5-FU/IFN alone group who achieved CR/PR without 3D-CRT and obtained prolonged survival (MST 34.3 months). These long-term survivors in the HAIC-5-FU/IFN alone group might balance out the benefit of additional 3D-CRT in the HAIC-5-FU/IFN plus radiotherapy group. With regard to the prognostic effect of 3D-CRT, radiotherapy and the associated reduction of VTT significantly improved the survival time in patients of non-CR/PR (intrahepatic response) group with VTT of Vv3 ($P = 0.028$, Fig. 4). In other words, patients who fail to show a response to HAIC-5-FU/IFN, 3D-CRT should be applied with the hope of improving survival. Conversely, the response to radiotherapy would be rather questionable in patients who show CR/PR response to HAIC-5-FU/IFN alone. Because the response of HCC with VTT to HAIC-5-FU/IFN cannot be predicted before treatment, it is important to monitor the patients on HAIC-5-FU/IFN for the response to such treatment as soon as possible, and introduce 3D-CRT to VTT to those who show non-CR/PR.

Despite the relatively high efficacy of the HAIC-5-FU/IFN regimen, the high incidence of extrahepatic metastasis is a poor prognostic sign. In the seven patients who were confirmed to be metastasis-free at baseline and developed extrahepatic metastasis during HAIC-5-FU/IFN treatment, the MST was 4.4 months after the detection of metastasis. In other words, the prognosis of these patients was similar to those who presented with extrahepatic metastasis before HAIC-5-FU/IFN (MST, 3.0 months). Various chemotherapies have been used for HCC extrahepatic metastasis though a standard regimen has not yet been established. Nevertheless, some investigators reported an objective response rate of 17–25% using systemic combination chemotherapy of S1 and IFN.[33,34]

Recent studies have praised the benefits of sorafenib tosilate in unresectable advanced HCC, reporting relatively long MST of 6.5–10.7 months and slowing of radiological progression in nearly 3 months.[35,36] While sorafenib seems to have survival benefits, the reported response rate is less than 2%. Compared with our results, with MST of 7.9 months, systemic RR of 24% and intrahepatic RR of 30% for advanced HCC with VTT, HAIC-5-FU/IFN seems to be characterized by higher response rate than sorafenib monotherapy, while MST was similar. Because the intrahepatic CR/PR patients by HAIC-5-FU/IFN could obtain significantly longer survival than the non-CR/PR patients (18.7 vs. 4.4 months, respectively), it might be meaningful to sort out HAIC-5-FU/IFN effective HCC patients who have potentially prolonged prognosis by HAIC-5-FU/IFN before introducing sorafenib treatment. There seemed to be a limitation of HAIC-5-FU/IFN that extrahepatic metastasis frequently occurred as a poor prognostic sign. In others, the benefits of sorafenib were reported to be consistent including patients with extrahepatic spread.[35,36] Sorafenib might be one of the most prospective modalities for extrahepatic metastasis after ineffective HAIC-5-FU/IFN.

The present study has certain limitations. These include data generated from a single institution, small population size and retrospective study design. For example, patients had a tendency to be elderly, HBV negative and HCV positive in comparison between the HAIC-5-FU/IFN alone group and the HAIC-5-FU/IFN plus 3D-CRT group (Table 7). There seemed to be no doubt about some clinicopathologic biases in patient background due to our study design. However, our results provide material for future large scale studies to determine the usefulness of the combination of HAIC-5-FU/IFN and 3D-CRT for advanced HCC with VTT.

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Hepatology Research

Hepatology Research 2011 doi: 10.1111/j.1872-034X.2011.00929.x

Original Article

Recent trend of clinical features in patients with hepatocellular carcinoma

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Aim: In this study, we evaluated the clinical characteristics of hepatocellular carcinoma (HCC) because the etiology of HCC has been changing recently.

Methods: Consecutive 1374 HCC patients at our institution from 1995 to 2009 were enrolled and clinical characteristics were investigated.

Results: Seventeen percent and 67% of HCC were related to hepatitis B virus (HBV-HCC) and hepatitis C virus (HCV-HCC), respectively. Fifteen percent of that was negative for hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (HCVAb) (NBNC-HCC). HCV-HCC tended to decrease and NBNC-HCC tended to increase in recent years. Patients with NBNC-HCC and HCV-HCC were significantly older than those with HBV-HCC. The complication rates of diabetes mellitus (DM), heavy alcohol consumption, hypertension, and hyperlipidemia in NBNC-HCC were significantly higher than those in other groups. Furthermore, the platelet counts and body mass index in NBNC-HCC were significantly higher than those of other groups. Among 209 NBNC-HCC patients, 58 patients underwent hepatic resection in which 29%, 36%, and 35% of those were based on non-alcoholic steatohepatitis (NASH), heavy alcohol consumption, and unknown etiology, respectively. DM was prevalent especially in NASH and heavy alcohol consumption. Cirrhosis was detected in 65%, 81%, and 15% in NASH-HCC, heavy alcohol consumption-HCC, and unknown etiology, respectively.

Conclusions: NBNC-HCC has gradually been increasing in recent years. The present study elucidated that the presence of NASH and metabolic syndrome were important risk factors for NBNC-HCC and suggests that these patients should receive surveillance for HCC development.

Key words: clinical characteristics, etiology, hepatocellular carcinoma, NBNC-HCC

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignant tumors in the world. HCC is presently the fifth most common cancer in the world, responsible for 500 000 deaths globally every year.[1–3] In Japan, it ranks third in men and fifth in women as a cause of death from malignancies and is 35 000 deaths every year.[4] The incidence of HCC varies according to the prevalence of its causative agents such as infection with hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, hemochromatosis, Budd-Chiari syndrome, and so on.[5,6]

The majority of HCCs are associated with chronic liver diseases (more than 70–80% of HCC is reported to be positive to hepatitis C virus (HCVAb) and 10–20% are positive to hepatitis B surface antigen (HBsAg) in Japan.[7] The remaining 5–10% of patients with HCC were categorized as negative for HBsAg and HCVAb (NBNC-HCC). Recently, the report has suggested that patients with NBNC-HCC are increasing more than those with HBV-HCC and with HCV-HCC.[8] Whereas other etiologic factors such as alcoholic liver disease (ALD) and autoimmune disease may be involved in NBNC-HCC, precise etiologies of liver diseases are yet to be elucidated.

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Received 24 June 2011; revision 6 October 2011; accepted 11 October 2011.

On the other hand, in recent years, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have received increasing attention for their relationship with cirrhosis and HCC.[9–13] NAFLD and NASH are clearly associated with the metabolic syndrome, comprising a cluster of interrelated metabolic risk factors such as elevated fasting glucose, central obesity, dyslipoproteinemia, and hypertension.[14–16] The prevalence of NAFLD is 10–30% in adults[17] and its prevalence is increasing in Japan as well as in Western countries because of the epidemic rise in obesity and diabetes mellitus (DM).

Furthermore, occult HBV infection was found in a considerable proportion of patients with HCV and also in NBNC-HCC. The relationship between occult HBV infection and HCC has been reported in several studies.[18,19] However, the relevancies between them remained controversial.[20–22] Therefore, we evaluated whether occult HBV infection leads to risk for NBNC-HCC.

The purpose of this study was to investigate the clinical characteristics of HCC to better understand the etiology of HCC that has been changing recently.

METHODS

Patients

WE RETROSPECTIVELY REVIEWED 1374 consecutive patients of HCC diagnosed at Hiroshima University Hospital from January 1995 to December 2009.

Anthropometric and laboratory evaluation

We reviewed epidemiological data (age, sex, history of blood transfusion, history of habitual alcohol consumption, height, weight). A detailed epidemiological questionnaire was administered during face-to-face interviews. Venous blood samples were taken in the morning following a 12-h overnight fast. Biochemical data (aspartate aminotransferase [AST]; alanine aminotransferase [ALT]; platelet counts, γ-glutamytranspeptidase [γ-GTP]; total cholesterol, blood glucose, hemoglobin A1c, HbA1c; antinuclear antibody [ANA] and anti-smooth muscle antibody [ASMA]) were measured using standard techniques for each patient at the time of diagnosis of HCC.

Anti-HCV in sera was assayed using the Anti-HCV-EIA Cobas Core Test (Hoffmann La Roche, Basel, Switzerland). HBsAg and anti-HBc in sera were assayed using the HBsAg-EIA Cobas Core Test (Hoffmann La Roche) and the Anti-HBc-EIA Cobas Core Test (Hoffmann La Roche), respectively. Qualitative detection of HCV RNA among anti-HCV-positive samples was performed using a thermocycler (Whatman Biometra, Goettingen, Germany) based on the nested polymerase chain reaction (PCR) method, as described before.[23]

HBV-HCC was defined as positive for HBsAg and negative for HCVAb, HCV-HCC was defined as positive for HCVAb and negative for HBsAg, NBNC-HCC was defined as negative for both HBsAg and HCVAb.

Heavy alcohol consumption was determined when alcohol intake was more than 80 g/day for 5 years.[24] The body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). The HbA1c (%) value was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by using the formula: A1c (%) = A1c (Japan Diabetes Society (JDS)) (%) + 0.4 %, considering the relational expression of A1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP).[25,26] DM was diagnosed according to the 2006 World Health Organization (WHO) criteria.[27]

Hyperlipidemia was diagnosed when the patient was being treated with lipid-lowering medications or had elevated levels of total cholesterol higher than 220 mg/dL and/or the triglyceride level was over 150 mg/dL. Hypertension was diagnosed when the patient was on antihypertensive medications and/or had a resting recumbent blood pressure of 130/85 mmHg or more on at least two occasions.

Histological diagnosis of AIH was performed by hepatologists and pathologists within our facility supported by findings of interface hepatitis, lymphoplasmacytic infiltration, and hepatic rosette formation. Using the combined clinicohistopathological data, AIH scores were calculated using the criteria introduced by the International Autoimmune Hepatitis Group (IAHG).[28,29]

Pathological evaluation

The pathological status of underlying liver disease was based on microscopic examination of the noncancerous part of the surgical specimen or biopsy specimen with hematoxylin-eosin and Azan staining. All liver tissue specimens were evaluated by two senior pathologists who were unaware of the laboratory data and the clinical course. Histological diagnosis of NASH was confirmed according to Brunt's criterion.[30–32] Steatosis was graded as follows: grade 1 (≥5% and <33% of

hepatocytes affected); grade 2 (33–66% of hepatocytes affected); or grade 3 (66 % < of hepatocytes affected). Necroinflammation was graded 0 to 3 (0, absent; 1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild-to-moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation). Fibrosis was graded 0 to 4 (0, absent; 1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis). Ballooing was graded 0 to 2 (0, none; 1, few balloon cells; 2, many cells/prominent ballooning).[31,32]

Diagnosis of HCC was based on the hypervascular staining pattern of the arterial phase and the hypovascular staining pattern of the portal phase, and confirmed by dynamic computed tomography, magnetic resonance imaging, or angiography. Tumors without enhancement upon imaging were diagnosed by fine-needle biopsy. HCC was classified as well differentiated, moderately differentiated, or poorly differentiated.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD). Fisher's exact probability test or the χ^2 were used to compare categorical data. Differences between two groups were measured using the Mann–Whitney *U*-test. A relationship between different continuous variables was measured by linear regression analysis. Furthermore, statistical differences among all the three groups were estimated using a post hoc multiple comparison test. All statistical analyses were performed using the SPSS software package (version 12.0 for Windows, SPSS Inc., Chicago, IL, USA), with $P < 0.05$ denoting statistically significance.

RESULTS

Trend of etiology of liver diseases in patients with HCC

AMONG 1374 PATIENTS with HCC from 1995 to 2009, 228 patients (16%) and 917 patients (67%) were related to HBV-HCC and HCV-HCC, respectively (Fig. 1). 209 patients (15%) were negative for HBsAg and HCVAb (NBNC-HCC). Twenty (2%) patients with HCC were positive for both HBsAg and HCVAb (BC-HCC). As shown in Figure 1, 194 patients (76%) were HCV-HCC from 1995 to1999, 251 patients (71%) from 2000 to 2004, and 472 patients (62%) from 2005 to 2009. 30 patients (20%) were HBV-HCC from 1995 to1999, 61 patients (17%) from 2000 to 2004, and 137 patients (18%) from 2005 to 2009. 26 patients (10%) were NBNC-HCC from 1995 to1999, 35 patients (10%) from 2000 to 2004, and 148 patients (19%) from 2005 to 2009. Seven patients (3%) were BC-HCC from 1995 to1999, six patients (2%) from 2000 to 2004, and seven patients (1%) from 2005 to 2009. Whereas rate of HCV-HCC patients tended to decrease, rate and number of NBNC-HCC tended to increase, especially in recent years.

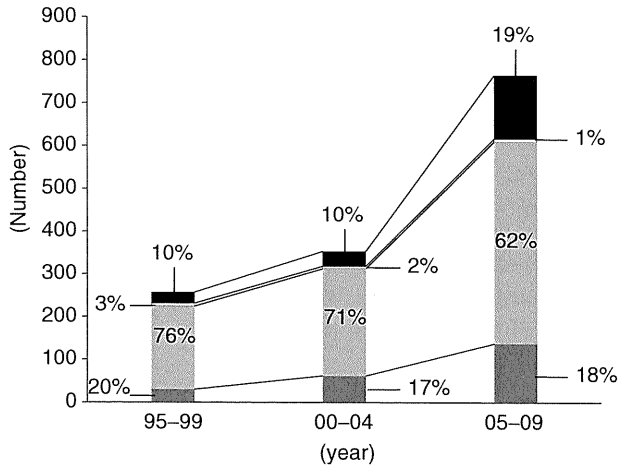

Figure 1 Trend of etiology of liver diseases in patients with hepatocellular carcinoma (HCC). We show the distribution of the trend of etiology of liver disease in patients with HCC every 5 years, among 1374 patients from 1995 to 2009. HCV-HCC tended to decrease and NBNC-HCC tended to increase, especially in recent years. (■): NBNC; (□): B+C; (▒): HCV; (▓): HBV.

Characteristics of patients with HCC based on etiology of liver diseases

Twenty patients with BC-HCC were excluded from the analysis. Characteristics of remaining 1354 patients with HCC were shown in Table 1. Patients with NBNC-HCC consisted of 164 males and 45 females. The mean age was 68 years old, 9% had blood transfusion, 60% had heavy alcohol consumption, and 52% were positive for HBcAb. The mean body mass index (BMI) was 24.6 kg/m^2, 48% of patients had DM, 38% had hypertension, and 11% had hyperlipidemia. The mean platelet counts were 14.6 × 10^4/μL and 4% were autoimmune hepatitis. On tumor node metastasis (TNM) staging for HCC according to the criteria of the Liver Cancer Study Group of Japan, 15 patients were categorized as stage I, 79 as stage II, 43 as stage III, 33 as IVa, and 38 as IVb.

Patients with HBV-HCC consisted of 191 males and 37 females. The mean age was 57 years old, 13% had

Table 1 Characteristics of patients with hepatocellular carcinoma (HCC) based on etiology of liver disease

	Total $n = 1354$	NBNC $n = 209$	HBV $n = 228$	HCV $n = 917$	*P*-value NBNC vs. HBV	*P*-value NBNC vs. HCV
Age (years)	65.9 ± 9.9	67.3 ± 10.2	56.6 ± 9.6	67.9 ± 8.5	<0.001	N.S
Sex (male/female)	985/369	164/45	191/37	630/287	NS	N.S
History of blood transfusion	334(25%)	19(9%)	30(13%)	285(32%)	NS	0.006
Heavy alcohol consumption	539(40%)	125(60%)	87(38%)	327(36%)	<0.001	<0.001
Antibody to hepatitis B core antigen	607(45%)	109(52%)	228(100%)	312(34%)	<0.001	<0.001
Body mass index (kg/m^2)	22.9 ± 3.4	24.8 ± 3.7	22.8 ± 3.6	22.4 ± 3.2	0.006	<0.001
Diabetes mellitus	386(29%)	100(48%)	37(16%)	249(27%)	<0.001	<0.001
Hypertension	396(29%)	77(38%)	31(14%)	287(31%)	<0.001	0.018
Hyperlipidemia	61(5%)	24(11%)	8(4%)	29(3%)	0.018	<0.001
Platelet counts (×10^4/μL, median)	12.6 ± 11.5	15.8 ± 8.4	13.0 ± 6.7	11.7 ± 12.9	0.01	0.008
Autoimmune hepatitis	13(1.0%)	9(4%)	0(0%)	4(0.4%)	0.003	<0.001
HCC stage† (I/II/III/IVa/IVb)	253/479/337/155/90	15/79/43/33/38	36/68/49/32/4	202/332/245/90/48	N.S	<0.001
No. HCC tumors (single/multiple)	632/722	95/114	97/131	440/477	N.S	N.S
Maximum tumor size (mm, median)	57.3 ± 319.3	54.3 ± 40.7	48.3 ± 42.4	26.5 ± 38.4	0.021	<0.001
Portal vein invasion (0/1/2/3/4)	1115/17/53/77/87	149/6/11/22/18	164/2/12/20/28	802/9/30/35/41	N.S	<0.001

Values are mean ± standard deviation (SD).
†Determined according to the criteria set by the Liver Cancer Study Group of Japan.
HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, negative for hepatitis B surface antigen and antibody to hepatitis C virus; NS, not significant.

blood transfusion, 38% had heavy alcohol consumption, 100% were positive for HBcAb. The mean BMI was 22.8 kg/m^2, 16% of patients had DM, 14% had hypertension, and 4% had hyperlipidemia. The mean platelet counts were 11.9×10^4/μL and none had autoimmune hepatitis. On TNM staging for HCC, 36 patients were categorized as stage I, 68 as stage II, 49 as stage III, 32 as IVa, and 4 as IVb.

Patients with HCV-HCC consisted of 630 males and 69 females. The mean age was 69 years old, 32% had blood transfusion, 36% had heavy alcohol consumption, and 34% were positive for HBcAb. The mean BMI was 22.4 kg/m^2, 27% of patients had DM, 31% had hypertension, and 3% had hyperlipidemia. The mean platelet counts were 9.9×10^4/μL and 0.4% were autoimmune hepatitis. On TNM staging for HCC, 202 patients were categorized as stage I, 332 as stage II, 245 as stage III, 90 as IVa, and 48 as IVb.

Patients with NBNC-HCC were significantly older than those with HBV-HCC ($P < 0.001$), but there was no significant difference compared with those with HCV-HCC. Patients of HCC with any etiology were male predominant, but male to female ratio was not significantly different for all groups. The complication rates of DM ($P < 0.001$), heavy alcohol consumption ($P < 0.001$), hypertension, and hyperlipidemia in patients with NBNC-HCC were significantly higher than those in other groups. Furthermore, BMI and the platelet counts in NBNC-HCC patients were significantly higher than those of other groups. The maximum tumor size in NBNC HCC was significantly larger than those in other groups; also HCC stage was significantly higher than those of with HCV-HCC ($P < 0.001$).

Clinical characteristics of NBNC-HCC patients with liver resection

Among 209 patients with NBNC-HCC, we studied clinical features of 58 patients who underwent hepatic resection. The patients consisted of 45 males and 13 females. Among NBNC-HCC patients, 29% of those were NASH, 36% of those were heavy alcohol consumption, and 35% of those were unknown etiology (Table 2). BMI in unknown group was significantly lower than those of other groups. The platelet counts in the heavy alcohol consumption group were significantly lower than those of other groups. Cirrhosis was detected in 65%, 67%, and 15% of patients with NASH, heavy alcohol consumption, and unknown etiology, respectively. The prevalence of cirrhosis in NASH ($P = 0.009$) and

Table 2 Characteristics of patients with NBNC-HCC

	NASH $n = 17$	Heavy alcohol consumption $n = 21$	Unknown $n = 20$	*P*-value		
				NASH vs Heavy alcohol consumption	NASH vs Unknown	Heavy alcohol consumption vs Unknown
Age (years)	66.3 ± 10.6	66.2 ± 7.2	65.5 ± 13.5	NS	NS	NS
Sex (male/female)	11/6	21/0	13/7	0.012	NS	0.009
Body mass index (kg/m^2)	24.8 ± 3.2	24.3 ± 2.7	22.0 ± 3.4	NS	0.024	0.03
Antibody to hepatitis B core antigen	9 (53%)	6 (29%)	11 (55%)	NS	NS	NS
Diabetes mellitus	9 (53%)	13 (62%)	8 (40%)	NS	NS	NS
Platelet counts (×10^4/μL)	17.3 ± 7.5	13.6 ± 5.2	20.7 ± 9.1	0.024	NS	0.009
AST (IU/L)	34.2 ± 14.2	32.7 ± 11.5	32.6 ± 15.1	NS	NS	NS
ALT (IU/L)	33.8 ± 21.5	35.2 ± 26.9	28.3 ± 15.7	NS	NS	NS
γ-GTP (IU/L)	126.1 ± 158.4	101.9 ± 97.2	115.6 ± 118.9	NS	NS	NS
Total cholesterol (mg/dL)	203.6 ± 34.2	181.8 ± 31.9	185.1 ± 30.7	NS	NS	NS
Cirrhosis	11 (65%)	14 (67%)	3 (15%)	NS	0.009	0.003

Values are mean ± standard deviation (SD).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamytranspeptidase; NASH, non-alcoholic steatohepatitis; NS, not significant.

heavy alcohol consumption ($P = 0.003$) groups were significantly higher than those in the unknown group. The patients with unknown etiology consisted of 13 males and seven females; the median age of patients was 65 years old. Forty percent of patients had DM, BMI was 21 kg/m^2, 55% of those were positive for HBcAb. Whereas 25%, and 15% of those with unknown etiology showed liver fibrosis (F1, F2), and cirrhosis, respectively, 60% of those did not show any fibrosis in the liver.

Clinical characteristics of NASH-HCC patients

Clinical characteristics of the 17 patients with NASH-HCC are shown in Table 3. Among 58 patients, 29% of those had a histological diagnosis of NASH. The patients consisted of 11 males and six females, the mean ages of patients was 67 years old. 53% of patients had DM, mean BMI was 25 kg/m^2, 53% were positive for HBcAb. 6%, 23%, 6%, and 65% of NASH patients were stage 1,

Table 3 Characteristics of patients with NASH-related HCC

	Total $n = 17$	Stage 1, 2 $n = 5$	Stage 3, 4 $n = 12$	Stage 1,2 vs Stage 3,4
Age (years)	67 ± 10.5	70.8 ± 14.1	66.0 ± 9.0	NS
Sex (male/female)	11/6	3/2	8/4	NS
Body mass index (kg/m^2)	25.3 ± 2.6	25.6 ± 3.4	25.7 ± 2.4	NS
Antibody to hepatitis B core antigen	9 (53%)	3 (60%)	6 (50%)	NS
Diabetes mellitus	9 (53%)	4 (80%)	5 (42%)	NS
Platelet counts (×10^4/μL)	18.0 ± 5.6	22.8 ± 4.1	14.8 ± 5.0	0.024
Fibrosis Stage (Brunt's criterion) Stage 1/2/3/4	1 (6%)/4(23%)/1(6%)/11(65%)			
Steatosis Grade1/2/3	11 (65%)/6(35%)/0			

Values are mean ± SD.
HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; NS, not significant.

2, 3, and 4, respectively. As shown in Table 3 and 29% of NASH-HCC patients were early stage NASH (stage 1, 2) and 61% of those were advanced stage NASH (stage 3,4). The mean of platelet counts in early stage NASH and in advanced stage NASH was $22.8 \times 10^4/\mu L$ and $14.8 \times 10^4/\mu L$. The platelet counts in advanced stage NASH were significantly lower than early stage NASH ($P = 0.024$).

DISCUSSION

IN THE PRESENT study, we retrospectively assessed the clinical characteristics of HCC. We found that 67% of patients were HCV-HCC from 1995 to1999, 71% from 2000 to 2004, and 62% from 2005 to 2009. Ten percent of patients were NBNC-HCC from 1995 to1999, 10% from 2000 to 2004, and 19% from 2005 to 2009. Patients with NBNC-HCC were significantly older than those with HBV-HCC, but there was no significant difference compared with those with HCV-HCC. The complication rates of DM, heavy alcohol consumption, hypertension, and hyperlipidemia in patients with NBNC-HCC were significantly higher than those in other groups. Furthermore, the platelet counts and BMI in NBNC-HCC patients was significantly higher than those of other groups. HBcAb were positive in 52% and 34% of patients with NBNC-HCC and HCV-HCC, respectively. The number of patients and the rate of NBNC-HCC have been gradually increasing especially in recent years, culminating in 20% of all HCC patients. In general, our findings were in agreement with the reported findings that people with obesity, DM or heavy alcohol consumption and metabolic syndrome are at an increased risk of developing HCC.[33–37] Further, the maximum tumor size was significantly larger than those in other groups and HCC stage with NBNC-HCC was significantly higher than those with HCV-HCC. Most of NBNC-HCC cases in our study were discovered in advanced stages compared with those of B-HCC and C-HCC. This is because patients with NBNC-HCC do not receive regular HCC surveillance whereas HBV-HCC and HCV HCC patients received regular HCC surveillance. Therefore, even in patients without obvious hepatic viral infection, patients with alcoholic liver diseases and those with NAFLD accompanied with metabolic syndrome should receive surveillance for HCC development.

Our findings showed that 29% of NBNC patients who underwent hepatic resection were NASH, 36% of those were heavy alcohol consumption, and 35% of those were unknown etiology. 29% of NASH-HCC was early stage NASH and 71% of those were advanced stage NASH. BMI and cirrhosis in the NASH group was higher than those of unknown groups. Cirrhosis was detected in 65% of patients with NASH though the platelet counts are comparatively not decreased. Furthermore, the platelet counts in advanced stage NASH were significantly lower than early stage NASH. Recently, Hashimoto *et al.* have reported that advanced fibrosis are important risk factors for HCC.[13] The severity of liver fibrosis must be estimated to determine the prognosis, for surveillance, and for optimal treatment of NAFLD, similar to the situation for other liver diseases such as chronic hepatitis C.[38] Taken together with our findings, advanced fibrosis is surely an important risk factor for HCC. On the other hand, our finding that HCC developed even in early stage NASH indicates the need for regular HCC surveillance.

Heavy alcohol consumption comprised 36% of NBNC-HCC in our study. The platelet counts in the heavy alcohol consumption group were significantly lower than those in other groups and cirrhosis was higher than those in unknown groups. Heavy alcohol consumption is known to be an important risk factor as reported.[33] Reported mechanisms related to the development of HCC in heavy alcohol consumption are that alcohol-induced oxidative stress may increase the susceptibility to cirrhosis especially in heavy alcohol consumption complicated with DM, DNA damage, and further the development of HCC.[33] In line with these reports, our findings support higher prevalence of cirrhosis in heavy alcohol consumption.

Unknown etiology of the liver comprised 35% of NBNC-HCC in our study. Liver function tests were within normal range, BMI and cirrhosis in unknown etiology group was lower than those of other groups. In histology, 85% of patients showed normal liver. The underlying cause of liver diseases was not detected in this patient group. Occult HBV infection may be involved in this patient population and may contribute to the development of HCC.

HBV infections are usually diagnosed by the detection of HBsAg, and the disappearance of HBsAg indicates the clearance of HBV. Recently, more advanced techniques using polymerase chain reaction (PCR) have revealed that HBV-DNA is detectable in the serum or liver tissue from HBsAg-negative subjects positive or negative for currently available HBV markers in general medicine. The condition of such subjects is commonly called an "occult HBV infection". Occult HBV infection is highly prevalent in chronic carriers of HCV[39] and has a relevant

clinical impact in accelerating the progression of liver fibrosis, perhaps resulting in cirrhosis. Many studies have reported that occult HBV infections may be correlated with the development of HCC in patients with chronic hepatitis C.[18,19,39] Based on the clinical findings that higher presence of HBcAb in NBNC-HCC in our study, occult HBV infection might be, at least in part, involved in the development of HCC in NBNC-HCC patient group. So far, the role of an occult HBV infection in HCC development is still a matter of controversy in relation to patients with viral hepatitis and NBNC-HCC. Nevertheless, we should focus on screening for patients with occult HBV infection who also have anti-HBs and anti-HBc seropositivity by using highly sensitive detection methods. Accordingly, it might be useful to examine HBV-DNA in this patient population until the causative role of occult HBV infection will be elucidated.

Limitations of our study are that not all the pathological aspects of background liver were available in NBNC-HCC group and that the study population may not reflect the entire patients with HCC in Japan.

In conclusion, NBNC-HCC has gradually been increasing in recent years, culminating in 20% of all HCC patients. The present study elucidated that the presence of NASH, DM, and heavy alcohol consumption were important risk factors for NBNC-HCC and suggests that these patients should receive surveillance for HCC development.

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Hepatology Research

Hepatology Research 2011; 41: 1046–1056 doi: 10.1111/j.1872-034X.2011.00857.x

Original Article

Clinical outcome of esophageal varices after hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with major portal vein tumor thrombus

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Aim: To analyze the clinical outcome of esophageal varices (EV) after hepatic arterial infusion chemotherapy (HAIC) in patients with advanced hepatocellular carcinoma (HCC) and major portal vein tumor thrombus (Vp3/4).

Methods: The study subjects were 45 consecutive patients who received HAIC for HCC with Vp3/4 between January 2005 and December 2009. HAIC comprised the combination therapy of intra-arterial 5-FU with interferon-α (5-FU/IFN) in 23 patients and low-dose cisplatin plus 5-FU (FP) in 22. Radiotherapy (RT) was also provided in 19 patients for portal vein tumor thrombosis. Aggravation rate for EV and overall survival rate were analyzed.

Results: The aggravation rates for EV were 47% and 64% at 12 and 24 months, respectively. The survival rates were 47% and 33% at 12 and 24 months, respectively. The response rates to 5-FU/IFN and FP were 35% and 41%, while the disease control rates in these two groups were 57% and 50%, respectively. There were no significant differences in the objective response and disease control between 5-FU/IFN and FP. Multivariate analysis identified size of EV (F2/F3) ($HR = 7.554$, $P = 0.006$) and HCC disease control ($HR = 5.948$, $P = 0.015$) as significant and independent determinants of aggravation of EV, and HCC disease control ($HR = 12.233$, $P < 0.001$), metastasis from HCC ($HR = 11.469$, $P = 0.001$), ascites ($HR = 8.825$, $P = 0.003$) and low serum albumin ($HR = 4.953$, $P = 0.026$) as determinants of overall survival. RT for portal vein tumor thrombosis tended to reduce the aggravation rate for EV in patients with these risk factors.

Conclusions: Hepatocellular carcinoma disease control was the most significant and independent factor for aggravation of EV and overall survival in HCC patients with major portal vein tumor thrombosis treated with HAIC.

Key words: esophageal varices, hepatic arterial infusion chemotherapy, hepatocellular carcinoma, portal vein tumor thrombosis, radiotherapy

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the commonest malignancies worldwide.[1–3] The causes of death in patients with HCC are cancer-related; including hepatic failure and massive bleeding from esophageal varices (EV). Development of new diagnostic techniques and advancements in therapeutic modalities have gradually improved the prognosis of HCC patients.[4–8] However, the prognosis of patients with advanced HCC and portal vein tumor thrombosis (PVTT) is still poor.[9–13] PVTT is associated with widespread intrahepatic and extrahepatic dissemination by the spread of tumor cells through the portal tract. Recent advances in implantable drug delivery systems have facilitated repeated arterial infusion of chemotherapeutic agents. Because hepatic arterial infusion

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Received 23 February 2011; revision 8 June 2011; accepted 13 June 2011.

chemotherapy (HAIC) increases local tissue drug concentrations and consequently reduces the side effects of anticancer agents, this modality is suitable for HCC patients with PVTT and poor hepatic reserve. Several groups[14,15] reported favorable results with low-dose cisplatin plus 5-FU (FP) for advanced HCC, especially those with PVTT in the first branch (Vp3) or in the main trunk (Vp4), though the prognosis of HCC patients with Vp3/4 is poor. Recent studies[16–19] have also reported the survival benefits of the combination therapy of intra-arterial 5-FU with interferon-α (IFN-α) (5-FU/IFN) for advanced HCC with Vp3/4; the response rate to the latter therapy in HCC with Vp3/4 is about 30–50%.[16–19] However, portal hypertension, which is commonly present in HCC with Vp3/4, is associated with aggravation of the condition, due to bleeding from EV,[20] and poor prognosis of these patients. Moreover, the mortality rate in association with the first episode of variceal bleeding remains high (20–35%), and bleeding from EV is extremely traumatic.[21–24] It is reported that PVTT and large HCC are independent risk factors for bleeding from EV in patients with HCC,[20] and that the EV-aggravation rate in patients with HCC was higher than in those without HCC. Thus, the combination of portal hypertension and EV in HCC patients with Vp3/4 potentially increases the aggravation rate. To date, there is no standardized treatment for EV in HCC patients with Vp3/4. Prophylactic therapy for EV may be needed to reduce death from variceal bleeding and add survival benefits to patients with HCC. Before one can determine the most appropriate type and timing of prophylactic therapy, the factors related to variceal bleeding should be evaluated and defined. The aims of the present study were (i) retrospective analysis of the clinical outcome of EV during HAIC for HCC with Vp3/4; (ii) identification of the factors associated with aggravation and survival rates for EV in HCC patients with Vp3/4; and (iii) set up a strategy for treatment of these patients.

METHODS

Patients

FORTY-FIVE CONSECUTIVE PATIENTS who underwent HAIC for HCC with Vp3/4 at Hiroshima University Hospital between January 2005 and December 2009 were enrolled in this cohort study. We analyzed retrospectively the clinical course of EV in these patients. Endoscopic findings of the EV were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices.[25] The form of EV was classified as complete eradication after treatment (F0), small straight (F1), enlarged tortuous (F2), or large coiled-shaped (F3) varices. The positive red color (RC) sign represented the presence of dark red spots on the mucosa of the lower esophagus detected on endoscopy. RC was classified into four grades in order to evaluate the risk of hemorrhage and provide a rough estimate of intravascular pressure within the EV: RC0: no mucosal coloring (negative RC sign); RC1: a few localized red spots; RC2: between RC1 and RC3; and RC3: several mucosal red spots throughout the circumference of the lower esophagus. HCCs were classified according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer by Liver Cancer Study Group of Japan.[26] The institutional review board approved this study, which was based on the Declaration of Helsinki as declared by the World Health Organization. Each patient gave informed consent before the study.

Treatment protocol

Hepatic arterial infusion chemotherapy

Patients with advanced HCC received repeated arterial infusions of anticancer agents via the injection port. One course of chemotherapy represented 2 weeks and comprised either 5-FU/IFN or FP. In both regimens, 5-FU (300 mg/m^2/day; Kyowa Hakko, Tokyo) was administered within 5 h using a mechanical infusion pump on days 1–5 of the first and second weeks (5 g per course). Recombinant IFN α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan) at 3×10^6 U (3MU), or natural IFN-α (OIF, Otsuka Pharmaceuticals, Tokyo) at 5×10^6 U (5 MU), was administered intramuscularly on days 1, 3 and 5 of each week (total dose, 18 and 30 MU, respectively). Alternatively, low-dose CDDP (6 mg/m^2/day; Randa, Nippon Kayaku, Tokyo) was administered first followed by 5-FU at the above dose and schedule. In principle, treatment was repeated several times unless PS changed to three or four during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course. The regimen of HAIC varied according to the study period; the 5-FU/IFN was used between January 2005 and December 2007, and FP was used between January 2008 and December 2009.

Radiotherapy

Among the 45 patients, 19 received three-dimensional (3D) conformal radiotherapy (3D-CRT), high-energy photon beam irradiation using 18, 10 or 6 MV, deliv-

ered by a 3D conformal technique (CLINAC 2300 C/D or CLINAC iX linear accelerators, Varian Medical Systems Inc., Palo Alto, CA, USA), at the Division of Radiation Oncology at our hospital. The planning computed tomography (CT) determined the gross tumor volume (GTV) representing the PVTT only. The clinical target volume (CTV) represented the GTV plus intrahepatic tumor forming the basal part of PVTT. The planning target volume (PTV) represented the CTV plus a 10–20-mm margin in all directions for internal motion and set-up error. Four to five portal fields were used. The outlined target volume, total liver tissue and at risk structures, including the spinal cord, both kidneys and nearby intestinal tract targets, were transferred to the treatment planning system (Pinnacle 3, Philips Medical Systems, Eindhoven, the Netherlands) with reference to the diagnostic enhanced CT images. The prescribed dose was 30, 39 or 45 Gy, in accordance with the dose-volume constraint of normal tissue and liver function. At least 95% of the prescribed dose targeted 95% of the PTV. The decision to use or not to use 3D-CRT was left to the attending physician. Indeed, the use of radiotherapy (RT) varied according to the study period; HAIC alone was used between January 2005 and June 2007, whereas HAIC combined with RT was used between July 2007 and December 2009.

Evaluation of response to HAIC and RT

Follow-up endoscopy after the start of HAIC for HCC was performed every 3–6 months. The EV-related endoscopic findings were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices[25] and were compared with the findings before the start of HAIC (baseline). Worsening of the F and RC sign relative to baseline or bleeding on follow-up endoscopy was regarded as aggravation of EV. We defined aggravation of EV as the primary endpoint and survival as the secondary endpoint. Data were analyzed in October 2010.

The response to HCC therapy was assessed with contrast-enhanced CT and tumor markers, such as α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), at 1–2 months after completion of the first course of the treatment, and then every 2–3 months. The response was defined according to the response evaluation criteria for solid tumors (RECIST version 1.1).[27] We evaluated the response to the therapies for PVTT and intrahepatic tumor as well as the overall response. Adverse events were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE version 4.0).

Table 1 Clinical characteristics of patients

Gender (male/female)	38/7
Age (≤65/>65) (years)†	64 (35–79)
Varices size (F0/F1/F2/F3)‡	13/22/8/2
Red color sign (RC0/RC1/RC2/RC3)‡	34/9/2/0
Platelet count ($\leq 12 \times 10^4 / > 12 \times 10^4$) (/μL)	18/27
T. bilirubin (≤1.0/>1.0) (mg/dL)	25/20
Albumin (≤3.5/>3.5) (g/dL)	18/27
Prothrombin time activity (≤70/>70) (%)	6/39
Ascites (yes/no)	9/36
Tumor size (mm)†	75 (18–140)
Size of HCC relative to whole liver (≤50/>50) (%)	28/17
Vp (3/4)§	29/16
Vv (yes/no)	34/11
Metastasis from HCC (yes/no)	13/32
Etiology (HBV/HCV/NBNC)	16/19/10
HAIC regimen (low-dose FP/5-FU-IFN)	22/23
RT for PVTT (yes/no)	19/26

†Data are median values (range). ‡Classification of esophageal varices: F0 no varices, F1 small straight, F2 enlarged tortuous, F3 large coiled-shaped, RC0 negative red color sign, RC1 a few localized red spots, RC2 between RC1 and RC3, RC3 several mucosal spots throughout the circumference. §PVTT grade: Vp3, tumor thrombus in the first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.
HAIC, hepatic arterial infusion chemotherapy; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NBNC, infection without HBV or HCV; PVTT, portal vein tumor thrombus; RT, radiotherapy; Vv, tumor thrombus in the hepatic vein.

Statistical analysis

The cumulative aggravation and survival rates were determined using the Kaplan–Meier method with The Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Significance was tested using a generalized log-rank test and *t*-test. The independent determinants of the cumulative aggravation and survival rates were compared using a Cox proportional hazards model. A P-value < 0.05 was regarded as statistically significant.

RESULTS

Clinical and endoscopic findings

TABLE 1 LISTS THE clinical characteristics of patients. Based on the endoscopic findings of EV, 13 patients were classified as F0, 22 as F1, 8 as F2, and 2 patients as F3. Furthermore, the RC sign findings were classified as RC0 in 34 patients, RC1 in nine, and RC2 in two patients. The median tumor size for the entire group was

Table 2 Response and disease control rates of hepatocellular carcinoma (HCC) to hepatic arterial infusion chemotherapy (HAIC) with or without radiotherapy (RT)

	Response of PVTT			Response of intrahepatic HCC		
	HAIC combined with RT	HAIC alone	*P*-value	HAIC combined with RT	HAIC alone	*P*-value
CR	2	4		1	4	
PR	10	4		8	4	
SD	7	3		4	3	
PD	0	15		6	15	
CR + PR	63%	31%	0.03	47%	31%	0.25
CR + PR + SD	100%	42%	<0.0001	68%	42%	0.08

CR, complete response; PD, progressive disease; PR, partial response; PVTT, portal vein tumor thrombus; SD, stable disease.

75 mm. The size of the HCC tumor relative to the whole liver was ≤50% in 28 patients and >50% in 17 patients. The severity of portal vein tumor thrombosis was Vp3 in 29 patients and Vp4 in 16.

Response to treatment

The median number of HAIC treatment courses was four in 23 patients of the 5-FU/IFN group and four courses in 22 patients of the FP group. With regard to the response to HAIC among patients of the 5-FU/IFN group, 3, 5, 5, and 10 were classified as complete response, partial response, stable disease, and progressive disease, respectively. The respective patients for the FP group were 2, 7, 2, and 11. Thus, the response rates of the 5-FU/IFN and FP groups were 35% and 41%, respectively, while the disease control rates of these groups were 57% and 50%, respectively. The response and disease control rates were not significantly different between the two regimens. The response and disease control rates for all patients were 38% and 53%, respectively. The response and disease control rates of PVTT were 63% and 100%, respectively, for those treated with HAIC plus RT and 31% and 42%, respectively, for those treated with HAIC alone (Table 2). There were significant differences in these rates between the two groups ($P = 0.03$, <0.0001).

The response and disease control rates of intrahepatic HCC were 47% and 68%, for those who received HAIC plus RT and 31% and 42%, respectively, for those who received HAIC alone (Table 2). There were no differences in these rates between the two groups ($P = 0.25$, 0.08).

Aggravation rates for esophageal varices

Aggravation was recognized in 26 patients. Aggravation according to the F factor and RC sign was noted in 13 patients, and according to variceal bleeding in 13 patients. The median follow-up period was 18 months. The cumulative aggravation rates for EV were 39%, 47%, and 64% at 6, 12, and 24 months, respectively, for all patients (Fig. 1a). The cumulative bleeding rates for EV were 25%, 29%, and 39% at 6, 12, and 24 months, respectively, for all patients. Table 3 shows the factors that correlated with the cumulative aggravation rate by univariate analysis. The overall aggravation rate correlated significantly with varices size ($P < 0.0001$), RC sign ($P < 0.0001$), serum albumin ($P = 0.0333$), ascites ($P = 0.0041$), size of HCC relative to the whole liver ($P = 0.0210$), metastasis from HCC ($P = 0.0018$), and disease control of HCC ($P < 0.0001$).

The above factors were entered into multivariate analysis, which identified varices size ($P = 0.006$) and disease control of HCC ($P = 0.015$) as significant and independent factors of overall aggravation (Table 4). The cumulative aggravation rates at 12 and 24 months were 90% and 90%, for patients with F2/F3, and 33, and 55%, respectively, for patients with F0/F1 (Fig. 1b). There was a significant difference in cumulative aggravation rate between the two groups ($P < 0.0001$). The cumulative aggravation rates at 12 and 24 months were 21% and 46%, respectively, for patients of the disease control group, and 77% and 77%, respectively, for patients of the non-disease control group (Fig. 1c). There was a significant difference in the cumulative aggravation rate between the two groups ($P < 0.0001$).

Effect of radiotherapy in patients at risk for aggravation of esophageal varices

Radiotherapy did not correlate with aggravation of EV on univariate analysis for all patients. However, analysis

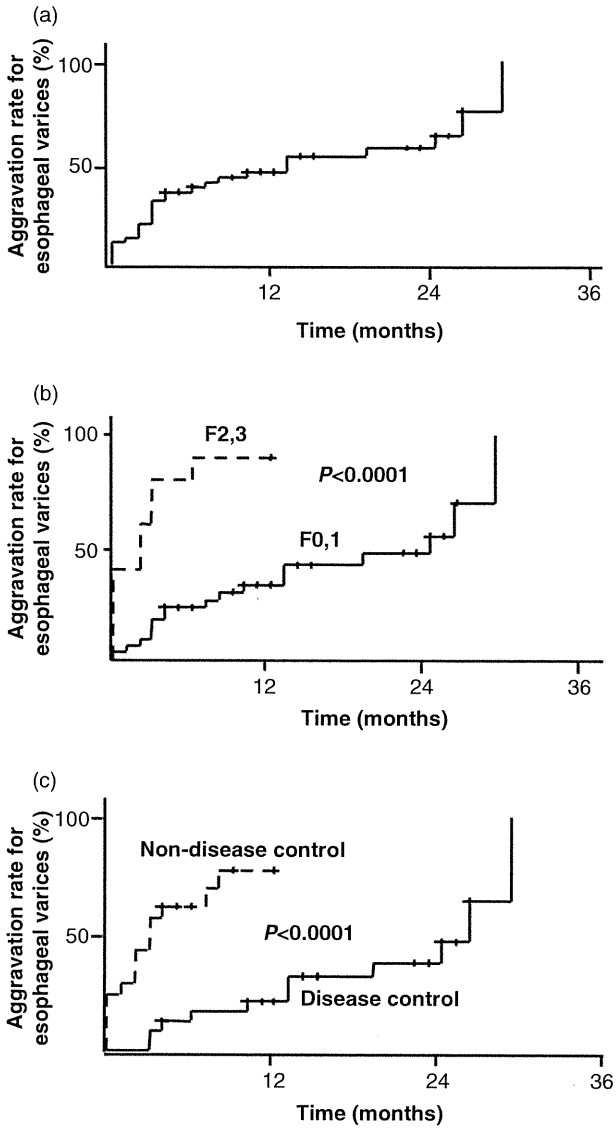

Figure 1 Cumulative aggravation rates for esophageal varices in patients with Vp3/4 hepatocellular carcinoma (HCC). (a) Cumulative aggravation rates for esophageal varices using data of all patients. (b) Cumulative aggravation rate according to the size of esophageal varices (F grade). (c) Cumulative aggravation rate according to HCC disease control with hepatic arterial infusion chemotherapy (HAIC). Disease control: patients who responded to HAIC, non-disease control: patients who did not respond to HAIC.

of data of 32 patients with EV according to the F classification showed a significant difference in cumulative aggravation rate between the RT ($n = 14$) group and non-RT ($n = 18$) group ($P = 0.0044$, Fig. 2a). Moreover, for 28 patients who showed no response to HAIC, the cumulative aggravation rate was significantly different between the RT ($n = 10$) and non-RT ($n = 18$) groups ($P = 0.0465$, Fig. 2b).

Figures 3a and b are representative figures showing improvement of PVTT and EV, respectively. Figures 4a and b are representative figures showing aggravation of PVTT and EV, respectively.

Table 3 Results of univariate analysis for the relationship between cumulative aggravation rate and various clinicopathological variables

Gender (male/female)	0.4449
Age (≤65/>65) (years)	0.0969
Varices size (F0, 1/F2, 3)†	<0.0001
Red color sign (0/1–3)†	<0.0001
Platelet count ($\leq 15 \times 10^4 / > 15 \times 10^4$) (/μL)	0.1987
T. bilirubin (≤1.0/>1.0) (mg/dL)	0.5258
Albumin (≤3.5/>3.5) (g/dL)	0.0333
Prothrombin time activity (≤70/>70) (%)	0.3468
Ascites (yes/no)	0.0041
Tumor size (≤70/>70) (mm)	0.3936
Size of HCC relative to whole liver (≤50/>50) (%)	0.0210
Vp (3/4)†	0.4542
Vv (yes/no)	0.7653
Metastasis from HCC (yes/no)	0.0018
HCC treatment protocol (low-dose FP/5-FU-IFN)	0.3591
Radiotherapy (yes/no)	0.0892
Disease control of HCC (yes/no)	<0.0001

†See Table 1 for classification of endoscopic findings and of portal vein tumor thrombus (PVTT) grade and abbreviations.

Overall survival

The cumulative survival rates were 69%, 47%, and 33% at 6, 12, and 24 months, respectively, for all patients (Fig. 5a). The median follow-up period was 19 months. Univariate analysis showed that survival rate correlated

Table 4 Determinants of cumulative aggravation rate for esophageal varices by multivariate analysis

	Factor	Hazard ratio	95% confidence interval	*P*-value
Varices size	F2/3	7.554	1.571–14.155	0.006
	F0/1	1		
Disease control of HCC	Non-disease control	5.948	1.282–9.795	0.015
	Disease control	1		

HCC, hepatocellular carcinoma.

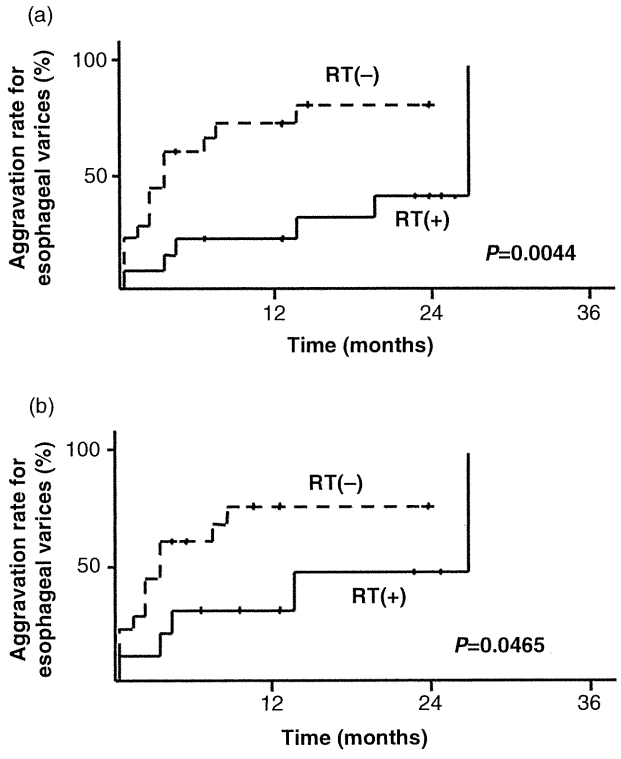

Figure 2 Cumulative aggravation rates for esophageal varices in patients with Vp3/4 hepatocellular carcinoma (HCC) treated with or without irradiation. (a) Cumulative aggravation rates in patients with F factor > F0. (b) Cumulative aggravation rates in non-responders to hepatic arterial infusion chemotherapy (HAIC), according to radiotherapy. RT(+): patients who received radiotherapy, RT(–): patients who did not receive radiotherapy.

significantly with varices size ($P = 0.0004$), RC sign ($P = 0.0001$), serum albumin ($P = 0.0061$), ascites ($P < 0.0001$), size of HCC relative to the whole liver ($P = 0.0003$), metastasis from HCC ($P < 0.0001$), and disease control of HCC ($P < 0.0001$) (Table 5). The above factors were entered in multivariate analysis, which identified disease control of HCC ($P < 0.001$), metastasis from HCC ($P = 0.001$), ascites ($P = 0.003$) and serum albumin ($P = 0.026$) as significant and independent factors of overall survival (Table 6), but not factors related to esophageal varices. The survival rates at 12 and 24 months were 82% and 61%, respectively, for patients of the disease control group, and 6% and 0%, respectively, for patients of the non-disease control group. There were significant differences in cumulative survival rates between two groups ($P < 0.0001$) (Fig. 5b).

Further analysis showed that the survival rates at 12 and 24 months were 26% and 13%, respectively, for patients of the EV rupture group, and 55% and 40%, respectively, for patients of the non-EV rupture group (Fig. 5c). There was a significant difference in the cumulative survival rate between the latter two groups ($P = 0.004$).

Effect of radiotherapy on overall survival in patients with poor prognostic factors

Univariate analysis showed no significant relationship between RT and overall survival, whereas multivariate analysis identified disease control of HCC, metastasis from HCC, ascites and serum albumin to be significant and independent factors of overall survival. However, the cumulative survival rate was different between the RT group and non-RT group ($P = 0.048$), for 35 patients with non-responders to HAIC, those with metastasis from HCC, ascites or serum albumin below 3.5 g/dL (Fig. 6).

Adverse events

The two major adverse events during HAIC were leukopenia in 17 patients (38%) and thrombocytopenia in 13 (29%). These complications were mostly CTCAE grade 1 or 2. Other less common side effects were vomiting in one patient (2%), abdominal pain in one (2%), and appetite loss in one (2%). These were all CTCAE grade 1 or 2. On the other hand, the 19 patients who received RT were classified according to liver functional reserve as no change in 16 patients, deterioration in two patients, and improvement in one patient. In other words, RT was not associated with worsening of liver function in this cohort.

DISCUSSION

THE MAIN FINDINGS of the present study were the following: (i) an extremely high aggravation rate for EV in HCC patients with Vp3/4; (ii) large size varices and lack of response to HAIC were two significant and independent factors that influenced the aggravation rate for EV; and (iii) factors related to EV such as the F factor did not influence overall survival, while HCC disease control was categorized as a significant factor for overall survival.

Analysis of data of all patients showed that the cumulative aggravation rates for EV were 47% and 64% at 12 and 24 months, respectively. Previous study showed

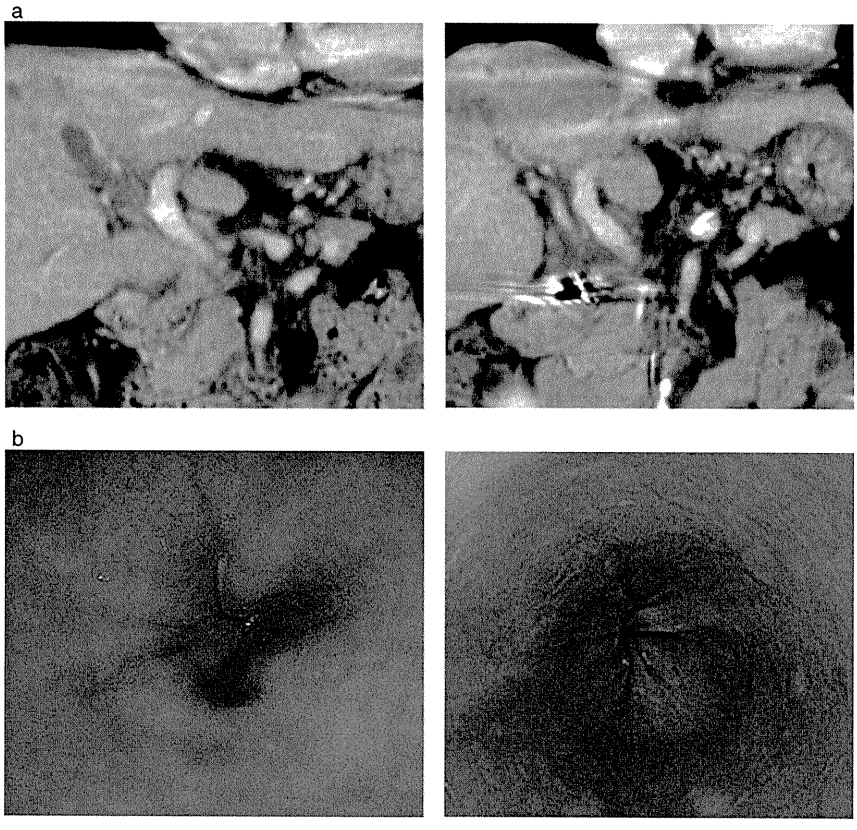

Figure 3 Representative figures showing improvement after the combination therapy of hepatic arterial infusion chemotherapy (HAIC) and radiotherapy (RT). The improvement from Vp3 to Vp2 in portal vein tumor thrombosis (PVTT) provided the reduction from F1 to F0 for varices size. (a) Improvement of PVTT. (b) Improvement of esophageal varices (EV). *Left:* before the combination therapy, *right:* after the combination therapy.

Table 5 Results of univariate analysis for the relationship between cumulative survival rate and various clinicopathological variables

Gender (male/female)	0.1914
Age (≤65/>65) (years)	0.8363
Varices size (F0, 1/F2, 3)†	0.0004
Red color sign (0/1–3)†	0.0001
Platelet count ($\leq 15 \times 10^4 / > 15 \times 10^4$) (/μL)	0.2844
T. bilirubin (≤1.0/>1.0) (mg/dL)	0.8501
Albumin (≤3.5/>3.5) (g/dL)	0.0061
Prothrombin time activity (≤70/>70) (%)	0.8449
Ascites (yes/no)	<0.0001
Tumor size (≤70/>70) (mm)	0.5367
Size of HCC relative to whole liver (≤50/>50) (%)	0.0003
Vp (3/4)†	0.4228
Vv (yes/no)	0.2654
Metastasis from HCC (yes/no)	<0.0001
HCC treatment protocol (low-dose FP/5-FU-IFN)	0.4816
Radiotherapy (yes/no)	0.2871
Disease control of HCC (yes/no)	<0.0001

†See Table 1 for classification of endoscopic findings and of portal vein tumor thrombus (PVTT) grade and abbreviations.

Table 6 Determinants of cumulative survival rate of esophageal varices by multivariate analysis

	Factor	Hazard ratio	95% confidence interval	*P*-value
Disease control of HCC	Non-disease control	12.233	2.215–16.811	<0.001
	Disease control	1		
Metastasis from HCC	Yes	11.469	1.894–10.948	0.001
	No	1		
Ascites	Yes	8.825	1.796–17.390	0.003
	No	1		
Albumin (g/dL)	>3.5	4.953	1.132–7.008	0.026
	≤3.5	1		

HCC, hepatocellular carcinoma.

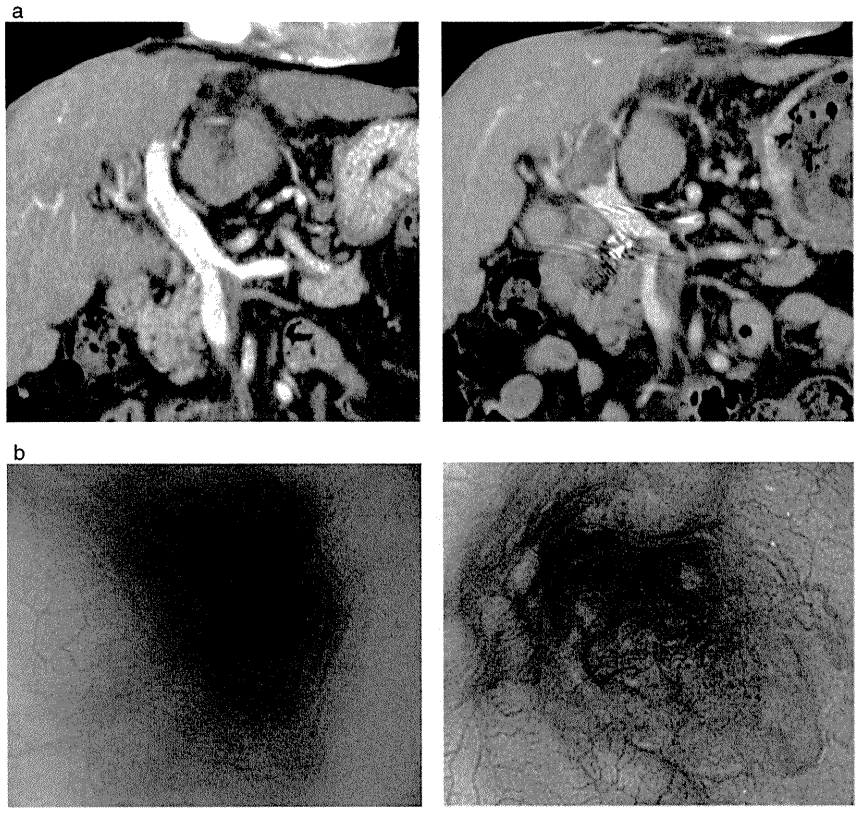

Figure 4 Representative figures showing aggravation after the combination therapy of hepatic arterial infusion chemotherapy (HAIC) and radiotherapy (RT). The aggravation from Vp3 to Vp4 in portal vein tumor thrombosis (PVTT) provided the increase from F1 to F2 for varices size. (a) Aggravation of PVTT. (b) Aggravation of esophageal varices (EV). *Left:* before the combination therapy, *right:* after the combination therapy.

that the proportions of LC patients free of HCC who were positive for the RC sign were 5%, 24% and 43%, at 1, 3, and 5 years, respectively.[20] The data showed that the aggravation rates for EV in HCC patients with Vp3/4 HCC were much higher than those in patients without HCC. In this study, varices size and the HCC disease control were significant and independent factors of overall aggravation for EV during the treatment (Table 4). These results suggest that disease control of HCC by HAIC reduced the aggravation of EV and the portal vein pressure. We analyzed retrospectively the clinical outcome of EV in HCC patients who received HAIC (low-dose FP therapy and 5-FU/IFN therapy). Previous studies reported that the response rate to those therapies in Vp3/4 HCC was less than ~50%.[23,24,28,29] Reduction of aggravation of EV is difficult without improvement in the response to HAIC in Vp3/4 HCC. Wu *et al.*[30] reported that Sorafenib, an oral multikinase inhibitor, could improve the outcome of variceal bleeding in patients with advanced Vp3/4 HCC. This new drug might provide better disease control and improve the aggravation of EV. However, Sorafenib might also increase the likelihood of hemorrhage from EV.

Our analysis showed that RT is not a serious factor in aggravation of EV and survival. However, Katamura *et al.*[31] showed that 5-FU/IFN-α combined with 3D-CRT for PVTT improved the response rate for PVTT and reduced the incidence of portal hypertension-related events. Our study also showed a significant difference in the cumulative aggravation rate between the RT and non-RT groups in patients non-responsive to HAIC or the F factor. Thus, patients who do not respond to HAIC should receive RT as complementary therapy. Moreover, our study showed a significant difference in the cumulative survival rate between the RT and non-RT groups in non-responders to HAIC or those with metastasis from HCC, ascites, or serum albumin below 3.5 g/dL. Thus, patients with these poor prognostic factors for overall survival (according to the results of multivariate analysis) might also benefit from RT as an additional therapy.

In addition to HCC disease control, varices size (F2/F3) was identified as an independent factor for aggravation of EV. Figure 2b shows that the cumulative aggravation rates at both 12 and 24 months were significantly higher in patients with large varices (F2/F3)

compared to those with small varices (F0/F1), suggesting the need for endoscopic treatment such as endoscopic injection sclerotherapy (EIS) or endscopic variceal legation (EVL) in patients with F-positive EV.

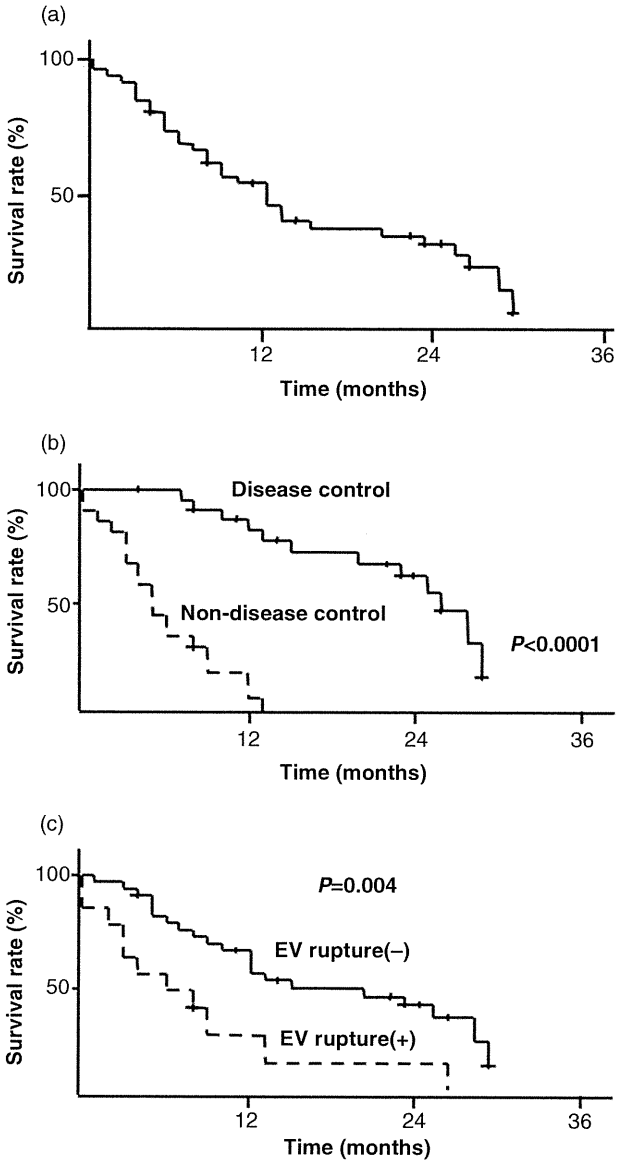

Figure 5 Cumulative survival rates for all patients with Vp3/4 hepatocellular carcinoma (HCC). (a) Cumulative survival rate of all patients. (b) Cumulative survival rate according to HCC disease control. Disease control with hepatic arterial infusion chemotherapy (HAIC): patients who responded to HAIC, non-disease control: patients who did not respond to HAIC. (c) Cumulative survival rate according to rupture of esophageal varices (EV). EV rupture(+): patients with rupture of esophageal varices, EV(–): patients with intact esophageal varices.

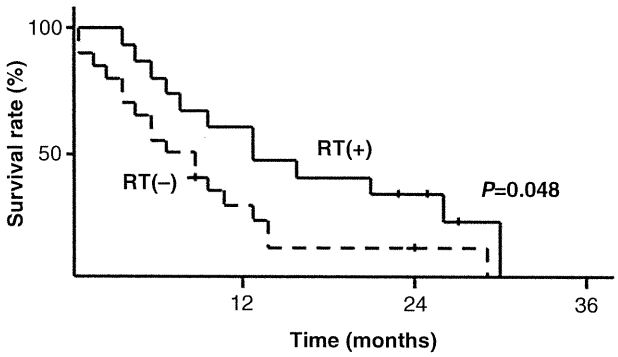

Figure 6 Cumulative survival rates of 35 patients with Vp3/4 hepatocellular carcinoma (HCC) who did not respond to hepatic arterial infusion chemotherapy (HAIC), or those with metastasis from HCC, ascites, or serum albumin below 3.5 g/dL according to radiotherapy (RT). RT(+): patients who received radiotherapy, RT(–): patients who did not receive radiotherapy.

EVL, which is less invasive than EIS, though it does not offer radical treatment, might be favorable for reduction of EV, considering the poor prognosis of HCC patients with Vp3/4. In this regard, radical treatment of EV might be considered after assessment of the response to HAIC. While treatment for EV is recommended for good responders to HAIC, it is not for poor responders. Taken together, we recommend the use of EVL for large EV (F2/F3) before HAIC in patients with Vp3/4 HCC to reduce varices size, followed by HAIC, although HCC disease control should be the most important in the treatment strategy for EV in these patients. On the other hand, HAIC should be provided for advanced HCC at first, while EV should not necessarily be treated since the bleeding rates of F0/F1 and RC0 in HCC patients with Vp3/4 are considered low. When HAIC produces an effective outcome, patients could undergo radical treatment for EV, especially those with F2/F3 and RC1/2/3. However, patients of the non-disease control could receive RT as an additional treatment without the need for treatment of EV (Fig. 7).

In conclusion, the present study demonstrated an extremely high aggravation rate of EV in HCC patients with Vp3/4. The results indicated that large-size varices and lack of response to HAIC are significant determinants of the aggravation rate of EV. In addition, the overall survival was significantly influenced by HCC disease control rather than by factors related to EV such as the F factor. These results emphasize the need for newer and more effective therapeutic modalities for the control of Vp3/4 HCC, and highlight the usefulness of

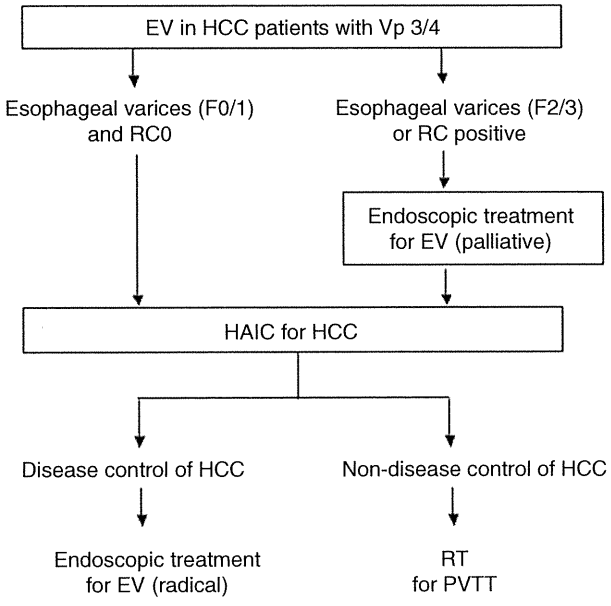

Figure 7 Treatment strategy for esophageal varices (EV) in hepatocellular carcinoma (HCC) patients with Vp3/4.

RT for poor responders to HAIC in the prevention of aggravation and bleeding of EV.

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