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Effect of occult hepatitis B virus infection on the early-onset of hepatocellular carcinoma in patients with hepatitis C virus infection

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Abstract. Although overt hepatitis B virus (HBV) infection promotes the onset of hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-infected patients, the effect of occult HBV infection remains unclear. The aim of this study was to investigate the effect of occult HBV infection on the early-onset of HCC in HCV-infected patients. A total of 173 HCC patients with HCV infection were enrolled and classified into 2 groups according to the median age of HCC onset: the early-onset group (n=91; 61.1±5.6 years) and the late-onset group (n=82; 73.8±3.7 years). Independent factors associated with the early-onset of HCC were assessed by multivariate analysis. In the overall analysis, independent risk factors for the early-onset of HCC were the white blood cell count and alanine aminotransferase level, but not the presence of HBV DNA. In a stratification analysis according to albumin levels of ≥3.5 g/dl, the presence of HBV DNA was a significant independent risk factor for the early-onset of HCC (OR 145.18, 95% CI 1.38-15296.61, P=0.036), whereas the presence of antibodies against hepatitis B core antigen was not found to be a risk factor. The presence of HBV DNA was not a risk factor for the early-onset of HCC in the overall analysis. However, its presence was an independent factor for the early-onset of HCC in HCV-infected patients with an albumin level of ≥3.5 g/dl. Thus, occult HBV infection may accelerate hepatocarcinogenesis in HCV-infected patients with relatively low carcinogenic potential.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It ranks third in men and fifth in women as the cause of death from malignancies in Japan (1). Chronic hepatitis C virus (HCV) infection is the major cause of HCC and accounts for ~60-70% of HCC cases in Japan (2). In addition to hepatic inflammation and subsequent fibrosis, various other factors including aging, obesity and diabetes mellitus are involved in the hepatocarcinogenesis in HCV-infected patients (3-5).

Co-infection of HCV with hepatitis B virus (HBV) is thought to synergistically increase the development of HCC (6). The status of HBV infection is evaluated by the presence of hepatitis B surface antigen (HBsAg), antibodies against hepatitis B core antigen (HBcAb), and HBV DNA. In some cases, HBV DNA can be detected in the serum or liver tissue of patients who are negative for HBsAg, a condition referred to as 'occult HBV infection' (7,8). In Japan, the prevalence of occult HBV infection in HCV-infected patients is reported

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Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; BMI, body mass index; WBC, white blood cell; HbA1c, hemoglobin A1c; AST, aspartate aminotransferase; ALT; alanine aminotransferase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HOMA, homeostasis model assessment; APRI, AST to platelet ratio index; AUROC, area under the receiver operating characteristic curve analysis; MAPK, mitogen activated protein kinase

Key words: latent HBV infection, hepatoma, liver cancer, oncogenesis, white blood cell

Table I. Nucleotide positions and sequences of TaqMan PCR primers and probes.

Primer/Probe	Sequence	Position
S-sense	TGTACAAAACCTTCGGACGGAAA	442-464
S-antisense	TGCGAAAGCCCAGGATGATG	485-504
S-probe	CTGCACTTGTATTCCC	465-480
C-sense	ACTGTGGTTTCACATTTCCTGTCTT	2072-2096
C-antisense	GGCATTTGGTGGTCTGTAAGC	2163-2183
C-probe	CCACACTCCAAAAGAC	2132-2147
X-sense	CTACTGTTCAAGCCTCCAAGCT	1729-1750
X-antisense	GCTCCAAATTCTTTATACGGGTCAATG	1778-1804
X-probe	AAGCCACCCAAGGCAC	1751-1766

Nucleotide positions are based on the sequence of hepatitis B virus subtype adr4 (GenBank accession no. X01587) (29).

to be between 37.7% and 90% (9-11). Occult HBV infection is associated with a poor response to interferon therapy for chronic hepatitis C (12,13) and is also known to accelerate the progression of liver fibrosis, resulting in cirrhosis in patients with HCV infection (9,14,15). Several previous studies have examined the impact of occult HBV infection on the development of HCC in HCV-infected patients, but no clear conclusions have emerged (14,16,17). Moreover, the effects of occult HBV infection on the early-onset of HCC have not been investigated in HCV-infected patients.

Albumin is produced by hepatocytes, and the level of serum albumin is used to evaluate hepatic function (18). Albumin plays a significant role in maintaining colloid osmotic pressure and transports drugs and endogenous substances including bilirubin and unesterified free fatty acids (19). In addition, albumin exerts antioxidative properties (19), and hypoalbuminemia has been shown to be an independent risk factor for mortality among residents of a hyperendemic area of HCV infection in Japan (20). A serum albumin level of ≥3.5 g/dl is an independent predictor of survival in HCC patients (21,22) and in cirrhotic patients with a serum albumin levels of <3.5 g/dl, branched-chain amino acids increase serum albumin levels and subsequently suppress hepatocarcinogenesis (23,24). Thus, the serum albumin level is an important factor in hepatocarcinogenesis.

The aim of this study is to investigate the impact of occult HBV infection on the early-onset of HCC in HCV-infected patients. We also performed a stratification analysis according to the serum albumin level.

Subjects and methods

Subjects. We conducted a retrospective study to investigate the effect of the presence of HBV DNA on the early-onset of HCC in HCV-infected patients. Between 1995 and 2011, 325 patients underwent hepatic resection at the Kurume University Hospital. The inclusion criteria were histologically proven HCC, a positive result for serum anti-HCV, and a negative result for serum HBsAg. Exclusion criteria were the presence of autoimmune hepatitis, primary biliary cirrhosis, and hemochromatosis, no test results for serum HBV DNA, and a histological diagnosis of combined hepatocellular and cholangiocellular carcinoma. Although 214 patients met the inclusion criteria, 41 patients had to be excluded because of one or more of these reasons. The remaining 173 HCC patients with HCV infection were therefore enrolled in this study and classified into 2 groups according to the median age of HCC onset: the early-onset group (n=91; 61.1±5.6 years) and the late-onset group (n=82; 73.8±3.7 years).

The study protocol was approved by the institutional review board, and informed consent for participation in the study was obtained from each subject. None of the subjects were institutionalized.

Data collection. Demographic data were collected at the time of hepatic resection including age, gender, and alcohol intake. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2).

Venous blood samples were taken in the morning after a 12-h overnight fast. The presence of serum anti-HCV, HBsAg, and HBcAb was tested using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital). Blood platelet count, white blood cell (WBC) count, prothrombin time %, plasma glucose levels; hemoglobin A1c (HbA1c) levels, and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total bilirubin, insulin, α-fetoprotein (AFP), and des-γ-carboxy prothrombin (DCP) were also measured using standard clinical methods. Insulin resistance was evaluated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment for insulin resistance (HOMA-IR), as previously described (25).

The stage of hepatic fibrosis was assessed using the AST-to-platelet ratio index (APRI), which is calculated as the serum AST level (U/l)/upper limit of normal AST (U/l) x100/platelet count ($x10^4$/ml). Patients with APRI values of ≤1.5 were diagnosed as having chronic hepatitis, and patients with APRI values >1.5 were diagnosed as having liver cirrhosis, as previously described (26). The degree of liver cirrhosis was categorized according to the Child-Pugh classification (27). Diabetes mellitus was diagnosed on the basis of fasting blood glucose levels >126 mg/dl or HbA1c levels >6.5%, in accordance with the Diagnostic Criteria for

Table II. Differences in the clinical characteristics between the early-onset and late-onset groups.

Variable	Reference value	Early-onset	Late-onset	P
Number of patients		91	82	
Age (years)		61.1±5.6	73.8±3.7	<0.001
AFP (ng/ml)	<8.7	1876±12163	769±3246	0.588
DCP (mAU/ml)	<40	1083±4120	1071±3845	0.378
Maximal HCC size (mm)	N/A	30.4±19.6	33.2±16.2	0.055
Gender (female/male)	N/A	23/68	20/62	0.893
BMI (kg/m^2)	18.5-22.0	23.6±3.6	22.4±3.2	0.045
Daily alcohol intake (none/0-60 g/>60 g)	N/A	21/42/14	23/36/10	0.676
Platelet count (x10^4/mm^3)	13-36	13.8±5.4	13.5±4.6	0.988
WBC count (/mm^3)	4000-9000	5009±1526	4420±1210	0.012
AST (U/l)	13-33	56.2±29.5	52.8±27.2	0.412
ALT (U/l)	6-30	62.2±40.9	51.7±31.7	0.104
Albumin (g/dl)	4.0-5.0	3.87±0.45	3.85±0.38	0.520
Prothrombin time (%)	70-130	90.0±11.2	91.7±12.2	0.272
Total bilirubin (mg/dl)	0.3-1.2	0.84±0.35	0.79±0.29	0.346
Chronic hepatitis/Child-Pugh class A/Child-Pugh class B	N/A	40/49/2	36/44/2	0.994
Complication of diabetes mellitus (yes/no)	N/A	30/61	20/62	0.214
Fasting blood glucose (mg/dl)	80-109	119±39	107±31	0.060
Insulin (μU/ml)	5-20	13.1±10.4	9.8±8.0	0.014
HOMA-IR	<2.5	3.05±2.47	2.11±1.03	0.622
HbA1c (%)	4.6-6.2	5.77±0.88	5.50±0.78	0.053
HBcAb positive/negative	N/A	49/42	50/32	0.344
HBV DNA positive/negative	N/A	6/85	3/79	0.385

Values are expressed as the mean ± SE. AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; BMI, body mass index; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment for insulin resistance; HbA1c, hemoglobin A1c; HBcAb, antibody for hepatitis B core antigen; HBV, hepatitis B virus; N/A, not applicable.

Diabetes Mellitus of the Japan Diabetes Society (28), or the use of antidiabetic agents.

Nucleic acid extraction from serum. Total nucleic acid was extracted from 300 μl of plasma using a commercially available kit (High Pure Viral Nucleic Acid kit; Roche Diagnostics, Tokyo, Japan) according to the manufacturer's instructions. The extracted nucleic acid was eluted in 25 μl of elution buffer.

PCR for HBV DNA. Serum HBV DNA was analyzed for the presence of HBs, HBc, and HBx (S, C and X) regions using TaqMan real-time PCR according to the manufacturer's instructions (TaqMan Fast Universal PCR Master mix; Applied Biosystems, Tokyo, Japan). The oligonucleotide primers and probes that were optimized for the HBV subtype adr4 (29) were specific for the S, X and C region sequences are listed in Table I. The full-length HBV DNA (GenBank accession no. X01587) (29) was used as an internal standard in the quantitative real-time detection PCR. We used 8 μl of nucleic acid-containing serum in our study for better sensitivity. The limit of sensitivity of our TaqMan Real-time PCR methods was 4.5 copies/well, and the detection limit of our tests was 45 copies/ml (1.7 log copies/ml). A real-time PCR assay (COBAS TaqMan HBV Auto; Roche Diagnostics) was also performed to detect the core region of HBV DNA (limit of quantification, 1.8 log copies/ml). The presence of HBV DNA was defined as any positivity of S, X or C region.

Statistical analysis. Data are expressed as the absolute value or the mean ± SD. Differences between the early-onset and late-onset groups were analyzed using the Mann-Whitney U test. A logistic regression model with the Firth's correction 30 was used for multivariate stepwise analysis to identify independent variables associated with the early-onset of HCC, as previously described (31,32). All P-values were 2-tailed, and a level of <0.05 was considered statistically significant. All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA) or R packages version 2.15.2 (URL http://www.R-project.org/).

Results

Univariate analysis between the early-onset and late-onset groups. AFP levels, DCP levels, and maximal HCC size did not differ between the early-onset and late-onset groups (Table II). Furthermore, although BMI, WBC count, and serum insulin levels were significantly higher in the early-onset group than in the late-onset group, there were no significant differences

Table III. Multivariate stepwise analysis for factors associated with the early-onset of hepatocellular carcinoma.

	Unit	Odds ratio	95% CI	P
HbA1c	1	1.37	0.91-2.07	0.136
BMI	1	1.08	0.98-1.19	0.133
ALT	10	1.10	1.00-1.21	0.045
DCP	20	0.99	0.98-1.00	0.091
WBC count	1000	1.35	1.06-1.73	0.014

All P-values were 2-tailed, and a level of <0.05 was considered statistically significant. HbA1c, hemoglobin A1c; BMI, body mass index; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; WBC, white blood cell.

in the daily alcohol intake, platelet count, prothrombin time, Child-Pugh classification, presence of diabetes mellitus as a comorbidity, fasting blood glucose level, HOMA-IR value, HbA1c levels, and the serum levels of AST, ALT, albumin, and total bilirubin (Table II). The presence of HBcAb and HBV DNA did not differ either between the early-onset and late-onset groups (Table II).

Multivariate stepwise analysis for early-onset of HCC. Multivariate stepwise analysis showed that the serum ALT level and WBC count were independent risk factors for the early-onset of HCC (OR 1.10; 95% CI 1.00-1.21; P=0.045 and OR 1.35; 95% CI 1.06-1.73; P=0.014, respectively; Table III), but not the presence of HBcAb or HBV DNA.

Stratification analysis according to serum albumin level. Differences in the clinical characteristics between patients with the albumin level of ≥3.5 g/dl and <3.5 g/dl were summarized in Table IV. There were no significant differences in AFP levels, DCP levels, and maximal HCC size between the albumin level of ≥3.5 g/dl and <3.5 g/dl groups (Table IV). In the albumin level of ≥3.5 g/dl group, a significant elevation was seen in platelet count, prothrombin time and the number of patients with chronic hepatitis and a significant depletion was seen in AST level than in the albumin level of <3.5 g/dl group. However, other biochemical parameters and the

Table IV. Differences in the clinical characteristics between patients with the albumin level of ≥3.5 g/dl and <3.5 g/dl.

		Albumin level of		
Variable	Reference value	≥3.5 g/dl	<3.5 g/dl	P
Number of patients		138	35	
Age (years)		67.8±8.1	67.9±6.7	0.895
AFP (ng/ml)	<8.7	786±3219	3262±18195	0.248
DCP (mAU/ml)	<40	854±3269	1961±5977	0.306
Maximal HCC size (mm)	N/A	30.6±15.9	36.8±23.8	0.171
Gender (female/male)	N/A	35/103	8/27	0.759
BMI (kg/m^2)	18.5-22.0	23.0±3.5	23.0±3.4	0.918
Daily alcohol intake (none/0-60 g/>60 g)	N/A	21/58/38	3/20/6	0.172
Platelet count (x10^4/mm^3)	13-36	14.3±4.9	11.3±4.8	0.001
WBC count (/mm^3)	4000-9000	4798±1395	4291±1331	0.052
AST (U/l)	13-33	51.2±26.2	67.1±32.6	0.001
ALT (U/l)	6-30	54.9±37.4	63.4±32.7	0.057
Albumin (g/dl)	4.0-5.0	4.02±0.28	3.23±0.20	<0.001
Prothrombin time (%)	70-130	91.6±12.0	88.0±10.0	0.038
Total bilirubin (mg/dl)	0.3-1.2	0.82±0.34	0.80±0.28	0.822
Chronic hepatitis/Child-Pugh class A/Child-Pugh class B	N/A	69/69/0	7/24/4	<0.001
Complication of diabetes mellitus (yes/no)	N/A	38/100	12/23	0.431
Fasting blood glucose (mg/dl)	80-109	112±37	121±49	0.694
Insulin (μU/ml)	5-20	10.1±6.4	17.6±16.3	0.063
HOMA-IR	<2.5	3.12±3.87	4.23±2.23	0.315
HbA1c (%)	4.6-6.2	5.61±0.79	5.68±1.04	0.905
HBcAb positive/negative	N/A	75/63	24/11	0.129
HBV DNA positive/negative	N/A	6/132	3/32	0.315

Values are expressed as the mean ± SE. AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; BMI, body mass index; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment for insulin resistance; HbA1c, hemoglobin A1c; HBcAb, antibody for hepatitis B core antigen; HBV, hepatitis B virus; N/A, not applicable.

Table V. Multivariate stepwise analysis for factors associated with the early-onset of hepatocellular carcinoma in patients with a serum albumin level of ≥3.5 g/dl.

	Unit	Odds ratio	95% CI	P
HBcAb	Positive	0.59	0.27-1.26	0.169
HBV DNA	Positive	145.18	1.38-15296.61	0.036
Prothrombin time	10	0.76	0.54-1.08	0.109
ALT	10	1.08	0.97-1.21	0.145
Albumin	0.1	1.17	1.01-1.36	0.036
DCP	20	0.99	0.98-1.00	0.037
Platelet count	1	0.92	0.84-1.02	0.107
WBC count	1000	1.64	1.15-2.35	0.006

All P-values were 2-tailed, and a level of <0.05 was considered statistically significant. HBcAb, antibody for hepatitis B core antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; WBC, white blood cell.

Table VI. Multivariate stepwise analysis for factors associated with the early-onset of hepatocellular carcinoma in patients with a serum albumin level of <3.5 g/dl.

	Unit	Odds ratio	95% CI	P
HbA1c	1	1.83	0.75-4.47	0.183
HBV DNA	Positive	0.00	0.00-2.96	0.093
AFP	20	1.39	1.01-1.93	0.045

All P-values were 2-tailed, and a level of <0.05 was considered statistically significant. HbA1c, hemoglobin A1c; HBV, hepatitis B virus; AFP, α-fetoprotein.

presence of HBcAb and HBV DNA did not differ between the albumin level of ≥3.5 g/dl and <3.5 g/dl groups (Table IV).

In patients with a serum albumin level of ≥3.5 g/dl, the WBC count and serum levels of albumin and DCP were identified as independent factors associated with the early-onset of HCC (OR 1.64; 95% CI 1.15-2.35; P=0.006, OR 1.17; 95% CI 1.01-1.36; P=0.036, and OR 0.99; 95% CI 0.98-1.00; P=0.037, respectively; Table V). Although the presence of HBcAb was not found to be a significant risk factor for the early-onset of HCC, the presence of HBV DNA was identified as a significant independent risk factor associated with the early-onset of HCC (OR 145.18; 95% CI 1.38-15296.61; P=0.036; Table VI).

In patients with a serum albumin level of <3.5 g/dl, the serum AFP level was the only significant risk factor found to be associated with the early-onset of HCC (Table V). The presence of HBcAb and HBV DNA was not found to be a significant risk factor for the early-onset of HCC.

Discussion

In the overall analysis, the presence of HBV DNA in serum was not identified as a risk factor for the early-onset of HCC in HCV-infected patients. However, a stratification analysis according to a serum albumin level of ≥3.5 g/dl revealed that the presence of HBV DNA was an independent factor for the early-onset of HCC. These findings suggest that occult HBV infection may accelerate hepatocarcinogenesis in HCV-infected patients with a relatively low carcinogenic potential.

Although co-infection of HCV and HBV is thought to synergistically increase the risk of HCC (6), the overall analysis in this study showed that occult HBV infection was not significantly associated with the early-onset of HCC in HCV-infected patients. Similarly, several studies conducted in Asia have also failed to show any significant effect of occult HBV infection in these patients (33-35). Recently, Lok *et al* (36) performed a nested case-control study using a large number of patients enrolled in the HALT-C cohort and reported no significant difference in the prevalence of occult HBV infection between HCC and non-HCC patients with HCV infection. Taken together, these results suggest that occult HBV infection may not be an intensive promoter of HCC development in the presence of a potent carcinogenic factor such as HCV infection.

In contrast with these previous studies and with our own findings for all patients, a stratification analysis according to a serum albumin level of ≥3.5 g/dl showed that occult HBV infection was an independent risk factor for the early-onset of HCC. In patients with occult HBV infection, it is unclear whether a presence of HBV DNA is due to full-length HBV DNA replicated from covalently closed circular DNA in hepatocytes or fragmented HBV DNA integrated into the hepatocyte genome. However, the *HBx* gene is frequently integrated into cellular genes in HCC (37). The HBx protein upregulates the expression of proto-oncogenes including *c-jun*, *c-fos* and *c-myc*, all of which can promote hepatocarcinogenesis (38,39). In addition, albumin plays a crucial role in the development of various diseases, as it is a major antioxidant (19). In cirrhotic patients with a serum albumin level of <3.5 g/dl, branched-chain amino acids increase serum albumin levels, and this subsequently suppresses hepatocarcinogenesis (23,24). In this study, we found a significant association between occult HBV infection and the early-onset of HCC in patients with a serum albumin level of ≥3.5 g/dl, but not in patients with a serum albumin level of <3.5 g/dl. Taken together, these findings suggest that HBV DNA may promote hepatocarcinogenesis in HCV-infected patients with relatively low carcinogenic potential.

Although we designed this study to investigate the effect of HBV DNA on the early-onset of HCC in HCV-infected patients, we found instead that an elevated WBC count is an independent risk factor for the early-onset of HCC in HCV-infected patients. An elevated WBC count may reflect the consequences or underlying pathogenesis of the early-onset of HCC. One possible explanation is aging, because the WBC count declines in old age (40). Alternatively, an elevated WBC count still within the reference range is known to be associated with the development of various malignancies including gastric, colorectal, endometrial and lung cancers (41,42). The WBC count is a well-validated biomarker of inflammation. Chronic inflammation is a possible risk factor for hepatocarcinogenesis as it leads to the activation of receptors for chemokine and advanced glycation-end products (43,44). Another inflammation marker, C-reactive protein, is reported to be a diagnostic and prognostic marker of HCC (45,46). Taken together, these findings suggest that inflammation may promote the early-onset of HCC in HCV-infected patients.

A limitation of this study is that there were only a small number of HBV DNA-positive patients. Previous studies regarding occult HBV infection had a similar limitation (33,47,48). Since occult HBV infection is not frequently seen in HCV-infected patients with HCC, a multicenter study is needed to confirm our findings.

In conclusion, the presence of HBV DNA in serum was not a risk factor for the early-onset of HCC in HCV-infected patients. However, a stratification analysis based on a serum albumin level of ≥3.5 g/dl revealed that presence of HBV DNA in serum was an independent risk factor for the early-onset of HCC. These findings suggest that occult HBV infection may accelerate hepatocarcinogenesis in HCV-infected patients with relatively low carcinogenic potential.

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特集／肝炎から肝硬変・肝癌まで

肝炎から肝癌までのわが国の動向

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はじめに

わが国における慢性肝疾患の原因としてはＢ型肝炎とＣ型肝炎ウイルスに起因するものが全体の80％以上を占めると推測される。近年，これらのウイルス性肝疾患は感染に対する防御体制が進歩したことに加え，核酸アナログ製剤，インターフェロン製剤，プロテアーゼ阻害剤などが相次いで開発，改良されたことから治療方法が様変わりし，それに伴い治療成績も大きく改善してきた。一方，肝細胞癌による死亡は1975年から年々増加の一途をたどっていた。このうち女性患者の死亡は現在も相変わらず増加傾向にあるものの男性患者の死亡数が減少し始めたため，全体としては2002年をピークに近年ではやや減少に転じている[1)]。肝細胞癌の原因となる背景肝疾患に関しては，Ｂ型肝炎ウイルスによる肝疾患の占める割合は変わらないが，Ｃ型肝炎ウイルスの占める割合が低下傾向にあり，変わって，Ｂ，Ｃ型肝炎ウイルス陰性の肝細胞癌が増加傾向にある[2)]。肝細胞癌の治療に関しては，2009年に本邦でも認可された分子標的治療薬であるソラフェニブを用いた治療成績がいくつかの施設で公表され，分子標的治療薬の長所・問題点が次第に明らかにされつつある。

本稿では本邦におけるウイルス性慢性肝疾患，肝細胞癌の動向を紹介する。

Ⅰ．Ｂ型肝炎

Ｂ型肝炎は全世界で約４億人の感染者が存在しており，特にアジア地域は高浸淫地帯である。本邦では1985年に始まった母子感染防止事業によりＢ型肝炎ウイルス感染者は減少しているが，それでも未だに推計で約150万人の感染者が存在すると考えられている。その多くは無症候性キャリアであるが，約７万人程度が慢性肝炎，３万人が肝硬変や肝細胞癌を患っていると推定されている。

Ｂ型肝炎ウイルスは遺伝子の全塩基配列の８％以上の相違によりＡ型からＪ型（Ｉ型はＣ型の亜型）までの９種類の遺伝子型に分類されており，さらに４～８％の塩基配列の違いにより34のサブタイプが存在している。これら遺伝子型やサブタイプには地域特性があるばかりでなく，臨床像の違い，治療に対する反応性の違いなどにも関連していることが明らかとなってきた。たとえば，欧米にはGenotype A，Ｄが多く存在し，本邦を含めたアジアにはGenotype B，Ｃが多く分布している。このうちGenotype Aは急性肝炎から遷延化，慢性化しやすいが肝発癌の頻度は低いと言われている。またインターフェロン治療に対する感受性が高く，時にはHBs抗原のセロコンバージョンも起こることが報告されている。Genotype DはGenotype Aに比べてインターフェロン治療に対する感受性が低いと言われている。Genotype Bのうち日本型であるBjは予後良好であり，ほとんどのヒトは無症候性キャリアで一生を終えるがプレコア領域（1,896番目）に変異が入ると劇症化することがある。アジア型のBaは肝発癌のリスクが高いと言われている。Genotype Cは急性肝炎からの慢性化は希でありインターフェロン治療に対する感受性も低いと報告されている。さらに肝発癌のリスクが高く予後不良とされている。

現在，Ｂ型肝炎の治療は主にインターフェロンと核酸アナログ製剤で行われている。若年者においてはHBe抗原からHBe抗体へのセロコ

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ンバージョンを起こし薬剤の中止が期待できるインターフェロン療法が主体となる。2011年9月からペグインターフェロンα2aの48週間治療がHBe抗原陽性者のみならず陰性者に対しても可能となった。国内第Ⅱ/Ⅲ相試験の結果からすると，HBe抗原陽性例では35歳未満が治療の良い適応であり，HBe抗原陰性症例では35歳以上でもHBV DNA量が7.0 log copy/ml未満であれば治療効果が期待できると考えられる。現在本邦で認可されている核酸アナログ製剤はラミブジン，アデホビル，エンテカビルである。ラミブジンは耐性株の出現頻度が高いため耐性が出現しなくてもエンテカビルへの切り替えが推奨されている。また，耐性が出現した場合はアデホビルとの併用治療が行われる。エンテカビルは耐性株の出現率が低いためHBe抗原のセロコンバージョンの可能性の低い比較的年配者に対する治療の第一選択となっている。本邦では治験が始まった段階のテノホビルはアデホビルに似た構造を持っているが腎毒性がアデホビルに比べて少ないために大量投与が可能であり強い抗ウイルス効果が期待されている。AASLDやEASLではすでにエンテカビルと並んで第一選択薬として位置づけされている[3]。今後，本邦でもエンテカビルに対し耐性が生じた症例などに対する有効性を評価する必要がある。

母子感染の予防などによりHBV感染者は本邦において減少したが，国際交流の増加や性交渉の多様化によりGenotype Aによる水平感染が増加しており今後Genotype Aの成人感染からの慢性化の増加が問題となっている。さらに，B型肝炎の高浸淫地帯であるアジア諸国では未だにラミブジンが第一選択として使用されていることが多く，今後ラミブジン耐性株の本邦への流入が危惧される。これらの事に対処するためにもユニバーサルワクチンの導入は重要な検討課題と思われる。

Ⅱ．C　型　肝　炎

本邦における一般献血者のHCV抗体陽性率は1～2％であり，わが国には約150万～200万人のHCV感染者が存在すると言われている。HCV感染者の年齢分布をみると高齢者になるにつれて感染率が高くなる傾向がある。急性肝炎の発生に関しては，HCVのスクリーニングが導入されてから輸血後肝炎の発生はほとんどなく散発性の肝炎の発生率も低く横ばいである。C型慢性肝炎に対する治療は長らくペグインターフェロンとリバビリン併用療法が主力であったが本邦に多いGenotype 1b型，高ウイルス量の症例ではSVR率は約50％に留まっていた。ことに，*IL28B*のSNPがminor typeの症例ではSVRが得られにくいとされていた[4]。この為，HCVに直接作用するDAA（direct antiviral agents）と呼ばれる薬剤の開発が進み2011年11月に第一世代のプロテアーゼ阻害剤であるテラプレビルが認可された。この薬剤にペグインターフェロンとリバビリンを加えた3剤併用12週間＋ペグインターフェロンとリバビリン2剤併用12週間で治療が開始され，Genotype 1b型，高ウイルス量の症例でSVR率が73％に向上した。しかし，従来からのペグインターフェロンとリバビリンによる副作用に加えテラプレビル自体の貧血，皮膚症状，腎機能障害などの重篤な副作用発現の報告があり，治療完遂に難渋する症例も少なくない。この様な問題点を克服するために，第二世代のプロテアーゼ阻害剤にペグインターフェロンとリバビリンを併用した臨床試験が多数国内外で進行中である。第二世代のプロテアーゼ阻害剤の特徴としては，テラプレビルと比べ治療効果は大きくは変わらないものの，強い副作用が少ないことである。これらのDAAはここ1～2年の間に認可される予定である。しかし，これらの治療法は何れもインターフェロンを併用する治療法であるため，*IL28B*のSNPがminor typeの症例などインターフェロンに不応の症例の治癒効果が大幅に改善されることは期待しにくい。この問題点を解決するためgenotype 1b，高ウイルス症例に対しNS5A阻害剤であるダクラタスビルとプロテアーゼ阻害剤のアスナプレビルの併用試験がペグインターフェロン，リバビリン併用無効例21例とIFN治療が行えない患者22例に行われた。SVRは77％，副作用中止例は高ビリルビン血症1名，トランスアミナーゼ上昇2例という結果であった[5]。今後様々なDAAの組み合わせによる臨床試験が行われ，近い将来高齢者にも安全に施行できる抗ウイルス療法が確立されC型肝炎患者の減少が現実のものとなるこ

とが期待される。

Ⅲ．肝　細　胞　癌

本邦における肝細胞癌による死亡は男性が女性に比べ約２倍多いという特徴があり，1975年以降年々増加し2002年には人口10万人において27.5人まで達した。2002年以降女性は依然増加傾向にあるものの男性は2002年をピークに減少に転じ，全体でも2007年には人口10万人において26.6人と減少した。肝発癌の原因となる背景肝疾患はHCVによる慢性肝疾患が約70％，HBVによる慢性肝疾患が10％強を占める。背景肝疾患別の肝細胞癌患者数はHBV由来の例は変化なく，HCV由来の例が減少し，HBV，HCV陰性の症例が増加傾向にある。このうち多くは非アルコール性脂肪性肝炎などによると考えられている。1995年の調査では肝発癌の好発年齢は男性が60歳台前半，女性は男性に比べやや発癌年齢が高い傾向にあったが，その後の調査で発癌年齢は徐々に高齢化する傾向にあることが明らかとなった[6)]。

肝細胞癌はハイリスク　グループを設定しやすい腫瘍であり，われわれは定期的に腹部超音波検査，CTスキャン，MRIなどの画像診断とAFP，AFP-L3，PIVKA-Ⅱなどの腫瘍マーカーを定期的に検査することで肝細胞癌の早期発見に努めてきた[7)]。このサーベイランス　システムは肝細胞癌の早期発見に効果を発揮してきたが，超音波造影剤のソナゾイドとMRIの造影剤であるGd-EOB-DTPA（プリモビスト）が各々2007年，2008年に認可された。このうちソナゾイドによる超音波検査は肝細胞癌のスクリーニング，ステージング，局所再発の局在診断，Bモード超音波検査で認識できない結節の局在診断などに有用であると言われている。一方，Gd-EOB-DTPA MRI検査は従来のMRI検査やCTスキャンに比べて肝内の結節性病変の検出に効果を発揮する。今後スクリーニングに従来の画像診断に加えこれら新たな検査法を組み込むことでさらに肝細胞癌の早期発見が可能となることが期待される。

本邦における肝細胞癌の治療は基本的には2009年に改訂された“科学的根拠に基づく肝癌診療ガイドライン”に基づいて行われることが多い[8)]。このうち根治的治療は外科的切除（移植）の他，内科的治療としては主にラジオ波焼灼療法で行われている。外科的切除（移植）もラジオ波焼灼療法も導入されてから時間がたっておりその適応基準や治療成績はほぼ出そろった感がある。2009年の日本肝臓学会で行われたコンセンサスミーティングでは腫瘍径２cm以下の肝細胞癌はラジオ波焼灼療法を選択するという意見が多かった。一方，３cm前後の単発の腫瘍で肝予備能がChild-Pugh class Aであれば外科的切除を推奨する意見が多かった。ラジオ波焼灼療法と外科的切除に関しては現在無作為前向き比較試験（SURF試験）が全国で進行中でありこの結果の解析により，よりエビデンスに基づいた治療法の選択が可能になると考えられる。

肝移植に関しては脳死肝移植ではミラノ基準を遵守すべきであるという意見が多いのに対し，生体肝移植では必ずしもミラノ基準にこだわる必要がないとの考えが多いようである。レシピエントの背景肝に関してはChild-Pugh class Cに限定すべきという考えが支配的であり，これはChild-Pugh class A/Bの段階では，移植以外の治療法で対応可能との考えを反映しているものと思われる。

根治不能な進行肝細胞癌に対する治療は脈管浸潤がない場合は肝動脈化学塞栓術（TACE）がエビデンスのある治療法として世界中で広く行われている。門脈などへ高度に腫瘍が浸潤した症例やTACEができないほどの肝内多発症例などに対しては本邦ではシスプラチン＋5-FUもしくはインターフェロン皮下注射＋5-FUによる肝動注化学療法（HAIC）が行われている。いずれのHAICも奏効率は30～40％で，予後もほぼ同等である。しかし，HAICは本邦において積極的に行われているが，治療効果を対照群と比較した報告がないため欧米では受け入れられていないのが現状である。2007年に公表されたSHARP試験[9)]の結果により分子標的治療薬であるソラフェニブの進行肝細胞癌に対する有用性が証明され，本邦にも2009年に臨床での使用が認可された。現時点でのソラフェニブの治療対象症例は肝予備能がChild-Pugh class AでTACEやHAICが不応もしくは不能になった症例や肝外転移を認める症例である。SHARP試験以来症例の蓄積が進み，ソラフェニブ単独治

療では予後延長の効果がわずか 3 ヵ月であること，副作用が多く，こまめな薬剤の減量が必要なことなど様々な問題点も明らかとなってきた。今後しばらくはソラフェニブに勝る新規分子標的治療薬が認可される予定がないため，既存の治療法とソラフェニブとを組み合わせた治療法に更なる予後改善効果を期待したい。現在進行中の TACE とソラフェニブとの併用療法（TACTICS）や HAIC との併用療法（SILIUS）の結果次第で今後進行肝細胞癌の治療法が大きく変わる可能性がある。

お　わ　り　に

B 型肝炎は母子感染の予防方法が確立・実行されていることでウイルス保菌者の数が今後激減していくことが予想される。さらに，核酸アナログ製剤の登場により病勢のコントロールが容易になり肝硬変による肝不全や肝発癌に至る症例も減少することが予想される。同様に，C 型肝炎も感染予防の進歩とインターフェロン製剤，プロテアーゼ阻害剤の出現，さらに近い将来認可されるであろうポリメラーゼ阻害剤など薬剤の進歩およびインターフェロンを用いないプロテアーゼ阻害剤と NS5A 阻害剤併用療法の開発によるウイルス駆除の効率が改善され患者数は減少の一途をたどることが予想される。このため肝細胞癌に関しても本邦では自然に減少することが考えられるが，現在肝細胞癌に苦しんでいる患者や発癌直前の患者も数多くいることも事実である。また，海外においては，今後しばらくの間患者数が増加し続けることが予想される。このため，ソラフェニブに既存の治療法を併用した治療法の効果を早く検証し，エビデンスのある治療法として確立することが我々の責務と考える。

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Research Article

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Non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI are a risk factor for recurrence of HCC after hepatectomy

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Background & Aims: The gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) often depicts non-hypervascular hypointense hepatic nodules during the hepatobiliary phase in patients with hepatocellular carcinoma (HCC). It is unclear whether the presence of these nodules is associated with HCC recurrence after hepatectomy. We conducted a prospective observational study to investigate the impact of the presence of non-hypervascular hypointense hepatic nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI on the recurrence of HCC after hepatectomy.
Methods: A total of 77 patients who underwent hepatectomy for primary, non-recurrent, hypervascular HCC were prospectively followed up after hepatectomy. Post-operative recurrence rates were compared according to the presence of non-hypervascular hypointense nodules on preoperative Gd-EOB-DTPA-enhanced MRI.
Results: Recurrence rates after hepatectomy were higher in patients with non-hypervascular hypointense nodules (risk ratio 1.9396 [1.3615–2.7222]) and the presence of non-hypervascular hypointense nodules was an independent factor associated with postoperative recurrence (risk ratio 2.1767 [1.5089–3.1105]) along with HCC differentiation and portal vein invasion. While no differences were found in the rate of intrahepatic metastasis recurrence based on the preoperative presence of non-hypervascular hypointense hepatic nodules, the rate of multicentric recurrence was significantly higher in patients with preoperative non-hypervascular hypointense hepatic nodules.
Conclusions: Patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI are at higher risk of HCC recurrence after hepatectomy, mainly due to multicentric recurrence.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC is the third and fifth most common cause of death from cancer in men and women, respectively [3]. Tremendous efforts have been made to improve various imaging techniques, including ultrasonography (US), multidetector-row computed tomography (MDCT) [4,5], and magnetic resonance imaging (MRI) [6], for the detection of hepatic nodules, including small early-stage HCC tumors in high-risk patients under surveillance.

The liver-specific contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), which is taken up by hepatocytes, has been in clinical use for dynamic MRI studies since February 2008 in Japan. Gd-EOB-DTPA provides both dynamic and liver-specific hepatobiliary MR images [7–10]. In the hepatobiliary phase, hepatic lesions that lack normally functioning hepatocytes are imaged as an absence of hepatocyte-selective enhancement as compared with normal parenchyma [10,11]. The use of Gd-EOB-DTPA-enhanced MRI increases detection of concurrent non-hypervascular hepatic nodules as hypointense nodules during the hepatobiliary phase in patients with HCC. It is controversial whether the presence of these non-hypervascular hepatic nodules detected in patients with typical hypervascular HCC lesions has an impact on the recurrence of HCC after treatment.

In the present study, we attempted to evaluate the impact of concurrent non-hypervascular hepatic nodules detected as hypointense nodules during the hepatobiliary phase of Gd-EOB-

Cancer

Keywords: Hepatocellular carcinoma; Gd-EOB-DTPA-enhanced MRI; Non-hypervascular hypointense nodule; Hepatobiliary phase; Hepatectomy; Recurrence.
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Abbreviations: Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; HCC, hepatocellular carcinoma; US, ultrasonography; MDCT, multidetector-row computed tomography; TFE, turbo field echo; CTHA, computed tomography during hepatic arteriography.

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Table 1. Comparison of clinical characteristics of study patients based on the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (n = 77).

	Non-hypervascular hypointense nodule (+) (n = 18)	Non-hypervascular hypointense nodule (-) (n = 59)	*p* value
Age (mean ± SD, yr) (range)	65.8 ± 9.0 (46-76)	69.1 ± 7.0 (53-82)	0.2727
Sex (female/male)	3 (16.7)/15 (83.3)	18 (30.5)/41 (69.5)	0.3921
Etiology (HBV/HCV/non-HBV, non-HCV)	2 (11.1)/11 (61.1)/5 (27.8)	9 (15.3)/39 (66.1)/11 (18.6)	0.6796
Child-Pugh class (A/B)*	17 (94.4)/1 (5.6)	58 (98.3)/1 (1.7)	0.9474
Albumin (mean ± SD, g/dl)	3.91 ± 0.51	4.08 ± 0.32	0.1664
Total bilirubin (mean ± SD, mg/dl)	0.88 ± 0.36	0.84 ± 0.33	0.7296
15-minute ICG retention rate (%)	18.1 ± 5.4	16.0 ± 6.7	0.2405
Prothrombin (%)	95.3 ± 15.6	95.1 ± 11.2	0.9105
Platelet count (x10^3/ml)	132 ± 47	152 ± 66	0.5433
Tumor size (mean ± SD, cm) (range)	2.52 ± 0.99 (1.3-4.7)	2.84 ± 1.54 (1.0-8.6)	0.6600
Number of tumors (single/multiple)	15 (83.3)/3 (16.7)	53 (89.8)/6 (10.2)	0.7358
Portal vein invasion (absent/present)**	17 (94.4)/1 (5.6)	50 (84.7)/9 (15.3)	0.4989
Differentiation (well-/moderately or poorly)**	7 (38.9)/11 (61.1)	21 (35.6)/38 (64.4)	0.9999
Growth pattern (expansive/infiltrative)**	14 (77.8)/4 (22.2)	52 (88.1)/7 (11.9)	0.4718
Follow-up period (months) (median, range)	31.3 (9.4-53.9)	34.9 (8.5-55.4)	0.4200

Percentages are in parentheses.
HBV, hepatitis B virus; HCV, hepatitis C virus; ICG, indocyanine green test.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination based on resected specimens.

DTPA-enhanced MRI on postoperative recurrence in patients who underwent hepatectomy with curative intent for HCC.

Materials and methods

Patients, treatment and follow-up

This prospective study was conducted after the approval by the hospital institutional review board and carried out in compliance with the Helsinki Declaration. Patient enrollment was carried out between February 2008 and December 2011. A total of 102 patients underwent hepatectomy as a curative treatment for primary, non-recurrent HCC during the study period at Ogaki Municipal Hospital. Gd-EOB-DTPA-enhanced MRI could not be performed prior to hepatectomy in 25 patients, including 11 patients who had been referred from another institution only for hepatectomy and 14 patients who could not receive examination due to metal implants, history of allergy to contrast medium, tattoos, or claustrophobia. The remaining 77 patients who underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks prior to hepatectomy were studied. The initial diagnosis of HCC before treatment was based on appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. The final diagnosis of HCC was confirmed by pathologic diagnosis of resected specimens.

Decisions regarding individual treatments were based on Japanese treatment guidelines for HCC [14]. In all patients, HCC tumors were resected with ample margins; enucleation of tumors without margins was not performed.

After hepatectomy, all patients were prospectively followed from 8.5 months to 55.4 months (median follow-up, 34.1 months) until the end of September 2012 at our institution, with US and either MDCT or MRI every 3–6 months. Regular monitoring of serum tumor markers (alpha-fetoprotein, *Lens culinaris* agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin) was performed every 3 months. When an elevation in tumor markers was detected, additional imaging (usually MDCT or MRI) was performed to check for HCC recurrence. Recurrence was diagnosed by pathologic examination of resected specimens when patients underwent re-hepatectomy. In the remaining patients, HCC was diagnosed by appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. Recurrent HCC was categorized into two groups prior to the study, as intrahepatic metastasis recurrence or multicentric recurrence according to a previous study [15,16]. Intrahepatic metastasis recurrence was defined as recurrent tumors consisting of moderately or poorly differentiated HCC with the same or lower degree of differentiation than the primary tumors on pathologic examination or hypervascular tumor without non-hypervascular peripheral regions in a same hepatic segment on imaging examination. Multicentric recurrence was defined according to previously reported criteria with some modifications [17,18] as follows: (i) the recurrent tumor consists of well-differentiated HCC occurring in a different hepatic segment, than moderately or poorly differentiated pre-existing HCCs; (ii) both the primary and recurrent tumors are well-differentiated HCCs; and (iii) the recurrent tumor contained regions of dysplastic nodules in peripheral areas based on pathologic examination or contained non-hypervascular regions in peripheral areas of hypervascular tumor on imaging examination.

Preoperative imaging examinations of liver nodules by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI and confirmation of non-hypervascular hypointense hepatic nodules

All patients underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks of hepatectomy. MRI was performed using a 1.5-T whole-body MRI system (Intera Achieva 1.5T NOVA; Philips Medical Systems) with a phased-array body coil as the receiver coil. T1-weighted sequences were acquired with the following parameters: T1-weighted turbo field echo (TFE) in-phase and opposed-phase transverse (TE, opposed-phase 2.3, in-phase 4.6; flip angle, 12°; matrix size, 256 × 512; scan percentage, 70) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. After intravenous injection of Gd-EOB-DTPA (Primovist; Bayer Schering Pharma, Osaka, Japan), T1-weighted transverse gradient-echo sequences (high-resolution isotropic volume examination [THRIVE] with spectral presaturation with inversion recovery [SPIR], 4/1.8; flip angle, 12°; matrix size, 256 × 512; scan percentage, 78.54) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view were obtained. Gd-EOB-DTPA was administered intravenously as a bolus at a rate of 2 ml/s (0.1 ml/kg, maximum dose of 10 ml) through an intravenous cubital line (20–22 gauge), which was flushed with 20 ml of saline using a power injector (Sonic Shot; Nemoto Kyorindo, Tokyo, Japan). The timing for dynamic arterial phase imaging was determined using

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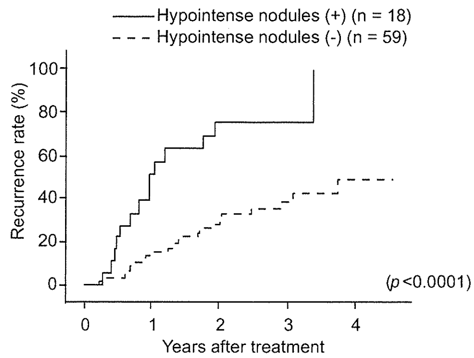

Patients at risk					
Hypointense nodules (+)	18	17	14	7	2
Hypointense nodules (-)	59	58	47	29	16

Fig. 1. Overall recurrence rate after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI.

MR fluoroscopic bolus detection of the descending aorta (Bolus Trak; Philips Medical Systems). The mean delay times (time interval between the start of bolus administration and the start of image acquisition) for the arterial, portal, and delayed phases were 20, 60, and 180 s, respectively. Immediately after the dynamic study, a respiration-triggered single-shot T2-weighted sequence, with a reduction factor of 4 (1200/100; flip angle, 90°; matrix size, 400 × 512) with 7-mm section thickness, a 1-mm intersection gap, and a 38-cm field of view, was obtained with SPIR. The 20-min-delayed hepatobiliary phase [19] was obtained with a T1-weighted TFE sequence (TR/TE, 4/1.8; flip angle, 12°; matrix size, 256 × 512) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. All the sequences were obtained with parallel imaging (SENSE). Hypointense hepatic nodules during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI were nodules greater than 3.5 mm with low-intensity.

Prior to hepatectomy, all patients underwent CT during hepatic arteriography (CTHA) [20–22] to evaluate the intranodular blood supply, and to confirm the hypervascularity of HCC lesions and the lack of hypervascularity of non-hypervascular hepatic nodules.

All imaging findings were evaluated by a radiologist (Y.S.) and a hepatologist (H.T.) independently, blind to the clinical data. When the imaging assessment was discordant between two reviewers, consensus was made through the discussion.

Statistical analyses

Differences in percentages between groups were analyzed using the Chi-square test. Differences in mean quantitative values were analyzed by the Mann–Whitney *U* test. The date of hepatectomy was defined as time zero for calculations of recurrence rates. In the analysis of the overall recurrence rate, patients in whom HCC did not recur were censored, and those in whom HCC recurred were not censored. In the analysis of the intrahepatic metastasis recurrence rate, patients in whom HCC did not recur or patients with multicentric HCC recurrence were censored, and those in whom HCC recurred as intrahepatic metastases were not censored. In the analysis of the multicentric recurrence rate, patients in whom HCC did not recur were censored and patients with multicentric HCC recurrence were not censored, while those in whom HCC recurred as intrahepatic metastases were excluded from the analysis. The Kaplan–Meier method [23] was used to calculate recurrence rates, and the log-rank test [24] was used to analyze differences.

The Cox proportional hazards model [25] was used for univariate and multivariate analyses of factors related to recurrence. Variables analyzed included patient age and sex, Child-Pugh class (A/B), tumor size, number of tumors (single/multiple), differentiation of resected HCC (well-differentiated/moderately or

Table 2. Univariate and multivariate analyses of factors associated with post-operative recurrence in HCC patients (n = 77).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	0.9943 (0.9535-1.0396)	0.7974	-	
Sex				
Male	1			
Female	1.0068 (0.6818-1.4290)	0.9711	-	
Child-Pugh class*				
A	1			
B	0.0428 (0.0198-1.5669)	0.2068	-	
Tumor size	0.9376 (0.7179-1.1700)	0.5935	-	
Number of tumors				
Single	1			
Multiple	1.0419 (0.5669-1.6643)	0.8792	-	
Differentiation**				
Well-	1		1	
Moderately/poorly	1.5871 (1.0958-2.4354)	0.0134	1.6536 (1.1381-2.5445)	0.0073
Growth pattern**				
Expansive	1			
Infiltrative	1.1101 (0.6798-1.6625)	0.6487	-	
Portal vein invasion**				
Absent	1		1	
Present	1.5659 (1.0161-2.2813)	0.0428	1.7818 (1.1388-2.6597)	0.0134
Non-hypervascular hypointense nodules				
Absent	1		1	
Present	1.9396 (1.3615-2.7222)	0.0004	2.1767 (1.5089-3.1105)	0.0001

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

poorly differentiated), growth pattern of resected HCC (expansive growth/infiltrative growth), portal vein invasion of resected HCC (absent/present), and presence of non-hypervascular hypointense nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (absent/present). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC). All p values were derived from 2-tailed tests, with $p < 0.05$ accepted as statistically significant.

Results

Patients characteristics and imaging findings

Patients consisted of 56 males and 21 females with a mean age of 68.3 ± 7.6 years (range, 46–82 years). A total of 40 non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI in 28 of 77 patients (36.4%). The size of non-hypervascular hypointense nodules was 1.17 ± 0.38 cm (range, 0.4–2.1 cm). Two of 40 non-hypervascular hypointense hepatic nodules (5.0%) were identified by T2-weighted sequence as high-intensity nodules. The other 38 non-hypervascular hypointense hepatic nodules were not identified either by T1- and T2-weighted sequences. Two nodules were located in segment II of the liver, 7 in III, 1 in IV, 10 in V, 6 in VI, 4 in VII, and 10 in VIII, respectively. Among 28 patients with non-hypervascular hypointense nodules, 19 patients had one non-hypervascular hypointense nodule, 6 patients had 2 nodules, and the remaining 3 patients had 3 nodules. Non-hypervascular hypointense nodules were resected along with HCC lesions during hepatectomy in 10 patients, because they were included within the intended area of resection. Therefore, we categorized these 10 patients and the 49 patients in whom non-hypervascular hypointense nodules were not detected by preoperative Gd-EOB-DTPA-enhanced MRI as the hypointense nodule (−) group and the remaining 18 patients who had residual hypointense nodules after hepatectomy as the hypointense nodule (+) group. Of 13 hypointense nodules in 10 patients resected along with HCC at hepatectomy, 3 nodules were diagnosed as well-differentiated HCC and the remaining 10 nodules were diagnosed as dysplastic nodules on pathologic examination.

Table 1 compares the preoperative characteristics of the study patients. No differences were found in patient age and sex, etiology, liver function, and tumor progression as evaluated by preoperative imaging examinations and by post-operative pathologic examinations. Multiple HCC nodules were resected in 6 patients (10.2%) of the hypointense nodule (−) group and 3 patients (16.7%) of the hypointense nodule (+) group, without difference in proportions. No difference was observed in the length of follow-up period.

Recurrence rate after hepatectomy according to the presence of non-hypervascular hypointense nodules detected during preoperative gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI

We determined the recurrence rate in patients after hepatectomy with curative intent based on the presence of non-hypervascular hypointense hepatic nodules identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (Fig. 1). The recurrence rate was significantly higher in patients in the hypointense nodule (+) group than in the hypointense nodule (−) group ($p < 0.0001$). In univariate analysis, HCC differentiation and portal vein invasion were identified as factors associated with the rate of recurrence after hepatectomy along with preoperative non-hypervascular hypointense nodules by Gd-EOB-DTPA-enhanced MRI. In multivariate analysis, these factors were confirmed to be independently associated with the rate of recurrence (Table 2). Among 18 patients in the hypointense nodule (+) group, recurrence was observed in 7 of 11 patients with one non-hypervascular hypointense nodule, whereas recurrence was observed in all 7 patients with multiple non-hypovascular hypointense nodules.

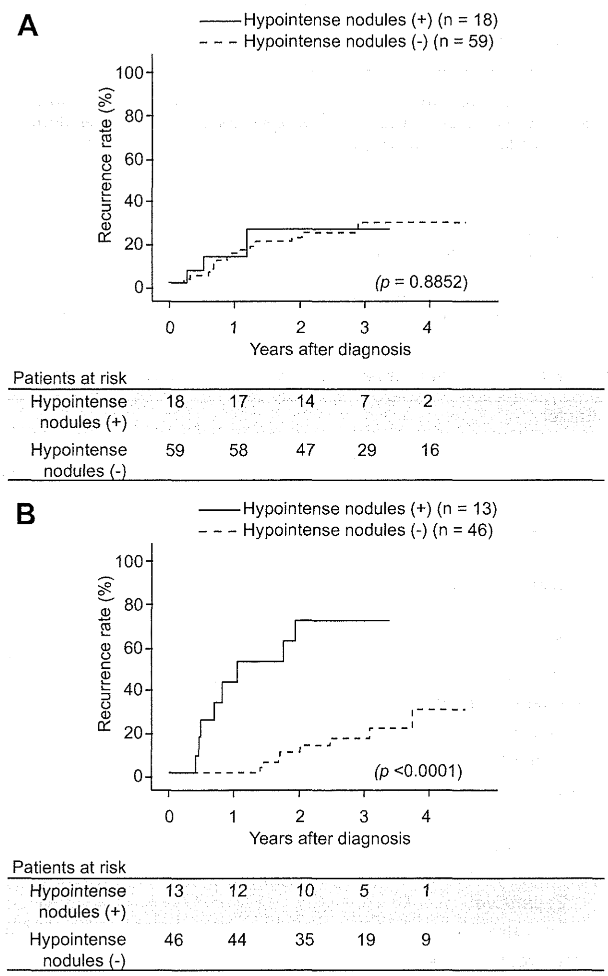

Fig. 2. Recurrence rate after hepatectomy according to the patterns of recurrence. (A) Rates of intrahepatic metastasis recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI. (B) Rates of multicentric recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI, among 59 patients, excluding 16 patients with intrahepatic metastasis recurrence.

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Table 3. Univariate and multivariate analyses of factors associated with post-operative intrahepatic metastasis recurrence in HCC patients (n = 77).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	0.9825 (0.9265-1.0470)	0.5743	-	
Sex				
Male	1			
Female	0.9022 (0.4784-1.5192)	0.7148	-	
Child-Pugh class*				
A	1			
B	0.0242 (0.0059-2.1819)	0.3573	-	
Tumor size	1.0051 (0.6929-1.3406)	0.9755	-	
Number of tumors				
Single	1			
Multiple	0.7038 (0.1655-1.5643)	0.4504	-	
Differentiation**				
Well-	1		1	
Moderately/poorly	1.7843 (1.0185-3.7176)	0.0424	1.6742 (0.9520-3.4993)	0.0769
Growth pattern**				
Expansive	1			
Infiltrative	0.9266 (0.3678-1.7453)	0.8365	-	
Portal vein invasion**				
Absent	1		1	
Present	2.1224 (1.2405-3.4608)	0.0079	2.0041 (1.1672-3.2828)	0.0138
Non-hypervascular hypointense nodules				
Absent	1			
Present	1.0474 (0.5012-1.8442)	0.8864	-	

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

Patterns of recurrence after hepatectomy according to the presence of non-hypervascular hypointense nodules detected during preoperative gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI

Among 30 patients with HCC recurrence after hepatectomy, 16 patients (53.3%) had intrahepatic metastasis recurrence and 14 patients (46.7%) had multicentric recurrence. There was no difference in the rate of intrahepatic metastasis recurrence between patients in the hypointense nodule (+) group and those in the hypointense nodule (−) group ($p = 0.8852$). In contrast, patients in the hypointense nodule (+) group had a significantly higher rate of multicentric recurrence than patients in the hypointense nodule (−) group ($p < 0.0001$, Fig. 2). Univariate and multivariate analyses revealed that portal vein invasion was independently associated with intrahepatic metastasis recurrence but preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI was not associated with intrahepatic metastasis recurrence (Table 3). The presence of preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI was the only factor associated with multicentric recurrence in univariate and multivariate analyses (Table 4). Among 8 HCCs that recurred multicentrically in the hypointense nodule (+) group, 6 nodules (75.0%) had existed as non-hypervascular hypointense hepatic nodules on Gd-EOB-DTPA-enhanced MRI before hepatectomy and progressed to hypervascular HCC tumors (Fig. 3), while the other 2 nodules (25.0%) newly occurred as multicentric recurrence after hepatectomy.

Discussion

Although one study reported that dysplastic nodules and early, well-differentiated HCC can be differentiated based on findings on Gd-EOB-DTPA uptake [26], differentiation of early, non-hypervascular HCC from dysplastic nodules within hypointense nodules is not actually feasible and controversial [27]. In addition, it is nearly impossible to characterize these hepatic nodules specifically using US or MDCT. Therefore, a histological diagnosis should be obtained with percutaneous liver biopsy under US guidance. However, this is not always possible due to the need for multiple samples and its invasive nature. Therefore, we did not resect these hepatic nodules during hepatectomy, except for nodules located within the hepatectomy field.

This study demonstrates a higher rate of recurrence of HCC in patients in whom non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI. This large difference in the recurrence rates indicated that the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI is a risk factor for recurrence of HCC after hepatectomy. Although we did not find differences in the rate of intrahepatic metastasis recurrence according to the non-hypervascular hypointense hepatic nodule status, we found a significantly higher rate of multicentric recurrence in patients with preoperative concurrent non-hypervascular hypointense hepatic nodules. In addition, the majority of multicentric recurrences involved the hypervascularization of non-hypervascular

Table 4. Univariate and multivariate analyses of factors associated with post-operative multicentric recurrence in HCC patients (n = 59).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	1.0047 (0.9359-1.0823)	0.8985	-	
Sex				
Male	1			
Female	1.0701 (0.5999-1.7781)	0.8038	-	
Child-Pugh class*				
A	1			
B	0.0664 (0.0176-5.7947)	0.7029	-	
Tumor size	0.9517 (0.6300-1.2943)	0.7801	-	
Number of tumors				
Single	1			
Multiple	1.1331 (0.4469-2.1714)	0.7510	-	
Differentiation**				
Well-	1			
Moderately/poorly	1.5198 (0.8959-2.8769)	0.1249	-	
Growth pattern**				
Expansive	1			
Infiltrative	1.3486 (0.7124-2.2884)	0.3270	-	
Portal vein invasion**				
Absent	1			
Present	1.2908 (0.5077-2.4730)	0.5312	-	
Non-hypervascular hypointense nodules				
Absent	1		1	
Present	2.8436 (1.6900-4.8407)	0.0002	2.8436 (1.6900-4.8407)	0.0002

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

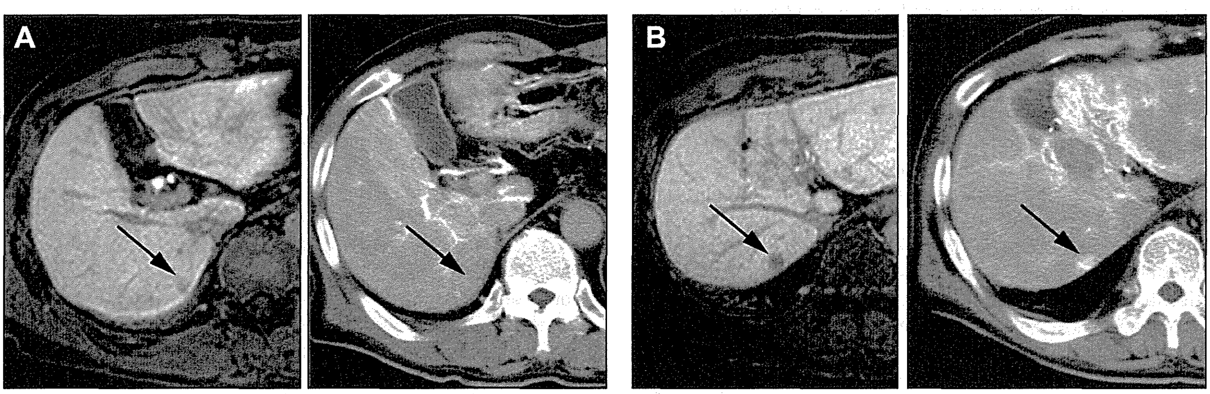

Fig. 3. Development of multicentric hepatocellular carcinoma in patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI. (A) Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (left panel) and computed tomography during hepatic arteriography (CTHA, right panel) before hepatectomy for hepatocellular carcinoma (HCC). In addition to the typical HCC located in segment VIII, a hypointense hepatic nodule was detected in segment VI during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (arrow). No hypervascular nodule was detected at this site by CTHA (arrow). (B) Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (left panel) and CTHA (right panel) 10 months after hepatectomy for HCC. The nodule detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI showed minute growth in size, with clearer margin, compared to preoperative image (arrow). The hypervascularity of this nodule was identified by CTHA (arrow). This nodule was resected by re-hepatectomy and was diagnosed as HCC pathologically.

hypointense hepatic nodules observed preoperatively with Gd-EOB-DTPA-enhanced MRI. It is controversial whether all non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI have the potential to progress to typical, hypervascular HCC. However, 26.5% of non-hypervascular hypointense nodules showed hypervascular spots with a long-term follow-up in our previous study [28]. In addition to the likelihood of non-hypervascular hypointense nodules

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progressing to HCC, the results of the present study suggest that the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI may indicate a high risk of multicentric recurrence of HCC after hepatectomy. Interestingly, multicentric recurrence was observed in all patients with multiple preoperative non-hypervascular hypointense nodules. While intrahepatic metastasis recurrence is considered as occurrence of metastasis of HCC that had been resected, multicentric recurrence is considered as new development of HCC that is not related to the resected HCC. Therefore, the presence of non-hypervascular hypointense nodules, especially multiple nodules, may indicate enhanced hepatocarcinogenesis even when the nodule itself does not progress to HCC.

There are several limitations to this study. The sample size was not large and the observation period was relatively short because Gd-EOB-DTPA has been in clinical use since February 2008 in Japan. In addition, the impact of the presence of non-hypervascular hypointense hepatic nodules on survival after hepatectomy was not analyzed because there were no patient deaths during the study period. However, we believe that our data should be shared with clinicians because of the markedly high rates of recurrence after hepatectomy in patients with preoperative non-hypervascular hypointense hepatic nodules. Further studies with more patients and a longer observation period are needed to confirm this observation. Furthermore, measures to suppress multicentric recurrence in patients with preoperative concurrent non-hypervascular hypointense hepatic nodules should be investigated in the future.

In conclusion, patients with preoperative concurrent non-hypervascular hypointense hepatic nodules, on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI, are at higher risk of HCC recurrence after hepatectomy. Clinicians should take this into consideration when determining the treatment modalities.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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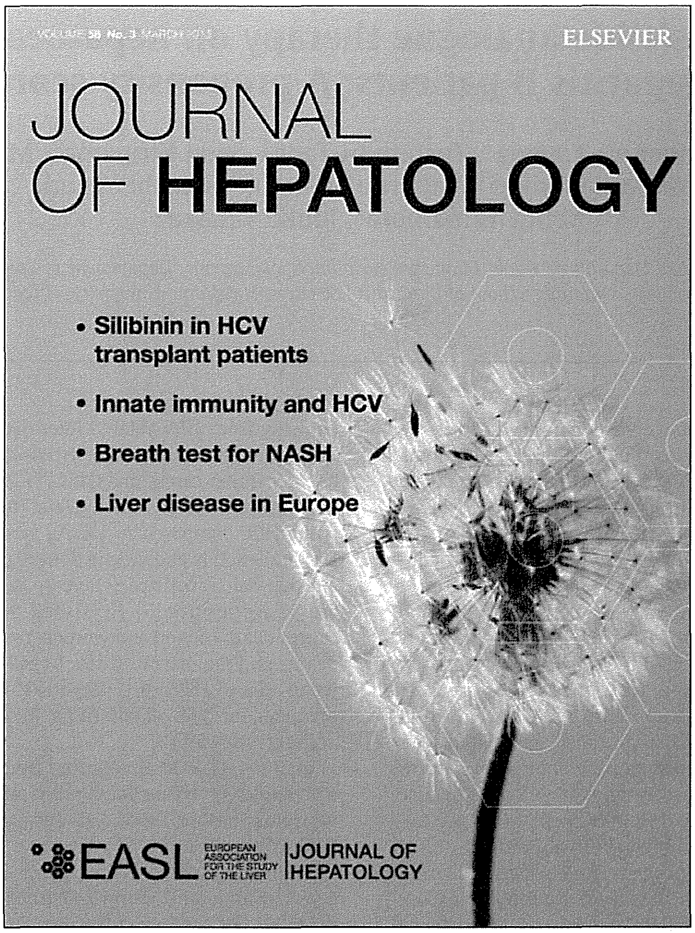
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Research Article

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Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: A propensity score analysis

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Background & Aims: Some patients with chronic hepatitis B virus (HBV) infection progress to hepatocellular carcinoma (HCC). However, the long-term effect of nucleos(t)ide analogue (NA) therapy on progression to HCC is unclear.
Methods: Therefore, we compared chronic hepatitis B patients who received NA therapy to those who did not, using a propensity analysis.
Results: Of 785 consecutive HBV carriers between 1998 and 2008, 117 patients who received NA therapy and 117 patients who did not, were selected by eligibility criteria and propensity score matching. Factors associated with the development of HCC were analyzed. In the follow-up period, HCC developed in 57 of 234 patients (24.4%). Factors significantly associated with the incidence of HCC, as determined by Cox proportional hazards models, include higher age (hazard ratio, 4.36 [95% confidence interval, 1.33–14.29], $p = 0.015$), NA treatment (0.28 [0.13–0.62], $p = 0.002$), basal core promoter (BCP) mutations (12.74 [1.74–93.11], $p = 0.012$), high HBV core-related antigen (HBcrAg) (2.77 [1.07–7.17], $p = 0.036$), and high gamma glutamyl transpeptidase levels (2.76 [1.49–5.12], $p = 0.001$).
Conclusions: NA therapy reduced the risk of HCC compared with untreated controls. Higher serum levels of HBcrAg and BCP mutations are associated with progression to HCC, independent of NA therapy.

Introduction

An estimated 350 million individuals worldwide are chronically infected with hepatitis B virus (HBV), of whom 1 million die annually from HBV-related liver disease [1]. Chronic HBV infection is recognized as a major risk factor for the development of hepatocellular carcinoma (HCC) [1,2]. Hepatitis B surface antigen (HBsAg)-positive patients have a 70-fold increased risk of developing HCC compared to HBsAg seronegative counterparts [3,4]. HBV infection is endemic in Southeast Asia, China, Taiwan, Korea, and sub-Saharan Africa, where up to 85–95% of patients with HCC are HBsAg positive [5]. HCC is the third and fifth leading cause of cancer death in men and women, respectively, and the number of deaths and the mortality rate from HCC have greatly increased in Japan since 1975 [6]. Hepatitis C virus (HCV)-related HCC accounts for 75% of all HCCs in Japan and HBV-related HCC accounts for 15% [6].

In 2004, Liaw *et al.* reported a significant reduction in HCC in 651 adults receiving lamivudine after adjustment for baseline variables (hazard ratio, 0.49 [95% confidence interval (95% CI), 0.25–0.99], $p = 0.047$) [7]. However, the results were not significant after exclusion of 5 patients who developed HCC within 1 year of randomization (0.47 [0.22–1.00], $p = 0.052$). Therefore, in 2009, the National Institutes of Health Consensus Development Conference concluded that there was insufficient evidence to assess whether nucleos(t)ide analogue (NA) therapy can prevent the development of HCC [8].

The long-term use of lamivudine has not been recommended because of tyrosine–methionine–aspartate–aspartate (YMDD) mutations, which have occasionally been associated with severe and even fatal flares of hepatitis [9,10]. Therefore, adefovir dipivoxil should be added immediately in patients with virological or biochemical breakthroughs or no response. Currently, there are 2 nucleoside agents (lamivudine, entecavir) and 1 nucleotide agent (adefovir dipivoxil) available for treatment of HBV infection in Japan. The agent with the higher genetic barrier to resistance, entecavir, is considered the initial drug of choice [11]. Recently, 3 studies on lamivudine suggested that long-term sustained viral suppression was associated with a reduced likelihood of developing HCC [12–14].

In this study, we sought to determine if NA therapy was associated with a reduction in the development of HCC. Since the validity of treatment effects in observational studies may be limited by selection bias and confounding factors, we performed a propensity analysis [15].

Keywords: HBcrAg; BCP; Gamma-GTP; Average integration value; HBV DNA.
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Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBcrAg, HBV core-related antigen; BCP, basal core promoter; gamma-GTP, gamma glutamyl transpeptidase.