Original Article

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Development of Hepatocellular Carcinoma after Successful Management of Esophageal Variceal Bleeding

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Key Words
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Background. Endoscopic and pharmacological treatments significantly reduce recurrent esophageal variceal bleeding and improve the prognosis of cirrhotic patients. This study was aimed to evaluate the incidence, risk factors, treatment and prognosis of hepatocellular carcinoma after esophageal variceal bleeding.

Methods. Patients with esophageal variceal bleeding underwent endoscopic or pharmacological treatments to arrest acute bleeding or prevent rebleeding. Concurrently, patients were followed by periodic serum α-fetoprotein examinations and ultrasonography, aiming at early detection and possible treatment of hepatocellular carcinoma.

Results. Hepatocellular carcinoma developed in 79 of 370 patients (21.4%) during the 10-year follow-up. The cumulative incidence of hepatocellular carcinoma was 21.1% at the end of the third year, 39.3% at the end of the fifth year and 53.0% at the end of the tenth year. Small tumors (≤ 3 cm) were found in 64 patients (81.0%, monofocal tumors in 46 patients). Age, hepatitis B virus, hepatitis C virus, and α-fetoprotein level > 20 ng/mL were factors associated with the risk of hepatocellular carcinoma development by multivariate analysis using Cox regression. Tumors were actively treated in 37 of 49 Child-Pugh A and B patients (75.5%) and 2 of 30 Child-Pugh C patients (6.7%). The median survival of all patients was 2.5 (range, 0.5 to 10.0) years. Development of hepatocellular carcinoma and serum bilirubin level were significantly associated with mortality in Child-Pugh A and B patients.

Conclusions. Patients with esophageal variceal bleeding had a high risk of developing hepatocellular carcinoma. Surveillance on hepatocellular carcinoma could detect most tumors with small size. Hepatocellular carcinoma had an adverse impact on the survival of patients without advanced cirrhosis.

Esophageal variceal bleeding (EVB) carries a mortality rate ranging from 30% to 50%.[1] Recurrent EVB is frequent after initial bleeding.[2] Treatment modalities including endoscopic variceal ligation, endoscopic injection sclerotherapy, and portal hypotensive medications are all effective in preventing recurrent EVB and significantly improve the prognosis of cirrhotic patients.[3-10]

Hepatocellular carcinoma (HCC) occurs in 1% to 5.8% of cirrhotic patients annually.[11-14] Early detection of HCC may increase the chances of early treatment and improve patients' survival.[15,16] Surveillance of HCC is generally accepted in cirrhotic patients. Cirrhotic patients with a history of EVB are also potential candidates suitable for screening for the possibility of HCC. However, to our knowledge, no study has been designed specifically to investigate this category. Therefore, we carried out this study to evaluate the incidence, risk factors, amenability of treatment and prognosis of HCC in cirrhotic patients after successful management of EVB.

METHODS

From February 1991 through August 2000, cirrhotic patients with EVB were consecutively recruited. Pa-

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tients were resuscitated and underwent endoscopic variceal ligation or endoscopic injection sclerotherapy when they had active bleeding. Their hepatic reserves were classified using Child-Pugh's classification.[17] The size of esophageal varices was graded based on Beppu's system.[18] After control of acute bleeding, endoscopic variceal ligation, endoscopic injection sclerotherapy or β-blockers plus nitrates were used to prevent rebleeding. The majority of the patients recruited in our previous trials for prevention of rebleeding [4,9,19] were enrolled in this study. Patients were excluded if they had any of the following conditions including a history of variceal bleeding, presence of HCC at enrollment or within 3 months after enrollment, major systemic disease other than cirrhosis, and died within 3 months after enrollment.

Diagnosis of cirrhosis

The diagnosis of cirrhosis was based on liver biopsy, clinical and biochemical examinations or image studies. Alcoholism was diagnosed according to a history of alcohol consumption (> 80 g per day) with serological study. Hepatitis B virus (HBV) surface antigen was determined using a commercial kit (Abbott Diagnostics, North Chicago, IL, USA). Antibody against hepatitis C virus (HCV) was determined using a second generation enzyme-linked immunoassay (Ortho Diagnostic System, Raritan, NJ, USA) from 1991 through 1999 and a third-generation enzyme-linked immunoassay (Abbott Diagnostics, North Chicago, IL, USA) after 1999. The diagnosis of primary biliary cirrhosis was made using positive anti-mitochondrial antibody results or characteristic liver biopsy.

Tumor surveillance

After successful management of EVB, surveillance including serum α-fetoprotein (AFP) and ultrasonography using a real-time instrument (Aloka SSD-2000 ultrasound system, Aloka Co., Ltd., Tokyo, Japan) was performed at 3- to 4-month intervals. When hepatic lesions were suggestive of malignancy or elevated AFP levels were present, computed tomography, magnetic resonance imaging or hepatic angiography was performed. Biopsies of the lesions were obtained when there was no contraindication and the patients agreed to them. The diagnosis of HCC was made according to liver biopsy, results from 2 positive image studies (ultrasonography, computed tomography, magnetic resonance imaging, and hepatic angiography) or an AFP level > 400 ng/mL with 1 positive image study.[20] Tumors were histologically typed according to the classification of the World Health Organization.[21]

Treatments of HCC

The treatment criteria for hepatectomy were: 1) unifocal nodule at the peripheral segment of liver; 2) an indocyanine green test < 15% at 15 minutes; and 3) no evidence of portal vein thrombosis or extrahepatic metastasis. If the tumors were unresectable or patients refused surgery, transarterial chemoembolization or percutaneous ethanol injection was used according to the characteristics of tumors and patients' choice. The treatment criteria for transarterial chemoembolization were: 1) tumor involvement of no more than 40% of the liver; 2) serum bilirubin level < 3 mg/dL, serum creatinine level < 2 mg/dL; and 3) no evidence of portal vein thrombosis or extrahepatic metastasis. The treatment criteria for percutaneous ethanol injection were: 1) no more than 3 tumors; 2) tumors smaller thaner 3 cm; 3) no ascites or small amount ascites, if present; 4) tumors were not subcapsular lesions; 5) serum bilirubin level < 3 mg/dL; and 6) no evidence of portal vein thrombosis or extrahepatic metastasis. When tumors were successfully treated, patients were on surveillance again by AFP and ultrasonography at 3-month intervals. If recurrent tumors were found, patients underwent transarterial chemoembolization or percutaneous ethanol injection until their liver reserves did not allow further treatment. Patients were on supportive care if treatments for HCC were not considered.

This protocol was approved by the Medical Ethics Committee of our hospital. All patients and their families gave their informed consent. The endpoint of this study was occurrence of HCC or death.

Statistical analysis

For continuous data suitable for normal testing, comparisons between patients with and without HCC were made using Student's *t*-test and results were given as mean ± s.d. Categorical data were analyzed using Chi-square test or Fisher's exact examination. A receiver operating

characteristics curve was constructed to identify the best cut-off level of AFP. Univariate analysis using Cox's regression with the Statistical Package for the Social Science (SPSS, SPSS Inc., Chicago, IL, USA) was performed to estimate the risk factors of HCC and mortality. All marginally significant factors ($p < 0.15$) were further tested by multi-variate analysis using Cox's regression. Kaplan-Meier analysis was used to estimate the cumulative incidence of HCC and the time from enrollment to death in patients with and without HCC. A log rank test was used to examine the differences of survival by the presence of HCC or not. All results were considered as statistically significant when a p value was less than 0.05.

RESULTS

A total of 582 patients were initially recruited. Two-hundred and twelve patients were excluded because of previous variceal bleeding (60 patients), death within 3 months (81 patients), coexistent HCC at enrollment (64 patients), detection of HCC within 3 months (2 patients), coexistent malignancy other than HCC (1 cervical cancer, 1 gastric cancer, 1 cholangiocarcinoma, 1 lymphoma) and uremia (1 patient). Only 370 patients entered this study. Their clinical features are presented in Table 1.

Development of HCC

Seventy-nine patients (21.4%) developed HCC during the 10-year follow-up. HCC was diagnosed using ultrasonography with computed tomography or magnetic resonance imaging in 45 patients with a serum AFP level < 400 ng/mL. Thirty-two tumors were detected by ultrasonography with a serum AFP level < 400 ng/mL but secondary images failed to make the diagnosis,

Table 1. Clinical characteristics of esophageal variceal bleeding patients at enrollment

	All patients (N = 370)	HCC (+) (N = 79)	HCC (-) (N = 291)	p value[§]
Age (years)	55.2 ± 13.6	58.7 ± 12.9	54.2 ± 13.6	0.40
Gender (male/female)	280/90	58/21	222/69	0.28
Etiology of cirrhosis				
HBV[†]	121	35	86	0.03
HCV[‡]	92	24	68	0.31
HBV[†] + HCV[‡]	13	2	11	0.59
Alcohol	106	13	93	0.007
Others[*]	38	5	33	0.19
Child-Pugh's class (A/B/C)	87/183/100	16/45/18	73/136/82	0.27
Albumin (mg/dL)	3.0 ± 0.6	2.9 ± 0.6	3.0 ± 0.6	0.76
Bilirubin (mg/dL)	2.4 ± 2.8	2.2 ± 2.5	2.5 ± 2.9	0.22
Prothrombin time-prolonged (sec)	3.8 ± 3.1	4.2 ± 3.1	3.9 ± 3.4	0.64
Ascites	244	51	194	0.70
Variceal size (F1/F2/F3)	4/114/252	2/16/61	2/98/191	0.06
Red wale marking (<++/≥++)	161/209	32/47	129/162	0.52
Encephalopathy (0/1/2)	256/105/9	57/21/1	199/84/8	0.83
Hematocrit (%)	27.8 ± 6.8	29.4 ± 6.7	27.3 ± 6.8	0.86
Blood urea nitrogen (mg/dL)	19.4 ± 12.0	19.4 ± 11.6	19.4 ± 12.1	0.89
Creatinine (mg/dL)	1.1 ± 0.8	1.2 ± 1.1	1.1 ± 0.7	0.89
α-fetoprotein (ng/mL)	11.4 ± 62.7	29.2 ± 131.1	6.6 ± 17.5	< 0.001
Diabetes mellitus	69	17	52	0.48
Endoscopic therapy	335	75	260	0.13

[†] Hepatitis B virus.
[‡] Hepatitis C virus.
[*] Cryptogenic cirrhosis in 36 patients and primary biliary cirrhosis in 2 patients.
[§] The difference between HCC (+) and HCC (-) patients.

which was confirmed by a liver biopsy. Two patients had a serum AFP level > 400 ng/mL but ultrasonography failed to detect the tumors. The diagnosis was established by computed tomography. The cumulative HCC occurrence rate was 21.1% at the end of the third year, 39.3% at the end of the fifth year and 53.0% at the end of the tenth year.

Risk factors and characteristics of HCC

HCC appeared in 35 of 121 patients (28.9%) with HBV infection, 24 of 92 patients (26.1%) with HCV infection, 2 of 13 patients (15.4%) with HBV and HCV infection, 13 of 106 alcoholic patients (12.3%), and 5 of 38 patients (13.1%) with other causes of cirrhosis. The cumulative incidence of HCC in patients with HBV, HCV, alcohol, and other causes of cirrhosis are shown in Fig. 1. HCC patients had a median serum AFP level of 3.1 (range, 3.0 to 1049.0) ng/mL at enrollment and 10.6 (range, 3.0 to 4780.0) ng/mL at diagnosis of HCC. Abnormal AFP levels (> 10 ng/mL) were found in 42 patients (53.2%) and 10 patients had AFP levels > 400 ng/mL at diagnosis of HCC. A receiver operating characteristics curve found 20 ng/mL to be the best cut-off value of AFP level which gave the lowest value significant at univariate analysis. Multi-variate analysis using Cox's regression showed that age (relative risk, 1.1; 95% CI: 1.0-1.1, $p = 0.001$), HBV (relative risk, 2.5; 95% CI: 1.4-4.7, $p = 0.003$), HCV (relative risk, 2.2; 95% CI: 1.1-4.3, $p = 0.03$), and serum AFP level > 20 ng/mL (relative risk: 2.6, 95% CI: 1.2-5.4, $p = 0.01$) were independent predictors of HCC (Table 2).

Tumors were unifocal in 54 patients, multifocal in 21 patients and infiltrative in 4 patients. The median

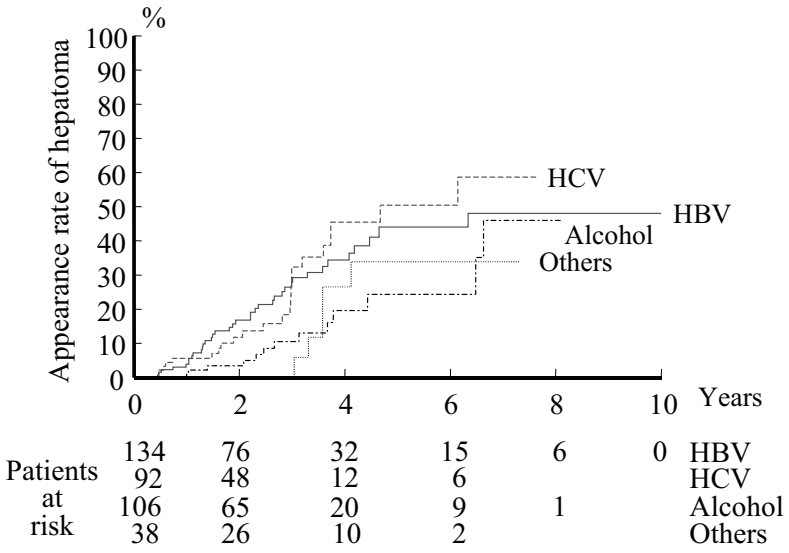

Fig. 1. Cumulative incidence of hepatocellular carcinoma in 370 cirrhotic patients following esophageal variceal bleeding classified by different etiologies of cirrhosis. HBV, viral hepatitis B; HCV, viral hepatitis C; HCC, hepatocellular carcinoma.

Table 2. Multi-variate analysis of risk factors associated with development of hepatocellular carcinoma in cirrhotic patients after management of esophageal variceal bleeding

Variables	Relative risk	*p* value	95% CI
Age	1.1	0.001	1.0 - 1.1
HBV	2.5	0.003	1.4 - 4.7
HCV	2.2	0.03	1.1 - 4.3
α-fetoprotein level > 20 ng/mL	2.6	0.01	1.2 - 5.4

size of nodular tumors was 2.0 (range, 1.0 to 6.0) cm. Tumors with a diameter equal or less than 3 cm were found in 62 patients (81.0%, monofocal tumors were found in 46 patients). Biopsies of tumors were obtained in 32 patients and classified as trabecular type (27 patients), acinar type (2 patients), scirrhous type (1 patient), and undifferentiated type (2 patients). The liver functions at diagnosis of HCC were Child-Pugh A in 19 patients, Child-Pugh B in 30 patients, and Child-Pugh C in 30 patients.

Treatments and prognosis of HCC

Based on the liver reserve at diagnosis of HCC, 37 of 49 Child-Pugh A and B patients (75.5%) received treatments for HCC. Three patients underwent hepatectomy. Sixteen patients received percutaneous ethanol injection. Eighteen patients received transarterial chemoembolization. Two patients refused transarterial chemoembolization. Ten patients could not receive treatments for HCC because of massive ascites (8 patients), massive ascites combined with deep jaundice (1 patient) and severe thrombocytopenia (1 patient). Two of 30 Child-Pugh C patients (6.7%) responded poorly to transarterial chemoembolization. Twenty-eight patients could not receive treatments for HCC because of deep jaundice (3 patients), massive ascites (13 patients), and combined massive ascites with deep jaundice (12 patients).

Survival

One-hundred and two patients (HBV-34 patients, HCV-31 patients, HBV+HCV-4 patients, alcoholism-23 patients, and other causes of cirrhosis-10 patients) died during the study period. The median survival of the whole group was 2.5 (range, 0.5 to 10.0) years. Thirty-three HCC patients die of tumor rupture (10 patients; 2 patients previously underwent transarterial chemoembolization, but 8 patients could not receive treatments for HCC), liver failure (8 patients), sepsis (8 patients), tumor progression (3 patients), EVB (2 patients), gastric ulcer bleeding (1 patient), and intraabdominal varix rupture (1 patient). Sixty-nine patients without HCC died of liver failure (21 patients), sepsis (21 patients), EVB (13 patients), gastric variceal bleeding (4 patients), intracranial hemorrhage (4 patients), acute myocardial infarction (2 patients), traffic accident (2 patients), heart failure (1 patient), and ischemic bowel disease (1 patient). HCC patients had a median survival of 3.5 (range, 0.5 to 9.0) years. Patients without HCC had a median survival of 2.3 (range, 0.5 to 10.0) years. Development of HCC (relative risk, 4.8; 95% CI: 3.0-7.8, $p < 0.001$) and Child-Pugh C liver reserve (relative risk, 2.7; 95% CI: 1.8-3.9, $p < 0.001$) were found to be independent predictors of mortality in the whole group of patients by Cox regression analysis with time-dependent factor. When only Child-Pugh A and B patients were analyzed, development of HCC (relative risk, 7.9; 95% CI: 4.5-13.9, $p < 0.001$) and serum bilirubin level (relative risk, 1.2; 95% CI: 1.0-1.4, $p = 0.02$) were found to be associated with mortality.

DISCUSSION

The cumulative incidence of HCC after successful management of EVB was 21.1% at the end of the third year, 39.3% at the end of the fifth year and 53.0% at the end of the tenth year. The occurrence rate of HCC was evidently higher in our study than in previous reports, which ranged from 15 to 20% at 5-year follow-up[20] but similar to that of a retrospective study on patients with advanced cirrhosis.[22] This suggests that HCC still occurs steadily after EVB if rebleeding can be effectively prevented. The high incidence of HCC might be because the occurrence of EVB usually indicates decompensation of cirrhosis and possibly a longer duration of the disease, which carries a greater risk of carcinogenesis.[12,13,23,24]

Consistent with previous studies,[12-14] our study showed that HBV, HCV, and age were risk factors for HCC in EVB patients. Integration of HBV DNA into hepatocyte chromosomal DNA, trans-activation of growth-regulating gene and functional inactivation of p53 gene by HBV X protein are proposed as mechanisms of hepatocarcinogenesis of HBV infection.[25,26] A process of dysregulation in cell cycle progression or dysregulation of aptosis may account for the carcinogenesis of HCV.[27] Also, HCV core protein has been shown to induce HCC in transgenic mice.[28] Alcoholic patients were at a lower risk of developing HCC as

compared with patients with other etiologies of cirrhosis. It is possible that alcoholic patients may be predisposed to die of liver failure before the occurrence of HCC.

Ultrasonography and serum AFP are used the most often for surveillance of HCC.[11,12,29,30] However, the sensitivity of abnormal AFP level (> 10 ng/mL) in detecting HCC was only 53.2%, and 12.6% of HCC patients had AFP levels > 400 ng/mL in this trial. About 80% of the tumors found in our patients were smaller than 3 cm. Nevertheless, we still observed 13 cases of nodular tumors larger than 3 cm and 4 cases of infiltrative tumors despite surveillance. Benvegnu *et al*. reported a strong association between infiltrative HCC and HBV.[31] This suggests that surveillance using ultrasonography and serum AFP examination still has limitations in the early detection of HCC. In addition, annual computed tomography or magnetic resonance imaging might be necessary for cirrhotic patients, especially those who have HBV infection.

The proportion of treatment in this trial (49.4%) was lower than that of a previous report, in which 68.8% of the tumors were treated using surgical or local treatments.[32] The low tumor resection rate (3.8%) in our patients is not surprising because it is proposed that hepatectomy for HCC is associated with high mortality and morbidity in cirrhotic patients.[33] When stratified by liver reserves at diagnosis of HCC, the proportion of treatment were 75.5% in Child-Pugh A and B patients while only 6.7% of Child-Pugh C patients received treatment for HCC. It is suggested that surveillance is beneficial for Child-Pugh A patients and controversial for Child-Pugh B patients, while Child-Pugh C patients should undergo liver transplantation.[20] However, the grafts are not worldwide readily available. Besides, transarterial chemoembolization or percutaneous ethanol injection could help to decrease the dropout rate if liver transplantation is considered.[34,35]

Our patients had a median survival of 2.5 years after management of EVB. Variceal bleeding-related death was significantly reduced in patients with and without HCC. HCC was associated with mortality in either all the patients or Child-Pugh A and B patients. Because early detection of HCC may result in timely liver transplantation or other appropriate treatments, patients without advanced cirrhosis should undergo surveillance of HCC after control of EVB in view of the high risk of HCC in these patients.

In conclusion, a very high incidence of HCC was observed in cirrhotic patients after EVB. Surveillance could detect most tumors in small size and made 75.5% of the tumors amenable to treatments in Child-Pugh A and B patients. However, Child-Pugh A and B patients still had an unfavorable survival if they developed HCC. This suggests that patients without advanced cirrhosis should be on surveillance for HCC if they survive EVB and more definitive treatment for HCC such as liver transplantation should be considered if HCC is detected.

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