youth, low platelet count, large tumor stage, and poorly differentiated HCC were independent risk factors for HCC recurrence. However, the percentages of relatively young patients and patients with active hepatitis and liver cirrhosis were higher in group A than in group B. Thus, each group exhibited different clinicopathologic risk factors for HCC recurrence; namely, relative youth, high AST activity, low platelet count, multiple tumors, large tumor size, portal invasion, liver cirrhosis, nonanatomic resection, and positive surgical margin in group A, and large tumor size and poorly differentiated HCC in group B. These different risk factors resulted in there being no difference in the tumor-free survival rates between the two groups.

We previously reported that the viral status, defined by a high viral load, the presence of HBeAg, the absence of the anti-HB envelope antibody, and the absence of precore mutant type HBV, as well as low platelet count and positive surgical margin, were risk factors for recurrence after resection of HCC in HBsAg-positive-patients.[36,37] Patients in the immune-clearance phase of chronic hepatitis B infection are at high risk of HCC recurrence after the operation. In this study, only group A included such patients, which explains why chronic hepatitis B status, tumor factors, and the type of operation affected recurrence, whereas in group B only tumor factors affected recurrence. This reflects the remission of chronic hepatitis B in group B. In group B, anatomic resection was performed mainly for large tumors. Because advanced age and diabetes mellitus limited the type of operation performed in group B, the operative methods might be based on tumor size and location, because anatomic resection was not performed for small HCC. This may explain why the type of operation was not a risk factor. Follow-up to detect HCC early is necessary for patients with previous or current chronic hepatitis B. Our findings also show that the strategies for treating HCC and preventing recurrence differ in these two groups. For group A patients, anatomic resection with a wide surgical margin (>5 mm) is recommended, as well as antiviral therapy, including lamivudine, and liver transplantation, in

selected cases. Conversely, for group B patients, effective adjuvant therapy for HCC recurrence is recommended because tumor characteristics were the risk factors for HCC recurrence.

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